Nutrition Management of Inherited Metabolic Diseases

Lessons from Metabolic University

Laurie E. Bernstein
Fran Rohr
Joanna R. Helm
Editors

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Preface

Nutrition Management of Inherited Metabolic Diseases: Lessons from Metabolic University is dedicated to the nutrition management of patients with inherited metabolic diseases (IMD). It presents a compilation of topics that have been taught at Metabolic University (MU), an interactive, didactic educational program that has trained over 350 metabolic dietitians/nutritionists, physicians, genetic counselors, and nurses since 2006. The purpose of MU, and thus the subject matter included within, is intended to assist the entry-level clinician with a broad understanding of the nutrition management of inherited metabolic diseases.

Each chapter in the book reflects both the author's literature review and insights from his or her clinical experience. For many disorders, there is no consensus in the literature regarding the nutritional management, likely because the incidence of IMD is low and randomized clinical trials on intervention strategies are rare. In addition, each disorder has a wide spectrum of disease severity. Recognizing that there are variations in practice, the precept of MU is that nutrition management of IMD is not a "cookbook." Rather, the key to management lies in understanding how the inactivity of an enzyme in a metabolic pathway determines which components of the diet must be restricted and which must be supplemented as well as the monitoring of appropriate biomarkers to make diet adjustments and ensure the goals of therapy are met. The goals of nutrition therapy are to correct the metabolic imbalance to lower the risk of morbidity and mortality associated with the disorder and to promote normal growth and development by providing adequate nutrition. Readers are encouraged to confer with their clinical teams with regard to management protocols specific to their institutions and to recognize that management of metabolic diseases is complex and guidance provided in the book may not apply to every clinical situation.

This book contains only subject matter covered at MU, and the chapters are authored by the experts who presented the material. Therefore, it is not a comprehensive treatise on IMD but rather a textbook on the most frequently encountered challenges in IMD nutrition. The book contains introductory chapters on nutrition and metabolism principles common to many metabolic disorders and disease-specific chapters on disorders of amino acid, fat, and carbohydrate metabolism. Appendix M contains an overview of nutrition management of IMD including those disorders for which there is not a specific chapter.

Feedback from MU participants in a published report regarding the efficacy and effectiveness of MU confirmed that they attended MU primarily to

obtain practical guidance on nutrition management for their IEM patients. Therefore, each nutrition chapter in the book highlights principles of nutrition management, how to initiate a diet, and biomarkers to monitor the diet. Diet calculations are another element of MU that appears at the end of each chapter. For some disorders, there is an online interactive tool that guides the reader through the steps of diet calculations.

The book is designed for day-to-day clinical use. We hope it helps you in your nutrition management of your patients with inborn errors of metabolism.

Aurora, CO, USA — Laurie E. Bernstein
Boston, MA, USA — Fran Rohr
Aurora, CO, USA — Joanna R. Helm

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- Metabolic University participants
- Our patients

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Part I

Background

Introduction to Genetics

1

Cynthia Freehauf

Contents

Core Messages

- Genes are short segments of DNA that carry information to make cellular proteins necessary for life.
- Mutations are heritable changes in the DNA nucleotide sequence of a gene.
- There are three broad categories of mutations: substitutions, insertions, and deletions.
- Molecular testing facilitates identification of genetic disease but carries risk for identification of uninterpretable and unsought information.
- Most single gene metabolic disorders are inherited as an autosomal recessive trait.

1.1 Background

The total number of cells in a human body is estimated to be 37.2 trillion [1]. In the nucleus of these trillions of cells are 46 chromosomes: one set of 23 chromosomes inherited from one's biological mother and a second set of 23 chromosomes inherited from one's biological father. Each chromosome is made up of tightly coiled strands of deoxyribonucleic acid (DNA). These strands of DNA contain genes that provide instructions, or codes, for making cellular proteins necessary for life. Proteins participate in a variety of biological processes and have a vast array of functions

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Box 1.1: Types of Cell Proteins

Function	Description	Example
Structural proteins	Provide support and structure for cells	Collagen
Messenger proteins	Transmit signals to coordinate biologic processes	Growth hormone
Antibodies	Detect and destroy microbes	IgG
Transport proteins	Bind and carry small molecules through the body	Carnitine transporter
Enzymes	Carry out thousands of chemical reactions required to keep the body working	Phenylalanine hydroxylase
Cell maintenance	Perform cell housekeeping duties, keeping the cell neat and orderly	Proteasome subunits

(Box 1.1). Each protein, when newly synthesized, starts as a linear chain of amino acids. Based on the properties of the amino acids in the chain, it then bends on itself forming a compact, functional structure with a size, shape, and set of chemical properties that enable it to perform its function.

1.2 From Genes to Proteins

The gene that encodes a particular protein determines the sequence of the amino acids in that protein. Genes, being short segments of DNA, are composed of four nucleotides: adenine (A), thymine (T), cytosine (C), and guanidine (G). The nucleotides function as chemical letters. Three adjacent nucleotides in a gene form a "codon," a unit of information denoting the specific amino acid to be incorporated into the amino acid chain (Fig. 1.1). The order of the nucleotides in a codon and the sequence of successive codons in a gene determine the sequence of the amino acids in the protein being made. This three-nucleotide genetic coding system was proposed and verified in the 1960s [2, 3]. It was then, and remains still, a profound discovery. Major contributors, Marshall Nirenberg, Har Gobind Khorna, and Robert Holley were awarded the Nobel Prize in medicine and physiology for their work on the project in 1968 [4].

Several important features of the three-nucleotide genetic coding system are illustrated in Table 1.1. First, the code is redundant but lacks ambiguity. For example, both TTC and TTT code for phenylalanine, thus the code is redundant; however, neither TTC or TTT code for any other amino acid, thus the code is not ambiguous. Secondly, there are three "stop codons," TAA, TAG, and TGA. These codons function as a punctuation mark, identifying the end of the gene. "Start codons" also exist. They do not act in isolation rather require additional factors. A start codon sets the "reading frame" for a gene. Thereafter each sequential set of three adjacent nucleotides form a codon.

Figure 1.2 depicts a short section of the gene phenylalanine hydroxylase, the emphasis here is on "short" as the gene is over 2,400 nucleotides long [5]. The first illustrated codon, ATG codes for the amino acid methionine (Met) and, is the start codon denoting the beginning of the reading frame for the protein; the second codon, TCC, codes for serine (Ser); and the third codon, ACT, codes for threonine (Thr) [5]. Look at the genetic code in Table 1.1 to determine which amino acid the last shown codon codes for in Fig. 1.2.

The amino acid sequence of a protein (as dictated by the nucleotide sequence of the gene) determines the final shape and chemical properties of the protein. Hydrophobic amino acids gravitate toward the center of the amino acid chain to avoid contact with water; hydrophilic amino acids move outward in search of water. In doing so a hydrophobic central core is created (Fig. 1.3). Positively charged amino acids then seek negatively charged amino acids, and accommodations are made for variances in amino acid shape and size. Subject to these intermolecular forces, the linear amino acid chain folds into a compact, three-dimensional tertiary structure.

The folding process is complex in the crowded cellular environment; hence, molecular chaperones assist. Chaperones are specialized molecules with cell housekeeping duties. They interact

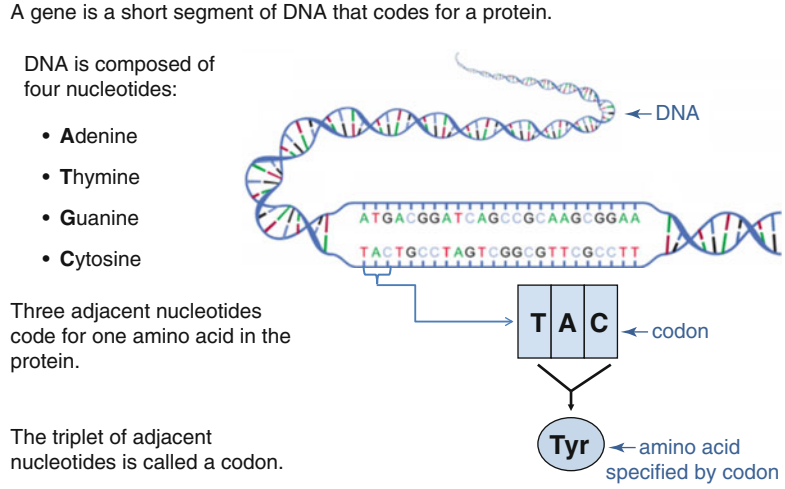

Fig. 1.1 In a gene, the DNA nucleotides function as letters spelling out which amino acids are to be incorporated into the protein

Table 1.1 Amino acid abbreviations and DNA codons

Genetic code			
	AA abbreviations		
Amino acid (AA)	3-letter	1-letter	DNA codons
Alanine	Ala	A	GCT, GCC, GCA, GCG
Arginine	Arg	R	CGT, CGC, CGA, CGG, AGA, AGG
Asparagine	Asn	N	AAT, AAC
Aspartic acid	Asp	D	GAT, GAC
Cysteine	Cys	C	TGT, TGC
Glutamine	Gln	Q	CAA, CAG
Glutamate	Glu	E	GAA, GAG
Glycine	Gly	G	GGT, GGC, GGA, GGG
Histidine	His	H	CAT, CAC
Isoleucine	Ile	I	ATT, ATC, ATA
Leucine	Leu	L	CTT, CTC, CTA, CTG, TTA, TTG
Lysine	Lys	K	AAA, AAG
Methionine	Met	M	ATG
Phenylalanine	Phe	F	TTT, TTC
Proline	Pro	P	CCT, CCC, CCA, CCG
Serine	Ser	S	TCT, TCC, TCA, TCG, AGT, AGC
Threonine	Thr	T	ACT, ACC, ACA, ACG
Tryptophan	Trp	W	TGG
Tyrosine	Tyr	Y	TAT, TAC
Valine	Val	V	GTT, GTC, GTA, GTG
Stop codons			TAA, TAG, TGA

with a newly synthesized unfolded or partially folded amino acid chain and promote folding and stabilization based on intermolecular forces. The resultant folded structure, by genetic design, is functional. A folded protein may function in the cell in isolation or it may connect with other proteins to form a functional unit. For example, phenylketonuria (PKU) is caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH). This enzyme has two forms. One is a homodimer made from the union of two identical amino acid chains, both encoded by the phenylalanine hydroxylase gene [6]. The other is a homotetramer made from the union of four identical amino acid chains, all encoded by the phenylalanine hydroxylase gene [6]. In contrast maple syrup urine disease (MSUD) is caused by a deficiency of the enzyme branched-chain keto acid dehydro-

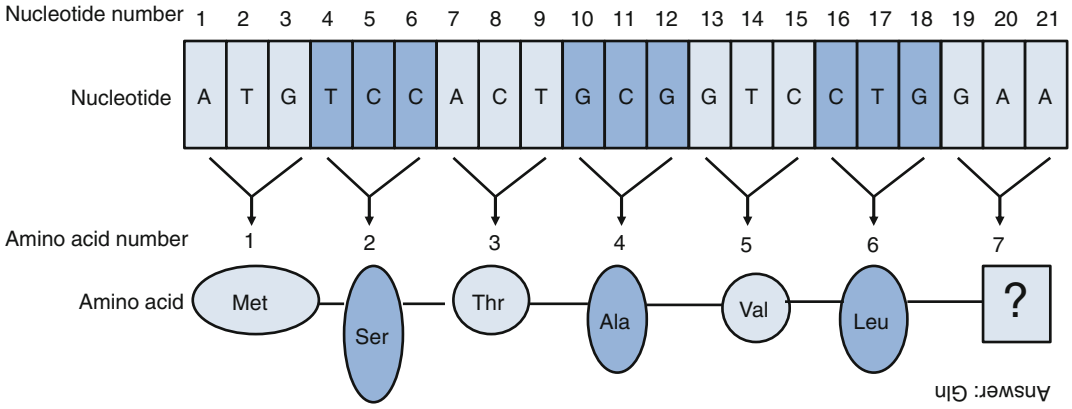

Fig. 1.2 A short segment of the gene for phenylalanine hydroxylase and the amino acid sequence of the protein generated from the gene. The successive triplet letter codons specify which amino acid is to be incorporated into the protein. Both the gene's nucleotides and the protein's amino acids are numbered sequentially to allow for reference to them

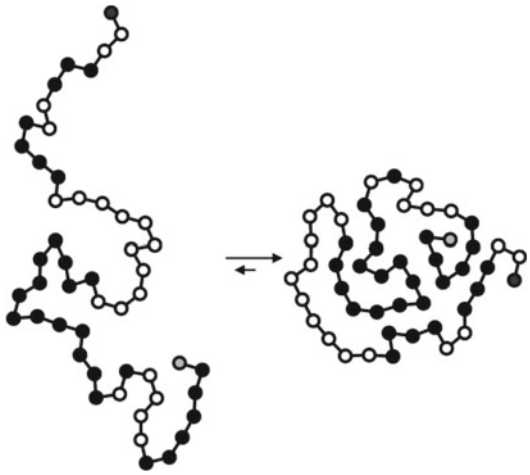

Fig. 1.3 The linear chain of amino acids (*left side*) assumes a compact, folded shape (*right side*) as hydrophobic amino acids (*dark dots*) gravitate toward the center core, promoting protein folding

genase (BKAD). This enzyme is a heterotetramer made up of four different proteins encoded by four different genes [7].

1.3 Genetic Mutations

A genetic mutation is a permanent, heritable change in the DNA nucleotide sequence of a gene. Depending on how the DNA sequence is modified, mutations can be categorized into three broad categories: substitution, insertion, and deletion.

1.3.1 Substitution Mutations

A substitution mutation results in the replacement of one nucleotide for another. This type of mutation alters one condon in the gene. Due to the redundancy in the genetic code, the altered codon may encode the same amino acid. In this case there will be no change to the amino acid sequence and no change to the synthesized protein. This is called a silent mutation (Table 1.2). More often, the altered codon codes for a different amino acid. This changes one amino acid in the protein. This is called a missense mutation (Table 1.2). Lastly, and relatively less often, a substitution mutation will generate a premature stop codon. The newly generated stop codon terminates reading of the gene and incorporation of amino acids into the protein. The result is a shortened, truncated protein. This is called a nonsense mutation (Table 1.2).

1.3.2 Insertion Mutations

An insertion mutation results in the addition of one or more DNA nucleotides to a gene. If the number of inserted nucleotides is three (or a

Table 1.2 A description of silent, missense, and nonsense mutations

Substitution Mutations				
Normal reference DNA and amino acid sequence.				
DNA nucleotides	C C A	A G T	T A C	T T T
Amino Acids	Pro	Ser	Tyr	Phe
Substitution, silent. No effect on protein sequence.				
DNA nucleotides	C C A	A G **C**	T A C	T T T
Amino Acids	Pro	Ser	Tyr	Phe
Substitution, missense. Results in an amino acid substitution.				
DNA nucleotides	C C A	**G** G T	T A C	T T T
Amino Acids	Pro	**Gly**	Tyr	Phe
Substitution, nonsense. Results in premature stop codon & termination of protein.				
DNA nucleotides	C C A	A G T	T A **G**	T T T
Amino Acids	Pro	Ser	**STOP**	

The reference DNA and amino acid sequence is noted on *top*. Underlined nucleotides indicate a nucleotide substitution. Changes in nucleotide sequence and amino acid sequence are noted in bolded *red*

number divisible by three), the result is an addition of one (or more) codon(s) to the gene and one (or more) amino acid(s) to the protein produced. This is called an in-frame insertion (Table 1.3).

If the number of inserted nucleotides is one, two, or another number not divisible by three, the codon reading frame is changed beginning at the point of the nucelotide(s) insertion. The impact of the mutation on the gene is therefor two fold: (1) the condon at the mutation site is altered by the insertion and (2) all codons distal to this are altered by the shift in codon reading frame. The protein is similarly affected; all amino acids at and distal to the mutation site are altered (Table 1.3). Frequently, change in codon reading frame results in generation of a premature stop codon.

1.3.3 Deletion Mutations

A deletion mutation results in the loss of one or more DNA nucleotides from a gene. The results are analogous to insertion mutations. If three

Table 1.3 A description of in-frame and frameshift insertion mutations

Insertion Mutations				
Normal reference DNA and amino acid sequence.				
DNA nucleotides	C C A	A G T	T A C	T T T
Amino Acids	Pro	Ser	Tyr	Phe
Insertion, in-frame. Results in the addition of one codon and one amino acid. No effect on subsequent codons or amino acids.				
DNA nucleotides	C C A	**G T A**	A G T	T A C
Amino Acids	Pro	**Val**	Ser	Tyr
Insertion, frameshift. Results in shift of reading frame. All codons and amino acids at and distal to the insertion are altered.				
DNA nucleotides	C C A	A G **G**	**T T A**	**C T T**
Amino Acids	Pro	**Arg**	**Leu**	**Leu**

The reference DNA and amino acid sequence is noted on *top*. Underlined nucleotides indicate a nucleotide substitution. Changes in nucleotide sequence and amino acid sequence are noted in bolded *red*

nucleotides (or a number divisible by three) are deleted, the result is a deletion of one (or more) codon(s) to the gene and one (or more) amino acid(s) in the protein being synthesized. This is called an in-frame deletion. If the number of deleted nucleotides is not divisible by three, the codon reading frame is changed. All codons at and distal to the mutation are altered as are their corresponding amino acids. This is called a frameshift deletion. As with insertion mutations, the shift in reading frame frequently results in the generation of a premature stop codon.

1.3.4 Mutation Effects

A mutation's effect on health and well-being is dependent upon the gene involved and the effect of the mutation on the protein it encodes. Possible effects include both gain of protein function and loss of protein function. In inherited metabolic disorders, the most common effect is loss of protein function. Loss of function may be due to an alteration of DNA sequences critical to the protein's activity or function. For example, a mutation may reduce or abolish the catalytic properties of an enzyme. Loss of function may also be due to mutations that drastically decrease the protein's abundance in the cell. This includes mutations that alter DNA sequences critical to protein folding. Improper or misfolded proteins are unstable proteins that are flagged as aberrant and rapidly destroyed by the cells' "garbage system," the proteasomes. Mutations that result in the loss of protein expression or that alter protein subcellular localization are other causes of decreased protein abundance.

Box 1.2: Genetic Mutation Nomenclature

Describing changes in a gene		
Example 1	*c.76A>C*	Denotes an A to C substitution at nucleotide 76 in the coding region for the gene
Example 2	*c.76delA*	Denotes deletion of A at nucleotide 76 in the coding region for the gene
Example 3	*c.76_78delACT*	Denotes deletion of ACT at nucleotides 76 to 78 in the coding region for the gene
Describing changes in a protein		
Example 4	*p.Arg22Ser*	Denotes a substitution of arginine for serine at amino acid 22 in the protein. Amino acid referenced by three-letter abbreviation
Example 5	*p.R22S*	Denotes a substitution of arginine for serine at amino acid 22 in the protein. Amino acid referenced by single-letter abbreviation
Example 6	*p.Arg22del*	Denotes a deletion of arginine at amino acid 22 in the protein

Ref. [15]

Missense mutations and small insertion/deletion mutations frequently result in protein misfolding. In fact, this is a common finding in PKU [8–10], medium-chain acyl-CoA dehydrogenase (MCAD deficiency) [11], and galactosemia [12, 13]. Nonsense mutations commonly lead to a short, truncated protein, or loss of protein expression. The frequency of the type of mutation varies with disease. In PKU the two most common types of mutations are missense mutations (60.1 %) and deletions (13.4 %) [14].

Mutations that fully abolish protein function are referred to as null mutations. Null mutations generally result in severe disease. Mutations that reduce but do not abolish protein function may result in less severe disease. For example, two null mutations in the enzyme phenylalanine hydroxylase result in classic PKU. A less severe mutation results in moderate or mild hyperphenylalaninemia.

1.4 Mutation Nomenclature

Mutations can be described based on the change in the gene and/or the change in the protein. Standard methods of reporting mutations have been developed [15]. A description of a mutation at the gene level identifies which nucleotide(s) has (have) been changed and type of mutation. Substitutions are denoted by the character ">" (meaning changed to), insertion by "ins", and deletion "del". A "c." preceding the mutation description indicates the change is relative to the DNA reference sequence for the coding portion of the gene (Box 1.2; example 1, 2, and 3).

Similarly, a description of a mutation at the protein level identifies which amino acid(s) has (have) been changed. Both three-letter (Arg = arginine) and single-letter (R = arginine) amino acid abbreviations may be used (Box 1.2; examples 4 and 5). The type of mutation is also described. Substitutions are denoted without the specific ">" character used to report DNA mutations. Deletion and insertion are denoted using "del" or "ins". A "p." preceding the mutation description indicates the change is relative to the protein's amino acid sequence (Box 1.2; examples 4, 5, and 6).

1.5 Molecular (DNA) Testing

The terms "molecular testing" and "DNA testing" are often used interchangeably. Both refer to testing techniques that allow for identification of nucleotides in a segment of DNA. This allows for the detection of the DNA sequence variation due to substitution, insertion, and deletion mutations, hence the ability to identify genetic disorders. This provides diagnostic information that allows for disease-specific treatment and knowledge of

recurrence risk. It also allows for treatment based on mutation effect. For example, chaperone therapy becomes a consideration if the disorder is caused by a missense mutation resulting in protein instability. Use of sapropterin dihydrochloride for treatment of PKU exemplifies this [16–18]. For disorders caused by nonsense (premature stop codon) mutations, drugs that read-through the stop codon and restore normal protein production are being developed and hold promise for the future.

A variety of molecular testing approaches and methods exist. Sequence analysis determines the precise order of the nucleotides in a segment of DNA. Sanger sequencing (developed by Frederic Sanger and colleagues in the 1970s) has long been the gold standard method [19, 20]. It is highly accurate and well suited for targeted molecular studies, for example, investigation of a mutation known to occur in the family or the investigation of one or two genes. Next-generation ("next-gen") sequencing (NGS) is relatively new to the scene. It utilizes techniques which dramatically decrease the time and cost of sequencing. This makes it an attractive option when the diagnostic differential is wide. NGS allows for large gene panel testing. These panels sequence known genetic causes for disorders with shared symptoms, such as neonatal onset seizure. A single panel can contain 100 genes or more, thereby eliminating the need for repeat rounds of single-gene testing. NGS also allows for the sequencing of all protein-encoding genes. This is referred to as exome (or whole exome) sequencing. Exome sequencing covers between 92 and 97 % of all 22,000 protein-coding genes. This approach is comprehensive and untargeted, allowing for the identification of unsuspected and unrecognized causes of genetic disease. While relatively new in use, the limited data suggests diagnostic utility [21].

Both Sanger and NGS have a limited ability to detect large deletions and duplications. Identification of such changes generally necessitates the use of other techniques. Options include array comparative genomic hybridization (aCGH) analysis, multiplex ligation-dependent probe amplification (MLPA) analysis, and quantitative PCR (qPCR) analysis. All three techniques allow for targeted, single-gene studies; aCGH analysis also allows for untargeted exome studies.

Dependent on testing method and approach used, there are risks for missing a clinically significant mutation, identifying a variant of unclear significance, and identifying incidental findings. Variants of unclear significance are generally novel DNA findings with unclear impact on protein function. This can make the relation of the discovered variant to the diagnosis ambiguous, neither confirming it nor ruling it out. Incidental findings are those that may be medically relevant but are unrelated to the primary reason for testing. This could be identification of carrier status for, susceptibility to, or presence of another disorder. Management of this unsought information raises moral and ethical challenges [22–25]. As one moves from single-gene testing to large gene panel testing to exome testing, the number of genes tested increases, while the focus of investigation decreases. With this, both the risk for identifying a variant of unclear significance and the risk for identifying incidental findings increase.

The challenge in genetic testing lies in selecting the approach that both minimizes the risks and maximizes the ability of the clinician to provide a diagnosis. Determination of the best testing approach requires assessment of the patient's clinical findings, family history, and the inherent advantages and disadvantages of the testing methods available. In all cases, pretest genetic counseling of the patient and the patient's family regarding the test's limitations and risk for

Box 1.3: Examples of Homozygous and Heterozygous States

State	Galactose-1-phosphate uridyltransferase (*GALT*) gene
Homozygous	
Not affected	Normal/Normal
Affected	p.Q188R/p.Q188R
Heterozygous	
Carrier, not affected	Normal/p.Q188R
Compound heterozygous, affected	p.Q188R/p.H319Q

uninterpretable and unexpected results is essential. Where there is risk for incidental findings, clear expectations for which results will and will not be shared with the patient should be established prior to testing [26].

1.6 Genotype and Phenotype

DNA testing determines the genotype, or genetic constitution, of an individual at the location(s) investigated. Since humans inherited a set of chromosomes from each of their biological parents, there are two copies or versions of most genes. The term allele refers to the version of a gene. If the two versions, or alleles, are identical, the person is described as being homozygous at that location. If the two alleles are different, they are described as being heterozygous at that location (Box 1.3). In the setting of autosomal recessive disorders, an individual who is heterozygous unaffected, with one normal and one disease-causing allele, is called a carrier. An individual who is heterozygous affected, having different mutations at each allele, is said to be compound heterozygous (Box 1.3).

In contrast to genotype, the term phenotype refers to the observable physical and biochemical characteristics of individuals. If genotype predicts phenotype (e.g., disease severity or responsiveness to therapy), the correlation between the two is strong and can be used to suggest outcome and drive the management of the disease.

The term phenocopy refers to an environmentally induced variation that closely resembles a genetically determined variation. For example, dietary vitamin B_{12} deficiency is a phenocopy of the inherited disorder, methylmalonic acidemia and homocystinuria due to cobalamin C disease. Both dietary vitamin B_{12} deficiency and cobalamin C disease have the biochemical findings of elevated plasma methylmalonic acid and homocysteine levels. Awareness of phenocopies is important as they provide an alternative explanation for clinical findings. For example, in the case of elevated

Box 1.4: Single-Gene Inheritance Patterns

Mode of inheritance	Features	Observations	Recurrence risk
Autosomal dominant	• Gene for disorder on numbered, non-sex chromosome • One copy of an altered gene is sufficient for disease	• Trait generally seen in each generation • An affected child has an affected parent • Males and females are equally affected	• An affected parent has a 50 % chance of passing the disease to their children with each pregnancy, independent of the child's sex
Autosomal recessive	• Gene for disorder on numbered, non-sex chromosome • Two copies of an altered gene are required for disease • Parents of an affected child are considered obligate carriers of the disease • Consanguineous couples are at an increase risk for having a child with a recessive disorder	• Trait generally seen in a child or in multiple children of unaffected parents • Affected children usually do not have affected parents • Males and females are equally affected	• Two carrier parents have a 25 % risk of having an affected child with each pregnancy, independent of the child's sex • The risk for an affected individual to have an affected child is dependent upon the carrier status of the individual's partner
X-linked	• Gene for disorder is on the X chromosome • Trait can be either dominant or recessive • Expression of X-linked recessive traits in women can be impacted by X-inactivation pattern	• Incidence of trait is higher in males than in females	• Affected fathers cannot pass X-linked traits to their sons • Carrier females (affected and non-affected) have a 50 % chance of passing down the altered gene to their sons and daughters

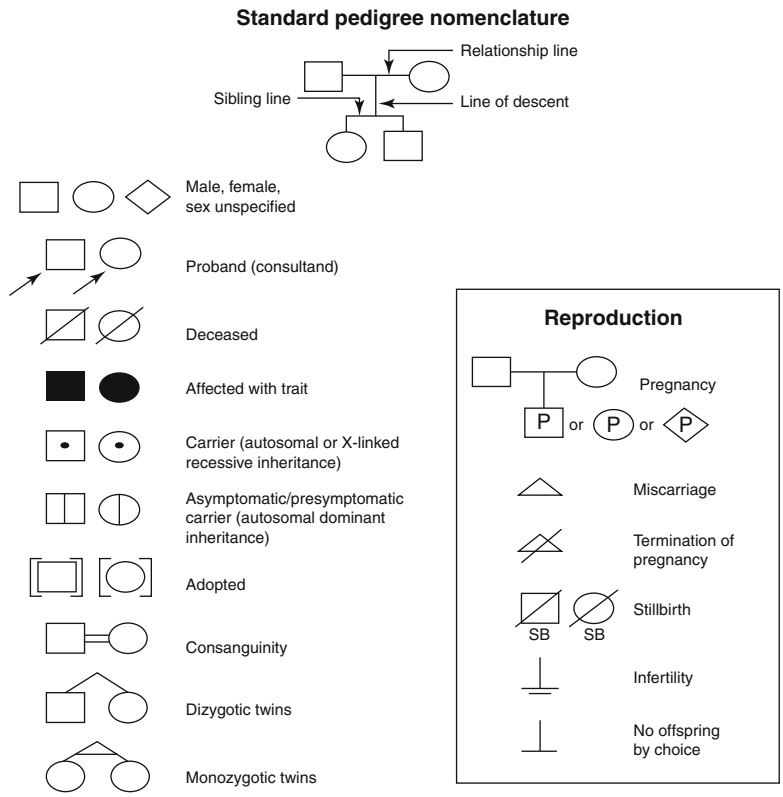

Fig. 1.4 Standard pedigree nomenclature. Common symbols are used to draw a pedigree (family tree). A pedigree shows relationships between family members and patterns of inheritance for certain traits and diseases. Figure source: The website of the National Cancer Institute (http://www.cancer.gov)

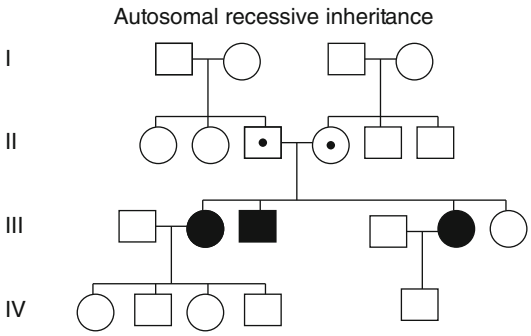

Fig. 1.5 An example of a pedigree. The clue that this is an autosomal recessive pattern lies in the occurrence of multiple affected children, of both female and male genders, being born to unaffected parents. Children born to carrier parents have a 25 % risk of being affected with the disorder

methylmalonic acid and elevated homocystinuria, one may want to exclude maternal vitamin B_{12} deficiency as a possible cause prior to testing for cobalamin C disease or other possible metabolic etiologies.

1.7 Single-Gene Inheritance Patterns and Pedigrees

There are three main patterns of single-gene inheritance (also called Mendelian inheritance, discovered by Gregor Mendel in the late 1800s): autosomal recessive, autosomal dominant, and X-linked (Box 1.4). In autosomal dominant and autosomal recessive inheritance, the gene for the disorder is located on one of the numbered 22 pairs of autosomes or non-sex-determining chromosomes. In the case of dominant inheritance, a single copy of the mutated gene results in disease. In the case of recessive inheritance, disease occurs only when two copies of the mutated gene are present. In X-linked inheritance, the gene for the disorder is on the X chromosome, not on one of the 22 autosomes. The trait can be either dominant or recessive. Mitochondrial and polygenetic inheritances exist as well but will not be addressed in this chapter.

X-linked recessive inheritance in females is impacted by a phenomenon known as X-inactivation or lyonization. A female's sex chromosomal constitution is XX; a male's sex chromosome constitution is in XY. In females, a recessive disease mutation on one X chromosome should not result in disease if the corresponding gene on the other X chromosome is normal. In actuality, the situation is more complicated. In females, one of the X chromosomes in each cell is randomly inactivated, a process known as X-inactivation. This is done to compensate for the double state of X chromosomes in females relative to males. If the inactivation pattern is skewed so that the majority of X chromosomes with the normal gene are inactivated, the individual may be affected. The greater the skewed inactivation pattern, the more severe the disease. Because of this, the clinical phenotype of females with an X-linked "recessive" disorder, such as ornithine transcarbamylase (OTC) deficiency, can range from unaffected to severely affected.

The majority of single-gene metabolic disorders is inherited as an autosomal recessive trait. For the most part, biochemical pathways are disrupted by mutations in genes encoding enzymes. These mutations adversely impact enzyme function. In the heterozygous state of one null mutation gene and one normal gene, enzyme activity is 50 % of normal. This is generally sufficient to maintain normal biologic function; hence, the trait is not seen.

A genetic family history or pedigree, detailing genetic relationships and medical history of family members, can help determine the inheritance pattern if it is unknown. Pedigrees can also identify individuals at risk for developing disease or passing on disease-causing genes. Standard symbols and terminology are used to identify individuals, relationships, and carrier or disease state [27] (Fig. 1.4). The use of symbols allows for a concise, graphic representation of a family's genetic health history [27]. It is a useful diagnostic and risk assessment tool (Fig. 1.5).

1.8 Summary

Our life and well-being depend upon the function of the thousands of proteins in each of our trillions of cells. Mutations in the genes that encode these proteins disrupt cellular function and can lead to disease. The symptoms of the disease and severity are dependent upon the protein and the degree to which it is impacted. Molecular testing allows for the identification of the underlying genetic change. It can predict disease severity, provide recurrence risk information, suggest and inform on likely pathophysiology, and provide strategies for intervention.

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Expanded Newborn Screening for Inherited Metabolic Diseases

2

Erica L. Wright

Contents

Core Messages

- The success of newborn screening for phenylketonuria (PKU) led to the expansion of newborn screening for other disorders, including many inborn errors of metabolism, thus preventing significant morbidity and mortality.
- Newborn screening is an integrated system requiring all aspects (birth hospital, newborn screening and confirmatory laboratories, primary care and specialty providers, and families) to work in conjunction with each other to ensure the best outcome of the patient.
- Limitations of newborn screening include timeliness of screening and diagnosis, false negatives, and disorders not included on the newborn screening panel.

2.1 Background

In the last 50 years, newborn screening has evolved to become one of the most successful public health initiatives and is considered the

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Fig. 2.1 Newborn heel-prick and spotting of blood onto filter paper for newborn screening (Photo courtesy of Erica Wright, MS, CGC)

gold standard as a population-based screening system. Prior to newborn screening, patients were diagnosed clinically only after symptoms manifested resulting in significant health and developmental sequelae, and even death. Other patients never received a diagnosis and therefore were never treated. Early attempts of population-based screening for inborn errors of metabolism began in the 1950s with phenylketonuria (PKU) after Dr. Horst Bickel showed that diet intervention resulted in improved outcome [2].

In 1957, Willard Centerwall developed the "diaper test" in which elevation of phenylalanine was detected in the urine of affected individuals with PKU by applying a ferric chloride solution to a wet diaper. This method was most often utilized in pediatric offices in older infants, thus still delaying the diagnosis and treatment of PKU [3, 4].

In 1959, Dr. Robert Guthrie developed a bacterial inhibition assay to detect elevation of phenylalanine in dried blood spots collected on filter paper [5, 6]. The presence of elevated phenylalanine in the blood spot resulted in bacterial growth. This method was sensitive enough to detect elevations of phenylalanine greater than 3–4 mg/dL (180–240 μmol/L). With an appropriate screening test available for early detection of PKU, Massachusetts became the first state in the United States to begin statewide newborn screening in 1963 (Fig. 2.1). By the late 1960s, newborn screening for PKU was mandated in a majority of the states.

The success of newborn screening for PKU led to the addition of other inborn errors of metabolism and endocrine disorders to the newborn screening panel. In fact, the bacterial inhibition technique was later implemented for newborn screening for maple syrup urine disease (elevated leucine) and homocystinuria (elevated methionine). Other laboratory techniques utilized in newborn screening include: enzymatic assays for other inborn errors of metabolism such as galactosemia and biotinidase deficiency, immunoassays for endocrine disorders, and electrophoresis for hemoglobinopathies [6]. Historically, each disorder required a separate screening test and was added to a panel after proving to meet the screening criteria set forth by World Health Organization in 1968 [1]. This report "Principles and Practice of Screening for Disease" authored by James Maxwell Glover Wilson and Gunner Jungner has been the standard of screening in the public health realm. In the United States, each individual state was at liberty to add disorders to their newborn screening panel and generally followed this criteria to determine if a disorder was suitable for inclusion on the newborn screening panel (Box 2.1).

Box 2.1: Criteria for Newborn Screening

Goal:

To provide early detection of children at increased risk for disorders in which promptly initiating treatment prevents a metabolic crisis and/or irreversible sequelae, thus improving outcome.

Principles of screening based on Wilson and Jungner's criteria [1]

1. The condition is an important health problem.
2. There is an acceptable treatment.
3. Facilities for diagnosis and treatment are available.
4. There is a recognizable latent or early symptomatic stage.
5. There is a suitable screening test.
6. The test is acceptable to the population.
7. The natural history of the condition is adequately understood.
8. There is an understanding of whom to treat as patients.
9. It is cost-effective.
10. Identification of affected patients is a continuing process.

2.2 Newborn Screening by Tandem Mass Spectrometry

In 1990, the application of tandem mass spectrometry (MS/MS) to the analysis of dried blood spots was first described by David Millington, which drastically changed the landscape of newborn screening [7]. During the 1990s, MS/MS proved to be a successful method for newborn screening with some states and private laboratories instituting the technology and better defining the sensitivity and specificity. MS/MS quantifies both amino acids and carnitine esters by using electrical and magnetic fields to separate and measure the mass of the charged particles. This allows for multiple biochemical parameters to be tested in a single dried blood spot, resulting in identification of over 30 inborn errors of metabolism including amino acidemias, urea cycle disorders, organic acidemias, and disorders of fatty-acid oxidation [8–11] (Table 2.1).

The case to add MS/MS to newborn screening was driven primarily by one disorder, medium-chain acyl-CoA dehydrogenase deficiency (MCAD). MCAD meets all of the traditional criteria for newborn screening. The prevalence of MCAD is similar to PKU with approximately 1 in 15,000 live births affected in the United States. MCAD typically presents in infants in late infancy, and/or during intercurrent illnesses with emesis or long periods of fasting. If not identified pre-symptomatically, mortality occurs in approximately 20 % of affected individuals. With appropriate management during illness, including glucose containing fluids and the avoidance of fasting, mortality is reduced essentially to 0 % [12, 13]. Screening for MCAD with MS/MS technology allows for the detection of multiple other inborn errors of metabolism simultaneously, without additional sampling or cost. Some disorders currently screened for by newborn screening do not meet the traditional criteria, either due to their rarity, limited understanding of natural history, or lack of an evidence-based effective treatment. Benefits argued in support of continued screening of these disorders include: the ability to initiate early treatment and possibly improve outcome, collection of long-term data on the course of the natural history of the disease, development of potential treatments, improved overall health of the affected individual, and genetic counseling for recurrence risks in future children [14, 15].

2.3 Standardization of Newborn Screening

By the start of the twenty-first century, with the advent of MS/MS newborn screening, the disorders included on the NBS panels varied from

Table 2.1 Disorders identified by newborn screening using tandem mass spectrometry (MS/MS)

Amino-acid disorders[a]	**Elevated analytes (amino acids)**[b]
Phenylketonuria (PKU) *or* hyperphenylalaninemia	Phenylalanine
Maple syrup urine disease (MSUD)	Leucine/isoleucine, valine
Homocystinuria (cystathionine synthase deficiency) *or* hypermethioninemia	Methionine
Tyrosinemia, type I and possibly type II or type III	Tyrosine (elevations may not be detectable on filter paper in the first days of life)
5-oxoprolinuria (glutathione synthetase deficiency)[a]	5-oxoproline
Urea cycle disorders[a]	
Citrullinemia	Citrulline
Argininosuccinic aciduria (ASA)	Citrulline, argininosuccinic acid
Argininemia[a]	Arginine
Fatty-acid oxidation disorders[a]	**Elevated analytes (acylcarnitines)**[b]
Short-chain acyl-CoA dehydrogenase deficiency (SCAD)	**C4**
Isobutyryl-CoA dehydrogenase deficiency (IBCD)	**C4**
Glutaric aciduria, type 2 (GAII) *or* multiple acyl-CoA dehydrogenase deficiency (MADD)	**C4**, **C5**, **C8**:**1**, C8, C12, C14, C16, C5-DC
Medium-/short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency (M/SCHAD)[a]	**C4-OH**
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	C6, **C8**, C10, C10:1
Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)	**C16-OH**, **C18**:**1-OH**
Trifunctional protein deficiency (TFPD)[a]	**C16-OH**, **C18**:**1-OH**
Very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	**C14**:**1**, C14, C16
Carnitine palmitoyltransferase deficiency, type 2 (CPTII)[a]	**C16**, **C18**:**1**, **C18**
Carnitine palmitoyltransferase deficiency, type 1A (CPT1A)[a]	**C0 elevated**, low C16, C18
Carnitine/acylcarnitine translocase deficiency (CACT)[a]	**C16**, **C18**:**1**, **C18**
Carnitine uptake defect (CUD)[a]	**Low C0** – may not be detected in first few days of life
Organic acid disorders[a]	**Analytes (acylcarnitines)**[b]
Propionic acidemia (PROP)[a]	**C3**
Methylmalonic acidemia (MMA)[a]	**C3**
Malonic aciduria (MA)[a]	**C3-DC**
Multiple carboxylase deficiency (MCD)	**C5-OH**
3-hydroxy 3-methylglutaric-CoA lyase deficiency (3HMG)	**C5-OH**
3-methylcrotonyl-CoA carboxylase deficiency (3MCC)	**C5-OH**
3-methylglutaconic aciduria (3MGA)	**C5-OH**
2-methylbutyryl-CoA dehydrogenase deficiency (2MBD)	**C5**
Isovaleric acidemia (IVA)	**C5**
Glutaric acidemia, type 1 (GAI)	**C5-DC**
Beta-ketothiolase deficiency (BKT)[a]	**C5**:**1**, C5-OH

MS/MS analytes are measured in micromoles/l (μmol/L)
Hydroxylation is designated (-OH); dicarboxylic acids are designated (-DC); unsaturation of fatty-acid is designated (:1)
[a]Some genotypes of these disorders may not be detected by newborn screening or are extremely rare (1:>250,000 live births)
[b]Primary MS/MS analyte(s) in **bold** type

state to state. Some states were quick to implement MS/MS and began screening for more than 40 disorders, while other states lagged behind and screened for only 3 disorders [16]. National organizations, such as the March of Dimes, as well as parent advocate groups, called for standardization of newborn screening panels across all states. At the federal level, the Health Resources and Services Administration commissioned the American College of Medical Genetics (ACMG)

to conduct an analysis of the scientific literature as well as gather expert opinion to develop a recommended uniform screening panel. In 2005, the recommended uniform panel was released with 29 disorders initially selected as part on the core panel [17]. Many of these core disorders were inborn errors of metabolism screened for by utilizing tandem mass spectrometry, resulting in the recommendation that all states were to perform MS/MS within 5 years of the establishment of the core panel. The process of adding disorders to the recommended uniform screening panel is now an undertaking of the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) as written through federal legislation [18]. New disorders are now nominated to the committee and undergo a lengthy evidence-based review process for inclusion on the recommended uniform screening panel (RUSP). Once recommended by the committee, the Secretary of Health and Human Services can either recommend or decline that a disorder is added to states' newborn panel [19, 20]. States currently retain the ability to add disorders at their own discretion.

2.4 The Newborn Screening Process

Newborn screening is an integrated system involving multiple entities including the birth hospital, public health department, the newborn screening lab, confirmatory laboratories, subspecialists, primary care providers, and families [21]. The process begins with the collection of blood via a heel prick, typically obtained at 24–48 h of age at the birth facility. The blood spots are collected on filter paper called the newborn screening card. The newborn screening card is then sent to either a state public health laboratory or a commercial laboratory. States with smaller birth frequencies might utilize another state's newborn screening lab or commercial lab. Newborns also get point of care screening at the birth hospital such as a hearing screen or pulse oximetry screening for heart defects. Once the lab has received the newborn screening card, the specimen is run in a timely manner with results optimally available within 24–48 h of receipt of the sample. If a specimen has an abnormal result, the public health department or subspecialists familiar with the flagged disorder conduct appropriate follow-up of the infant. In inborn errors of metabolism, timely follow-up is needed to ensure appropriate diagnosis and initiation of treatment prior to onset of symptoms. Appropriate biochemical studies will be requested on those infants with abnormal newborn screen results. In cases of severe disease or concerning newborn screening results, the infant may require immediate evaluation by a metabolic clinic to determine if the infant is symptomatic and if immediate treatment is necessary. Biochemical studies may prove to be diagnostic or additional studies such as gene sequencing or enzymatic studies may be indicated.

Terminology used in newborn screening includes true positive, false positive, and false negative (Box 2.2). An infant determined to have a disorder based on a positive newborn screening result and follow-up confirmatory studies is called a "true positive." An infant that has an abnormal newborn screening result but deemed not to have the disorder based on confirmatory studies is called a "false positive." An infant that has a normal newborn screen but later is determined to have a disorder is called a "false negative." Newborn screening programs attempt to identify all true positives while limiting false positives and false negatives by selecting appropriate cutoffs of the metabolites measured. Programs will track their "positive predictive values" as a quality indicator: a measurement of the true positives (the numerator) divided by the number of abnormal screens reported (the denominator) [6].

Box 2.2: Terminology Used in Newborn Screening

True positive = BOTH newborn screening and follow-up screening tests are POSITIVE

False positive = POSITIVE newborn screening but NEGATIVE follow-up screening test

False negative = NORMAL newborn screening but later determined to have the disorder

2.5 Limitations of Newborn Screening

2.5.1 Disorders that Present Early in Life

Some inborn errors of metabolism on the current newborn screening panel, such as organic acidemias and disorders of fatty-acid oxidation, have severe forms that may present as metabolic emergencies within the first days of life before newborn screening results are available. Timeliness of newborn screening is essential in order to limit morbidity and mortality. Turnaround time for newborn screening results will vary from state to state because most states utilize their own newborn screening labs as well as each have their own rules and regulations. Use of couriers instead of traditional mail for transport of samples to the newborn screening lab and testing of specimens on weekends improves turnaround time. A worst-case scenario is presented in the highlighted box above (Box 2.3).

2.5.2 Disorders that Have Risk of False Negatives

Newborn screening is not diagnostic. The cutoffs for a disorder to be flagged are established in order to ascertain all true positives while limiting the number of false positives. Metabolite levels (analytes) for affected infants may overlap significantly with unaffected infants. Case in point is the low-excretor phenotype of glutaric acidemia type I (GA-1) in whom affected individuals often do not excrete glutaric acid and 3-hydroxygluatric acid metabolites [14]. Newborn screening can miss other organic acidemias, aminoacidopathies, and fatty-acid oxidation disorders. Also, timing of the newborn screening will affect certain metabolites resulting in false positives. If a newborn screen specimen is obtained earlier than the recommended 24–48 h in aminoacidopathies, an affected infant may be missed, as the concentration of the metabolites have not yet become elevated in the blood to screen above the threshold. The opposite also holds true. If a newborn screen is obtained later, such as at a week or later

Box 2.3: Case Example of Delayed Newborn Screening

Mary, a newborn female infant, has a newborn screening card collected at 3 days of age on a Friday. The newborn screening card is dried overnight and placed in the hospital mail-room the following day, Saturday. The card is mailed from the hospital on Monday but does not get delivered to the newborn screening lab for analysis until Thursday.

The sample is run overnight and reported out to the local metabolic clinic on Friday with elevated propionylcarnitine. The differential diagnosis includes propionic acidemia, methylmalonic acidemias, maternal B_{12} deficiency, hyperbilirubinemia, and false-positive results.

The metabolic clinic immediately calls out the results to the provider of the infant, now 9 days old. The baby is in a neonatal intensive care unit due to prematurity but is reportedly doing well. However, the day the results are reported, the infant is no longer feeding well. The metabolic clinic requests that confirmatory testing of plasma acylcarnitine profile and urine organic acid screen be obtained.

Due to concern of the recent changes to the status of the infant, additional labs including a metabolic panel and plasma ammonia are recommended immediately. These labs show the baby is extremely acidotic as well as hyperammonemic. The infant is airlifted to a children's hospital for tertiary care by the metabolic team. However, due to the extent of the acidosis and hyperammonemia as well as the prematurity, care is terminated.

Postmortem studies indicate that the infant had methylmalonic acidemia, cobalamin A; a treatable form of B_{12} metabolism. Treatment with cobalamin injections and diet typically yields a good outcome.

Box 2.4: Case Example of a Missed Diagnosis During Newborn Screening

James, a 5-month-old male is brought to a local emergency room after his parents find him limp and lethargic in a crib in the morning. He had a 2-day history of a respiratory illness and had vomited once the night before. The infant is found to be extremely hypoglycemic with no ketones present. Further studies show him to be in liver failure with hyperammonemia. Parents report that his newborn screen was normal.

The baby is transported to the children's hospital for further tertiary care. The metabolic team is consulted and includes a disorder of fatty-acid oxidation on the differential diagnosis. The plasma acylcarnitine profile shows increased C16:OH and C18:1OH suggestive of either long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency or trifunctional protein deficiency. The infant is switched from breast milk to a metabolic formula low in long-chain fats and high in medium-chain triglycerides. The liver dysfunction significantly improves over the course of the hospital stay. At second glance of the newborn screen, the C16:OH and C18:1OH results were just below cutoff. The newborn screening lab has since changed the cutoffs.

of life, an affected individual with a long-chain fatty-acid oxidation disorder can be missed as long-chain carnitine esters decrease with age.

Some labs utilize second-tier testing to limit false positives while avoiding false negatives. Second-tier analysis is based on more conservative cutoffs, in order for more newborn screens to initially flag as abnormal. Abnormal newborn screens in tier one will undergo more specialized studies immediately from the same newborn screening card. Only those samples that have abnormal second-tier studies will be reported as abnormal. This limits the number of infants requiring additional confirmatory studies and thus parental anxiety [22] (Box 2.4).

The cutoffs for newborn screening are reevaluated by the newborn screening lab based on data collected and clinical experience. In recent years, worldwide collaboration has resulted in improved cutoff values based on cumulative data of true-positive cases thus improving sensitivity and specificity [23].

2.5.3 Metabolic Disorders Not Included on Newborn Screening

With the expansion of newborn screening in the last decade, it is common that many providers not familiar with inborn errors of metabolism are under the assumption that a "normal" newborn screen excludes inborn errors of metabolism in the differential diagnosis. While newborn screening is a very helpful tool, it is only one piece of the puzzle of a diagnostic work-up. If clinical concerns arise for an inborn error of metabolism, one should not rely on a newborn screening, but rather pursue further diagnostic work-up to investigate the possibility of a metabolic disorder (Box 2.5).

2.6 Future of Newborn Screening

The current trend of the expansion of newborn screening continues at a rapid pace. Many factors and contributors including technology, industry, researchers, parents, and politics drive this expansion. Multiple inborn errors of metabolism are currently being investigated for the potential of utilizing newborn screening for early diagnosis and initiation of treatment. The development of new technologies for screening opens up opportunity for new disorders to be added to the recommended uniform screening panel. For example, with the advent of whole-genome sequencing, newborn screening may change in the coming years. With the development of enzyme replacement therapies for lysosomal disorders, many of these disorders are now being considered for

Box 2.5: Case Example of a Metabolic Disorder Not Included on the Newborn Screening Panel

Camilla, a newborn female, was delivered in a forceps-assisted vaginal delivery after a normal pregnancy. The infant did well for the first 3 days of life, but began showing seizure-like activity. A CT scan showed a small trauma from the forceps-assisted birth including a small bleed and skull fracture. Laboratory studies obtained showed mild metabolic acidosis and mild hyperammonemia. The infant was transferred to the children's hospital for further tertiary care. Repeat plasma ammonia showed increasing hyperammonemia.

The metabolic team was consulted and obtained STAT biochemical labs including plasma acylcarnitine profile, plasma amino acids, urine organic acids, and urine orotic acid. Labs showed elevated orotic acid as well as a plasma amino-acid pattern consistent with ornithine transcarbamylase (OTC) deficiency. The newborn screen was normal. The infant was placed on a protein-restricted diet, supplemented with arginine, and started on nitrogen-scavenging medications.

OTC deficiency is the most common urea cycle disorder and is not routinely screened for on most states' newborn screening panel as it is difficult to establish cutoff for low concentrations of citrulline and arginine.

newborn screening. Parent advocacy groups' lobby for the addition of new disorders to states' newborn screening panels. Legislators may bypass the public health departments and pass laws mandating the addition of new disorders.

While newborn screening continues to progress forward, those in the field continue to advocate for strengthening the current system with improved testing and timely follow-up.

2.7 Resources

- http://www.babysfirsttest.org
- http://www.newsteps.org
- http://www.modimes.org
- http://www.newbornscreening.info
- http://www.savingbabies.org
- http://www.acmg.net/resources/policies/ACT/condition-analyte-links.htm

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Nutrition Education

3

Laurie E. Bernstein and Joanna R. Helm

Contents

Core Messages

- Health literacy is the degree to which individuals have the capacity to obtain, process, and understand basic information and services needed to make appropriate decisions regarding their health [1].
- Anticipatory guidance is an educational approach designed to help guide families and professionals in understanding what to expect during the current and/or approaching stage of development or treatment.
- There is a discrepancy between educational tools utilized in the clinic and the perceived effectiveness of these tools by the clinicians and patients.
- A different counseling approach and patient-provider interaction with the goal of improving sustainability of nutrition management is needed.

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3.1 Background

It is an exciting time to provide nutrition education in the field of inherited metabolic diseases. New products, therapies, and research breakthroughs are changing the landscape of how we educate our patients. Educational techniques founded on one-on-one counseling, collecting

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diet records, and measuring food are becoming antiquated as digital educational options continue to expand (Appendix B). Patients with inborn errors of metabolism have experienced changes in the concepts taught, such as the "diet for life" or "the simplified diet" for patients with phenylketonuria (PKU), or the action of pharmacological medications, such as Kuvan® (BioMarin Inc., Novato, California) in PKU or Ravicti® (Hyperion Therapeutics, Brisbane, California) in urea cycle disorders (UCD). Techniques and teaching tools are evolving into web-based programs and diet management applications for electronic devices. Even with the development and use of these tools, long-term dietary management remains a challenge for many patients and families [2–4]. This chapter addresses the current challenges in sustaining a metabolic diet, the utilization of educational tools and their perceived effectiveness, and a challenge to clinicians to rethink the educational tools and counseling approaches for their patients and families.

3.2 Patient Education

Patient education is defined by the Institute of Medicine as "the process by which health professionals and others impart information to patients that will alter their health behaviors or improve their health status" [1]. However, if a patient cannot understand the message being taught, regardless of the method of education, the education will be ineffective in producing the anticipated health behavior change. Health literacy is the degree to which individuals have the capacity to obtain, process, and understand basic information and services needed to make appropriate decisions regarding their health [1].

Health-care systems are becoming increasingly more complex with more medications, tests, and procedures requiring patients to take a proactive role in their health care. The brief time allotted for an appointment charges the clinician with ensuring the discussion and educational materials are understood by the patient (Box 3.1).

Box 3.1: Written and Verbal Instructions

- Written instructions
  - Provide continuity of the topic and message after the visit
- Verbal instructions
  - Can be complex
  - Delivered rapidly
  - Easily forgotten

Consistency in the messages provided by the health-care team is crucial in developing credibility and clarity for the patient. Patients expect clear communication or "patient education value" from their health-care team (Box 3.2). Clinicians anticipate increased patient motivation and "compliance" when, in their perception, the education and communication provided was effective. A patient may be more likely to respond positively to his or her treatment plan, resulting in fewer complications, when communication and education is clear. Patient satisfaction with health-care providers is also improved when the information imparted addresses the patient's concerns.

Box 3.2: Patient Education Value

The results of clear communication

- Increased compliance
- Improved patient outcomes
- Informed consent
- Improved utilization of services
- Increased patient satisfaction

Improved communication skills between the clinician and patient can lead to a more efficient use of medical services, which may result in fewer unnecessary phone calls and visits. An example of this scenario would be a late Friday afternoon phone call just before a long weekend. The patient has ran out of formula and is calling in a panic as to what to do. Is this scenario a result of poor communication between the patient and provider [5]?

3.3 Anticipatory Guidance

Anticipatory guidance is an educational approach designed to help guide families and professionals in understanding what to expect during the current and/or approaching stage of development or treatment. It is an important component of well-child care. The delivery system for anticipatory guidance is a flexible, individualized educational approach. Anticipatory guidance allows patients to receive information that is relevant to their current issues and also provides information on what to expect in the future [6, 7].

The anticipatory guidance tool "Eat Right Stay Bright™" was developed to provide a template for patient education with the goal of improving compliance and creating sustainability with restrictive metabolic diets. This particular anticipatory guidance tool is divided into four sections: (1) clinic encounter checklists, (2) experience and thoughts, (3) teaching aids and handouts, and (4) resources (Box 3.3).

Box 3.3: Eat Right Stay Bright™ Anticipatory Guidance Tool

1. Clinic encounter checklists
   - Forms to be used during clinic appointments to ensure that key issues are discussed and provide consistency between visits
2. Experience and thoughts
   - Insights from clinical experience as to what to expect and discuss
3. Teaching aids and handouts
   - Materials designed to assist in counseling and teaching
4. Resources
   - Useful teaching aids and information

http://www.ucdenver.edu/academics/colleges/medicalschool/departments/pediatrics/subs/genetics/clinical/IMDNutrition/Pages/IMDNutritionHome.aspx

Patients and families learn differently from one another. Providing verbal explanations about a disease and its treatment without a written explanation to reinforce the topic may confuse patients and make it difficult for them to retain the information. This may be especially true if a child has been recently diagnosed with a rare disorder and the family is overwhelmed. Handouts such as "altered neurochemistry" (Fig. 3.1) and "hyperphenylalaninemia" (Fig. 3.2) are examples of educational visual aids that can be used in conjunction with one-on-one counseling. How information is presented, taught, received, and retained by patients and families influences dietary compliance. Computer-based simulations and other interactive tools involving family-based learning are becoming increasingly popular.

3.4 Utilization and Perceived Effectiveness of Educational Tools

Bernstein et al. [8] addressed what types of patient education tools are currently used in international inherited metabolic disease clinics. A series of surveys were distributed to clinicians working with inherited metabolic disorders as well as to patients and families affected by PKU. The majority of clinicians (86.1 %) agreed that nutrition education affects dietary compliance in their patients. Perceived barriers to dietary compliance included embarrassment and/or frustration with the diet, poor family cohesion, difficulty in food preparation, and the inconvenience of the diet [8]. Clinicians from international clinics indicated one-on-one counseling is the most utilized educational tool regardless of patient age. The second most utilized educational tool reported was handouts and printed materials (Fig. 3.3).

Clinicians indicated that they perceive parents to be one of the primary educational sources up to the age of 12 and that the parent's role declines as the child enters adolescence and adulthood. This information reinforces how important it is to teach parents, as well as patients, in order to

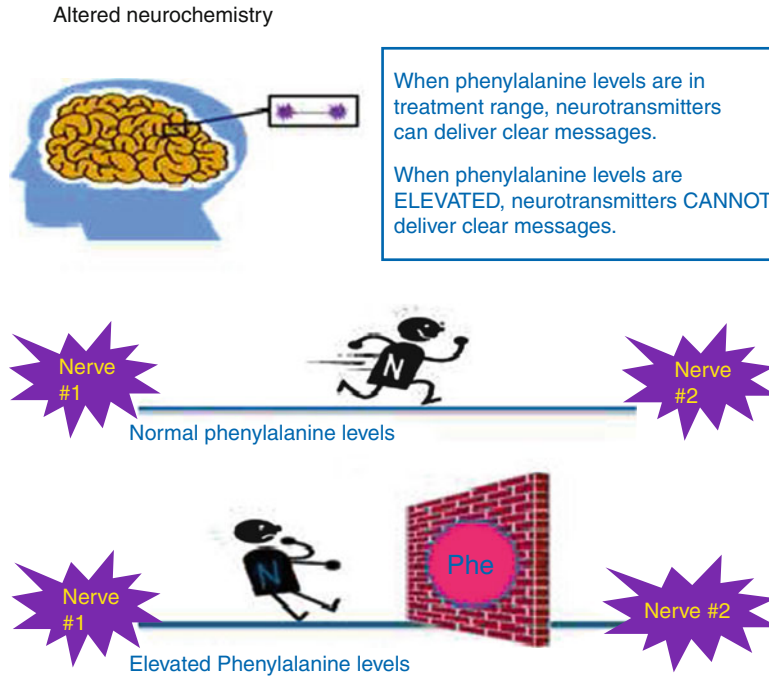

Fig. 3.1 Visual aid example from the anticipatory guidance tool, Eat Right Stay Bright™

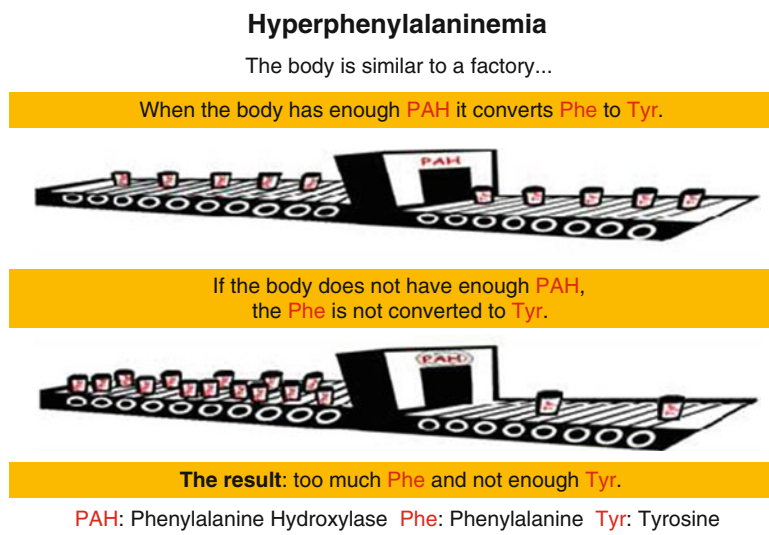

Fig. 3.2 Visual aid example from the anticipatory guidance tool, Eat Right Stay Bright™

establish a solid foundation of knowledge, reinforce educational objectives, and instill responsibility for the diet in their child at a young age (Fig. 3.4).

Early nutrition education provides the patient with a greater sense of control of his or her diet and allows parents to begin to transition the care of responsibility [9–11].

Bernstein et al. [8] also reported a discrepancy between the tools utilized in clinic and the clinician's perceived effectiveness of these tools. For example, printed materials were reported as the second most utilized educational tool, behind one-on-one counseling, yet clinicians view handouts as the least effective educational tool (Fig. 3.5).

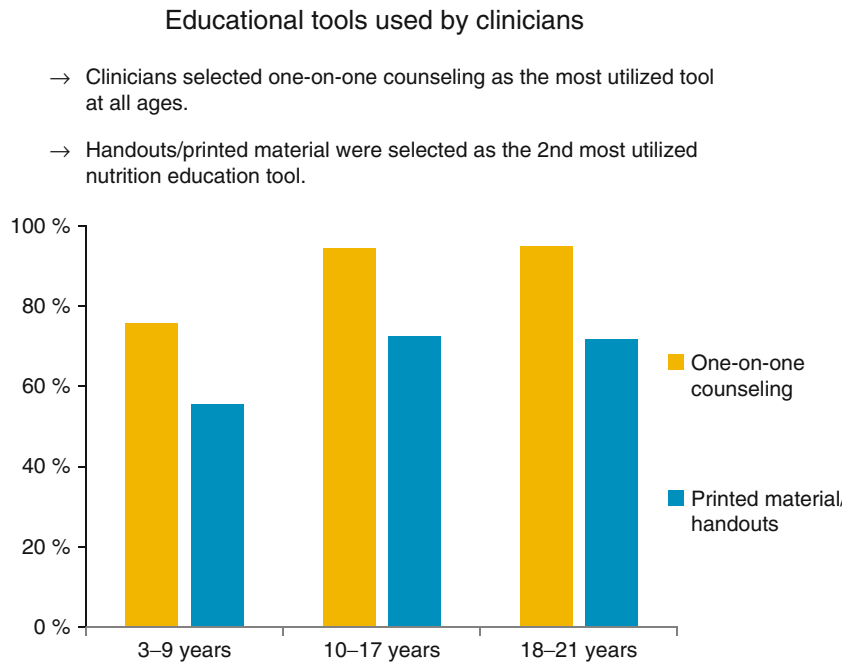

Fig. 3.3 One-on-one counseling and printed materials are the most common nutrition education tools used by clinicians based on responses from an international survey of metabolic clinics [8]

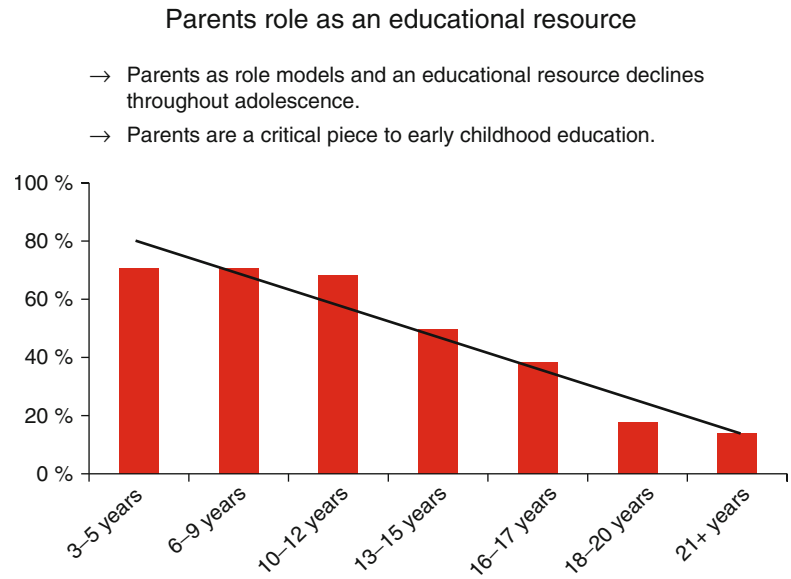

Fig. 3.4 Parents are viewed as an educational tool for patients with inherited metabolic disorders with the greatest percentage of impact during the formative years. The parent's role in education of the diet and disorder declines as the patient ages [8]

Conversely, group clinic is the least utilized educational tool by clinicians (Fig. 3.6). Yet clinicians perceive group clinic to be the most effective tool, second to one-on-one counseling, while only a quarter of international clinics currently offer group clinic (26 %) [8].

Patients and parents surveyed strongly agreed (96 and 98 %, respectively) there is a need for nutrition education in order to maintain compliance with the diet and improve overall health. Patients reported learning the most about the diet and disorder from family (Fig. 3.7). This

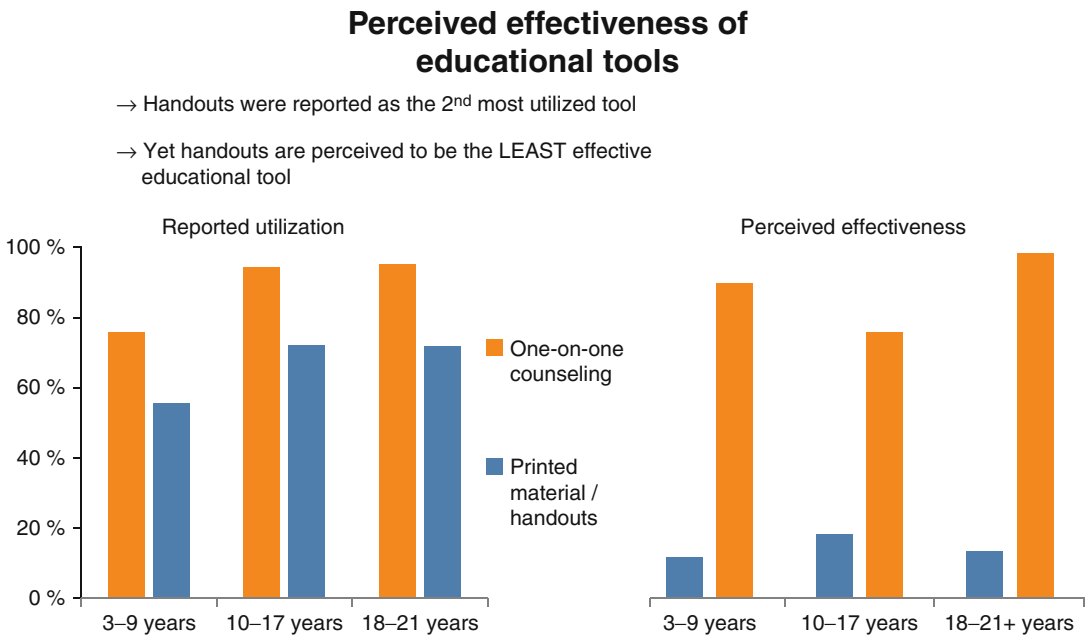

Fig. 3.5 Clinicians reported utilizing printed material nearly as much as one-on-one counseling (**a**) yet perceive these materials to be the least effective educational tools (**b**) [8]

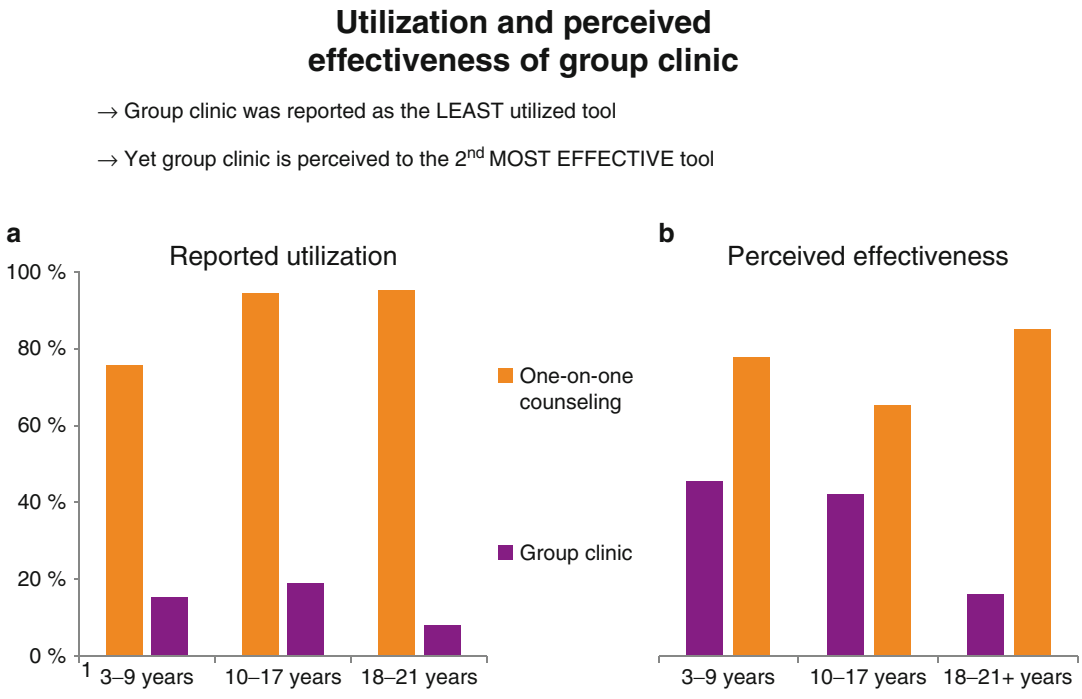

Fig. 3.6 Group clinic is the least utilized educational tool among international clinicians (**a**) but is perceived to be the most effective, second to one-on-one counseling (**b**) [8]

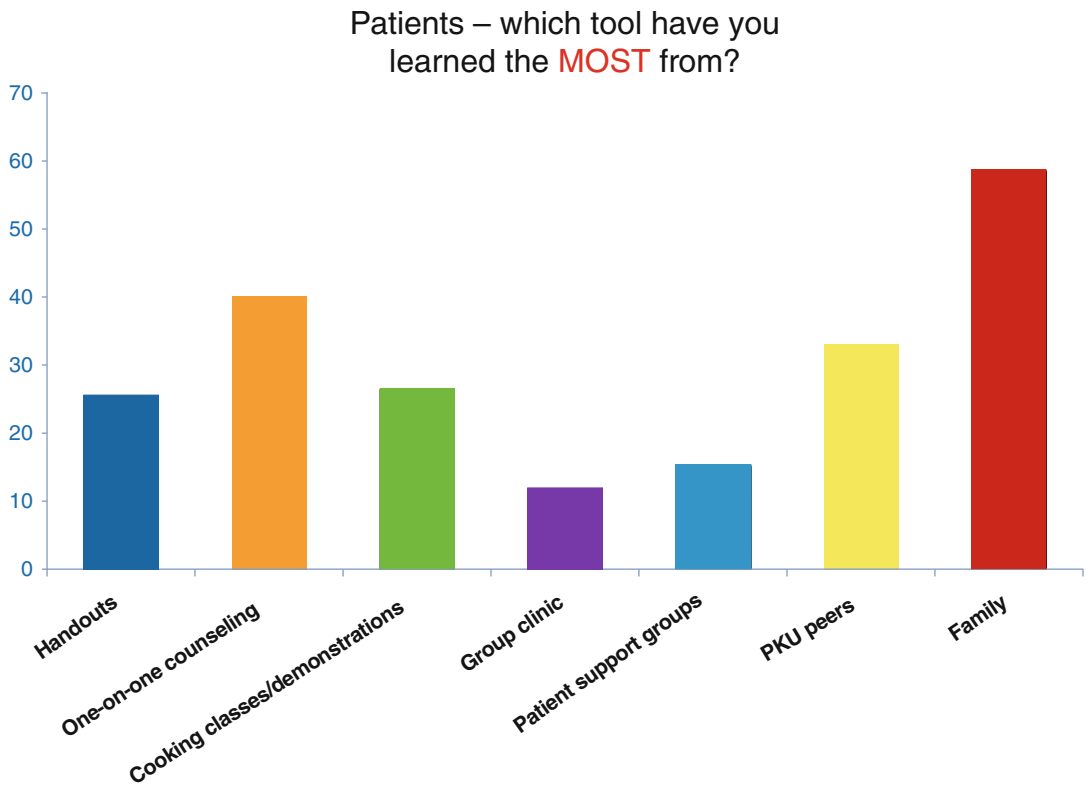

Fig. 3.7 Patients reported learning the most from their family about their diet [8]

data aligns with the responses from the clinicians, indicating that parents and family are an integral piece to patient learning during childhood. Patient responses also noted that printed material and handouts are the least effective tool for learning (Fig. 3.8). Based on these findings, the present approach to education does not overlap well with the needs of our patients and parents.

The words "compliance" and "adherence" both seem sticky and inflexible; the connotation of these terms may be setting our families up for failure. The words "sustainable" or "maintainable" may offer a greater sense of flexibility in the long-term management of restrictive metabolic diets. There is a need to look beyond the current practice and reassess how we, as clinicians and educators, approach our patients and families.

Box 3.4: Points to Consider when Providing Patient Education

- Health-care systems are directive in nature and generally characterized by clinicians giving advice and making recommendations.
- Well-intentioned clinicians tend to focus on information exchange and persuasion, which can often lead to patient resistance.
- Resistance is often interpreted as the patient being unmotivated and not fully participating in their care regimen.
- Clinic team members often fail to understand that resistance may represent the patient's ambivalence toward making the necessary health behavior change.

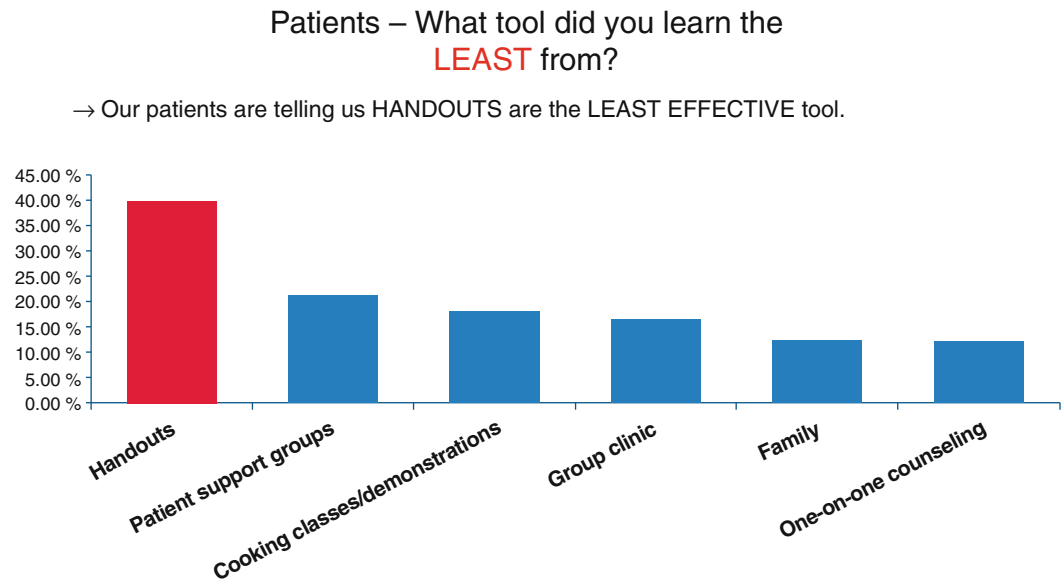

Fig. 3.8 Patients reported learning that handouts/printed materials are the least effective nutrition education tool [8]

3.5 Theoretical Models of Self-Management

Patients and families with chronic illness that require daily management make decisions and engage in behaviors that affect their health. Long-term outcomes depend on the effectiveness of self-management [12]. There are a number of well-researched, evidence-based self-management models. These include the Stanford Model, developed at Stanford University to evaluate a community-based, self-management program that assists people with chronic illness [13], the Flinders Model, developed by the Flinders Human Behaviour and Health Research Unit that utilizes one-on-one counseling for self-management and the care planning process [14], and Motivational Interviewing model that is a directive, patient-centered approach aimed at enhancing the motivation to change [15]. All three models provide benefits and have limitations with regard to creating a change in the management of chronic disease. It is important to keep in mind that patients with an inherited metabolic disease do not have the luxury to decide if they want to be compliant; the consequences of failing to follow medical advice can be irreversible and damaging.

3.6 A New Frontier in Nutrition Education

We live in a digital age where access to information is immediate and modern health care is becoming an electronic system in which series of data points and outcome measures too often define the condition of our patients. There is a disconnection between our "digital diagnosis" and the need for clinicians to actively listen to the undercurrent of emotion, and understand the complexities that make up an individual. Yet many clinicians may feel inept in their ability to adequately counsel and provide support to their patients. Radical Health™, a counseling and educational platform, is a new paradigm that may help to change the way in which clinicians engage and educate patients.

The word "radical" means "root" and the root of health care is a trusting patient-provider relationship. Radical Health™ is designed to teach dietitians how to see and hear their patients clearly without interpretations, explanations, rationalizations, and manipulation of their feelings or actions. Learning to become aware of judgments, age-old hurts, childhood angers, and reactive states enables clinicians to actively acknowledge these reactions and allow them to

disappear, thus providing a neutral environment in which strong relationships and effective education can flourish.

The intention of Radical Health™ is not to alter the content but to offer a different context of delivery and interaction with the goal of improving the long-term patient-provider relationship. Clinicians may often form "stories" about their patients based on knowledge of the family's background or their history of medical food consumption. The patient and family may come to clinic with a preconceived idea about what the clinician represents and what their encounter is "supposed to be like." If the clinician has an initial assumption of the patient's "story," the ability to have an open, honest, and engaging dialogue may be lost. The patient, now feeling as though no one is listening, because the clinician has not allowed for a neutral conversation to open, now withholds, or misrepresents, the truth in order to avoid embarrassment or in an attempt be viewed more positively. For example, a patient may change his or her answers to questions regarding frequency of medical food intake, amount of food being consumed, frequency of taking medications, or symptoms they may be experiencing.

Active listening can open channels to allow for honest conversations. Changing the clinic visit encounter and the mind-set of the clinician and patient will take time. In order for the healthcare field to improve the health and quality of life of our patients, we may need to consider changing our educational approaches to the management of chronic disease.

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Pathophysiology of Inherited Metabolic Disease

4

Peter R. Baker II

Contents

Core Messages

- The liver, brain, heart, muscle, and kidney are the organs most often affected by inborn errors of metabolism.
- As the primary site of amino acid, lipid, and glucose metabolism, the liver has a disproportionally high involvement in inborn errors of metabolisms.
- The brain, heart, and muscle have high energy demands and are therefore susceptible to disorders of fatty acid, ketone, or glucose metabolism.
- Several metabolic diseases affect kidney function leading to chronic complications including osteoporosis, hypertension, anemia, and electrolyte abnormalities.

4.1 Background

Inborn errors of metabolism are a large, diverse set of disorders in which genetic abnormalities at the cellular level result in pathophysiology at the tissue and organ level. For every disorder and corresponding enzyme affected, there may be one or (more often) more than one tissue and organ system involved. While the enzyme itself may be highly tissue specific, factors including systemic metabolite circulation and tissue energy requirements result in damage to the primary tissue as well

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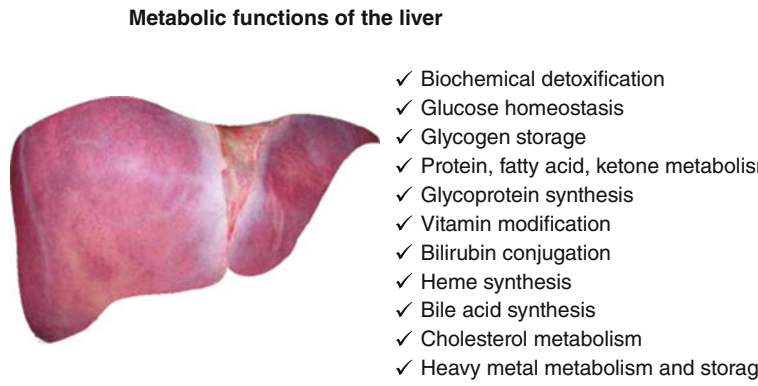

Fig. 4.1 Functions of the liver

as tissues far removed from the enzymatic defect. Therefore, inborn errors of metabolism typically have a multisystemic clinical presentation.

This chapter will explore the pathophysiology of key organs affected in various inherited metabolic disorders, specifically the normal structure and function of the liver, skeletal and cardiac muscle, kidney, and central nervous system.

The effects of physiologic damage to these organs at the biochemical level and highlights of key clinical and laboratory abnormalities associated with damage in the setting of metabolic disease will be examined. Finally a multisystemic perspective highlighting the role of each organ in particular inborn errors of metabolism are provided.

4.2 Pathophysiology of Organs

4.2.1 The Liver

The liver is arguably the most biochemically unique and multifunctional organ in the body. It sits in the right upper quadrant of the abdomen, with afferent blood flow from the intestine and systemic circulation and efferent flow to systemic circulation. As blood passes through the liver, it traverses the sinusoids, exposing the blood to hepatocytes. These cells, the basic functional cells of the liver, serve to detoxify exogenous metabolites through biochemical modifications carried out by various p450 enzymes. These enzymes facilitate excretion through conjugation with hydrophilic molecules like taurine, glycine, glucuronide, and sulfate. Additionally ammonia, a by-product of protein catabolism and potential neurotoxin, is turned into urea for excretion in the urine. Hepatocytes have primary synthetic function including the synthesis of bile, bile conjugates, cholesterol, proteins (including clotting factors and albumin), and glycoproteins as well as processing of metals (including iron and copper) and heme (Fig. 4.1).

Biochemically, the liver serves a primary role in glucose metabolism. Alanine is used to create glucose by gluconeogenesis, and glycogen (a branching glucose polymer) is stored for release of glucose in times of fasting. It also is involved in turning alternative carbohydrates including fructose and galactose into usable forms. The liver also plays a key role in lipid metabolism, ketogenesis, and energy metabolism in the fasting state. Further, in amino acid metabolism, it serves as an important organ of catabolism to allow utilization of amino acids as an alternative fuel source, via transamination and formation of keto acids. This again highlights the role of the liver in both energy metabolism and ammonia elimination. Finally, other amino acids, including glycine, are exclusively metabolized here.

In many inborn errors of metabolism, the liver's ability to accomplish some or most of these tasks may be diminished. Synthetic dysfunction can manifest by coagulopathy (including low clotting factors V, VII, VIII which results in prolongation of coagulation times (INR and partial prothrombin time)). Conversely, problems with glycosylation (including proteins C and S) can result in a hypercoagulable state. Clinically, coagulopathy manifests as excess bleeding and bruising, which can be complicated by ectopic

clot formation if there is a hypercoagulable component. Deficient production of albumin results in lower oncotic pressure in the blood, which in turn results in seeping of water from the blood into surrounding tissues and cavities. This seepage creates edema and, in severe cases, ascites and/or anasarca. The inability to make bile and conjugate bilirubin (the main heme breakdown product) results in high serum concentrations of bilirubin and jaundice, or a yellowing of the skin and eyes (which may be accompanied by itching). Failure to transport the bile to the intestine (via the gallbladder) results in cholestasis, which in turn may result in fat-soluble vitamin (A, D, E, K) malabsorption and steatorrhea (fatty stool). In specific disorders of metal metabolism, copper or iron may also be stored here (as well as other tissues), creating localized damage. This can also be found in disorders of heme catabolism (certain porphyrias). Cellular damage may be followed with elevated blood transaminase enzyme concentrations, including aspartate/alanine aminotransferase (AST, ALT) and gamma-glutamyl transferase (GGT), as well as synthetic markers of function including serum albumin, bilirubin, and coagulation times (Box 4.1).

Box 4.1: Manifestations and Laboratory Markers of Liver Failure

- Hepatocyte insufficiency/dysfunction
  - Hypoglycemia
  - Lactic acidosis
  - Hyperammonemia
  - Low albumin, edema
  - Abnormal or low coagulation factors (factor V, long INR/PT/PTT)
  - Failed bilirubin conjugation (jaundice)
- Hepatocyte lysis
  - Release of intracellular hepatocyte content
  - Elevated liver enzymes (AST, ALT, and GGT)
- Biliary dysfunction
  - Cholestasis (increased bilirubin, abnormal bile acids)
  - Intestinal malabsorption (fat and fat-soluble vitamins)

In energy metabolism, loss of liver function results in low blood sugar (as a result of dysfunctional gluconeogenesis and/or abnormal glycogen storage or release). This in turn may lead to elevations in gluconeogenic precursors alanine and lactate. Lipids and triglycerides, which cannot be converted to ketones, may be stored in the form of fatty vesicles or result in increased levels of circulating lipids (either as free fatty acids, triglycerides, or lipoproteins). Amino acid catabolism, especially in the setting of liver disease, may result in elevated amino acids in the serum. This includes branched-chain amino acids (leucine, isoleucine, and valine), tyrosine, homocysteine, and methionine. More critically, dysfunction of the urea cycle may lead to elevations in ammonia, glutamine, and glycine. The latter may also be elevated due to defects in the glycine cleavage complex, housed only in the liver and brain.

Chronic liver damage, by inflammation, toxic metabolite exposure, and/or intracellular storage, may result in a predictable path toward liver failure. This usually begins with hepatomegaly, contributed to by ectopic lipid storage or storage of material like mucopolysaccharides, oligosaccharides, or cholesterol. After years of exposure this gives way to fibrosis and eventually cirrhosis (Box 4.2). Most of the liver's volume becomes occupied by scar tissue, while patches of hepatocytes have limited functionality and a potential for oncogenic transformation (including hepatoblastoma or hepatocellular carcinoma). This results in vascular problems and many of the aforementioned functional abnormalities. Ultimately, and without transplant, long-standing and severe liver dysfunction can lead to death.

Box 4.2: Manifestations of Chronic Liver Insult

- *Hepatomegaly* – lipid, glycogen, or saccharide storage can lead to enlargement and slowly diminish liver function
- *Steatosis* – reversible fat storage
- *Fibrosis/cirrhosis* – scar tissue formation with portal hypertension, varicosities, bleeding, and splenomegaly

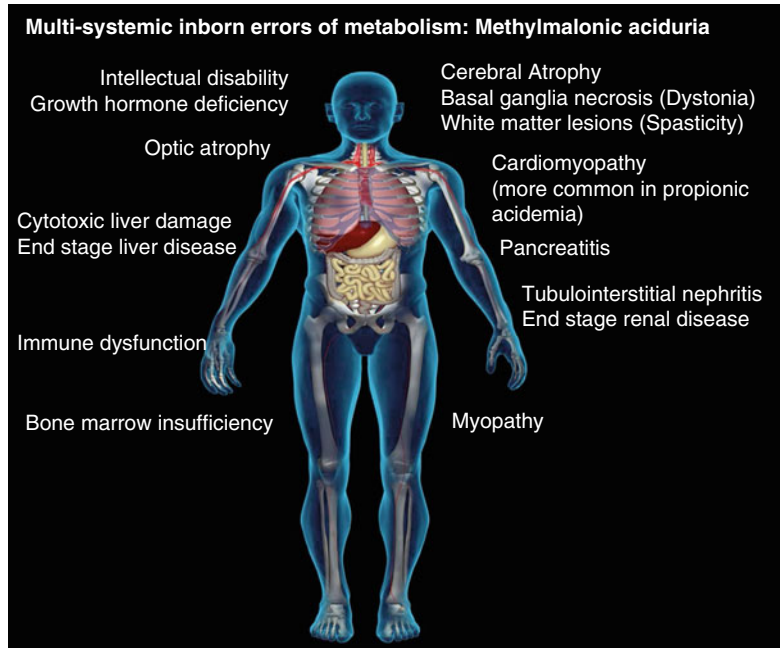

Fig. 4.2 Multisystemic complications due to methylmalonic acidemia

With the liver as a hub of metabolism, inborn errors of metabolism have a disproportionately high involvement of the liver itself. While other organ systems may be affected, one or more functions of the liver may be compromised, resulting in a spectrum of organ damage (Box 4.3) (Fig. 4.2). For example, in storage disorders like Wolman syndrome (or cholesteryl ester storage disease), several mucopolysaccharidoses, and Niemann-Pick disease (A, B, and C), storage material is trapped in macrophages, which are in turn trapped within the parenchyma of the liver. This leads to mechanical enlargement but can also lead to functional disturbances (manifesting as cellular damage, synthetic dysfunction, and cholestatic jaundice). In glycogen storage diseases (especially type I), patients have an enlarged liver, profound hypoglycemia, severe and chronic lactic acidosis, and increased long-term risk for liver cancer.

Box 4.3: Inborn Errors of Metabolism Associated with Liver Damage

Examples include:

- Tyrosinemia type 1
  - Hepatocellular dysfunction
  - Cirrhosis
- Galactosemia
  - Hepatomegaly
  - Hepatocellular dysfunction/cholestasis
- Glycogen storage diseases
  - Hepatomegaly
  - Cirrhosis (esp. GSD type IV)
- Fatty acid oxidation disorders
  - Hepatocellular damage (MCAD, VLCAD, LCHAD)
  - Cholestasis (LCHAD)
- Urea cycle disorders
  - Hepatocellular damage (ornithine transcarbamylase deficiency)
  - Steatosis/cholestasis (citrullinemia type II)
  - Fibrosis/cirrhosis (argininosuccinic aciduria)

Carbohydrate metabolism disorders like hereditary fructose intolerance and galactosemia result in acute, leading to chronic, liver damage through depletion of available ATP (by depleting the total phosphate pool) as well as aberrant glycosylation [1, 2]. Gluconeogenic defects like pyruvate carboxylase or 1,6-fructose-bis-phosphatase deficiency

manifest with hypoglycemia and profound lactic acidosis. In urea cycle disorders, targeted dysfunction of ammonia clearance may lead to direct hepatotoxic damage (as in ornithine transcarbamylase (OTC) deficiency) or long-term cirrhosis (as in argininosuccinic aciduria). Primary disorders of bile acid synthesis as well as disorders of bilirubin conjugation like Crigler-Najjar, Gilbert, Rotor, and Dubin-Johnson syndromes lead to hyperbilirubinemia and jaundice. Citrin deficiency (citrullinemia type II) causes cholestatic jaundice, as does the fatty acid oxidation disorder long-chain hydroxyl acyl-CoA dehydrogenase (LCHAD) deficiency. This disorder, along with other fatty acid oxidation and ketolytic defects, can also lead to direct cytotoxic damage in the liver manifesting as acute liver failure. Maple syrup urine disease (MSUD), in which branched-chain amino acids cannot be catabolized, causes cellular damage in the liver as well as a buildup of the toxic amino acid leucine that affects the brain. Toxic organic acid disorders, such as methylmalonic acidemia and propionic acidemia, lead to acute and chronic hepatocellular damage. Other amino acidopathies, such as tyrosinemia type 1, result in hepatocellular damage and abnormal synthetic function through toxic intermediates (e.g., succinylacetone) leading to cirrhosis and liver cancer risk early in life. Finally, the broad category of mitochondrial disorders, most specifically depletion disorders (e.g., *DGUOK* and *POLG1* defects), can lead to early and severe liver damage.

As a primary player in metabolism, many inborn errors affect liver function and health. Many harm the liver acutely, and some predispose to chronic liver disease. Unfortunately many of these disorders are not susceptible to dietary or medical therapies, making liver (or sometimes bone marrow) transplant the only means of treatment. Transplant can alternatively serve as a means of effective treatment. In disorders including ornithine transcarbamylase deficiency and GSD type Ia, liver transplant is thought to be curative. Bone marrow transplant helps with visceral symptoms in many storage disorders as well, providing an adjunct to enzyme or substrate replacement therapies.

4.2.2 The Muscle

Skeletal and cardiac muscles have unique properties making them susceptible to pathology in inborn errors of metabolism. As organs of high-energy utilization, they may be damaged in biochemical disorders of fatty acid, ketone, or glucose metabolism. By necessity, skeletal and cardiac muscles carry their own glucose stores in the form of glycogen, so in glycogen storage disorders they may be affected. While the muscle is a hearty tissue, it is susceptible to toxic damage as well.

Manifestations of metabolic disorders in the skeletal muscle include easy fatigue, exercise intolerance, myopathy (muscle weakness), pain, atrophy, and cellular breakdown (rhabdomyolysis). Biochemically, this is manifested in the serum with elevated creatine kinase (CK). Transaminase enzymes (ALT and AST) are elevated (as in the liver) with the exception of gamma-glutamyl transferase (GGT) that is found in the liver alone. Aldolase A, an enzyme in carbohydrate metabolism in the muscle, may also be elevated. This should not be confused with Aldolase B, the liver isoform that is deficient in hereditary fructose intolerance. In the urine, specifically in rhabdomyolysis, myoglobin can be detected. Finally, in some disorders of muscle metabolism, abnormal muscle pathology may be seen on biopsy. This may include storage material (e.g., glycogen), abnormal fiber distribution (largely nonspecific), or abnormal mitochondrial morphology or number indicative of a respiratory chain or mitochondrial depletion defect. Activity of the respiratory chain components may also provide clues to disorders of mitochondrial energy metabolism.

Manifestations and Laboratory Markers of Muscle Dysfunction

- Early and easy fatigue with exercise intolerance
- Myopathy (muscle weakness)
- Muscle pain/muscle atrophy
- Rhabdomyolysis (muscle cell breakdown)
- Elevated creatine kinase (CK)
- Myoglobinuria

- Elevated AST/ALT (not GGT)
- Aldolase A (not Aldolase B)
- Abnormal pathology of the muscle (requires a muscle biopsy)

Rhabdomyolysis may be seen in disorders of fatty acid oxidation, specifically in disorders of long-chain metabolism, as well as more subtly some muscle glycogenoses. Myopathy or muscle wasting may also manifest in these disorders as well as in primary mitochondrial defects. Muscle fatigue and exercise intolerance are found in energy metabolism disorders (including fatty acid oxidation disorders, carnitine metabolism defects, and primary mitochondrial defects). In glycogen storage disorders in which exercise intolerance may be the primary manifesting symptom, useful diagnostic tools include biopsy with evidence of glycogen storage or diastase resistance as well as the ischemic forearm test [3]. The test, useful in diagnosing muscular glycogen storage disorders, demonstrates ammonia production rising above lactate production in an exercised anaerobic muscle of affected patients. A rise in lactate without ammonia indicates myoadenylate deaminase deficiency, and a rise in both together is normal [4].

Cardiac muscle may have much of the same pathophysiology; however, laboratory values and clinical manifestations may differ. Depending on the inborn error of metabolism, effects on the heart may involve a weakened heart muscle seen as decreased ejection fraction on echocardiogram. The heart, in this case, may be large and floppy (dilated cardiomyopathy) or thick and rigid (hypertrophic cardiomyopathy). In either case the muscle is not strong enough to pump blood adequately. Damage to the heart muscle in both instances may manifest as elevated CK, lactate, and/or troponin levels in the blood. Basic natriuretic peptide (BNP) is also a sign of cardiac muscle strain indicative of myopathy. Finally, an electrocardiogram (ECG) may indicate hypertrophy as well as arrhythmogenic potential in individuals at risk.

Dilated cardiomyopathy can be found in many metabolic disorders including fatty acid oxidation defects, carnitine metabolism defects (e.g., CPT1 and primary carnitine deficiency), congenital disorders of glycosylation, and primary mitochondrial disorders [5]. Organic acidemias including propionic acidemia, 2-methyl-3-hydroxybutyric aciduria, and Barth syndrome (X-linked 3-methylglutaconic aciduria and neutropenia) all manifest with dilated cardiomyopathy. Nutritional deficiencies including carnitine and thiamin deficiency as well as storage disorders including some of the mucopolysaccharidoses can also present in this manner (Box 4.4).

Box 4.4: Dilated Cardiomyopathy Associated with Inborn Errors of Metabolism

- Energy defect
  - Primary carnitine deficiency (transporter)
  - Mitochondrial defects
  - Some fatty acid oxidation defects (VLCAD, LCHAD)
- Storage/transport
  - Congenital disorders of glycosylation
- Toxicity
  - Organic acid disorders
  - Nutritional
  - Dietary carnitine deficiency
  - Thiamin deficiency

Hypertrophic cardiomyopathy, a thick, rigid cardiac muscle, is also seen in inborn errors, with some overlap with disorders associated with a dilated phenotype. This includes carnitine deficiency, primary respiratory chain defects, Barth syndrome, several glycogenoses (e.g., GSD III, IV, and IX), and lysosomal storage disorders. In lysosomal storage disease the valves are typically more affected than the muscle itself. In the neonate there should be high suspicion for GSD type

II or Pompe disease. Tyrosinemia type 1, which mainly affects the liver and kidney, also manifests with hypertrophic cardiomyopathy. Conduction defects predisposing to arrhythmia are typically found in disorders of fatty acid oxidation (especially long-chain disorders, CPTII, and carnitine-acylcarnitine translocase deficiency), Kearns-Sayre, and other primary mitochondrial defects.

4.2.3 The Kidney

The kidneys act to filter toxins out of the blood for excretion in the urine. There are complex mechanisms to recover electrolytes, carbohydrates, and amino acids. The kidney is also an endocrine organ, regulating vitamin D metabolism and signaling red blood cell proliferation through erythropoietin. While each of these unique roles is not specifically tied to an inborn error of metabolism, the kidneys are affected by several disorders and may be the source of chronic complications of disease. Symptoms of chronic kidney disease include osteoporosis, hypertension, anemia, and electrolyte abnormalities with the primary means of therapy being hemodialysis or transplant (Box 4.5).

Box 4.5: Manifestations and Laboratory Markers of Kidney Dysfunction

- Decreased glomerular filtration
  - Renal insufficiency (electrolyte and pH imbalance, hypertension, uremia)
  - Proteinuria/hematuria
- Decreased tubular reabsorption
  - Generalized loss of amino acids, glucose, phosphate, bicarbonate (Fanconi syndrome)
  - Excessive urinary cysteine, ornithine, lysine, and arginine (cystinuria)
  - Excessive urinary ornithine, lysine, and arginine (lysinuric protein intolerance)
- Anemia and loss of bone density
  - Calcium and phosphorus imbalance
- Elevated serum creatinine, blood urea nitrogen
- Metabolic acidosis (decreased serum phosphate, serum bicarbonate)
- Glucosuria

Renal filtration involves blood flow to the kidney through renal arteries, filtering of small molecules through the glomerulus (a fine network of capillaries abutting renal tubules), and the passing of that filtrate past active and passive transporters to concentrate the urine and salvage small molecules for continued use in the body. Dysfunction in the proximal renal tubules, as may be found in mitochondrial diseases, may lead to renal Fanconi syndrome. This is an inability to resorb electrolytes, carbohydrates, and amino acids, resulting in low serum levels of sodium, potassium, bicarbonate, phosphorous, and glucose, as well as generalized aminoaciduria. Renal Fanconi syndrome is a major cause of renal tubular acidosis and may also be present in disorders of glycosylation, cystinosis (a lysosomal transport defect causing crystal accumulation systemically), galactosemia, and tyrosinemia type 1 [6].

Other, more specific, transporter dysfunctions lead to distinct inborn errors of metabolism. Oxaluria and cystinuria, defects in oxalate and cysteine transport, respectively, manifest with renal stones. Cystinuria specifically presents with cysteine, ornithine, lysine, and arginine in the urine. The latter should not be confused with cystinosis. Lysinuric protein intolerance (LPI) is a defect in the dibasic amino acid transporter. This results in a specific amino aciduria pattern (ornithine, lysine, and arginine), which in turn results in secondary inhibition of the urea cycle. Individuals affected by LPI are at risk for hyperammonemia and also have a unique susceptibility to macrophage activation syndrome, an exaggerated systemic inflammatory response, and alveolar proteinosis. Renal damage

secondary to other inborn errors of metabolism, and their circulating metabolites, can result in parenchymal damage and loss of renal function over time (Box 4.6).

Box 4.6: Inborn Errors of Metabolism Associated with Kidney Damage

Examples include:

- Tyrosinemia type 1
  - Glomerular/renal tubular dysfunction
- Methylmalonic acidemia
  - Interstitial nephropathy
- Galactosemia
  - Renal tubular dysfunction
- Glycogen storage disease type I
  - Glomerular/renal tubular dysfunction
- Fabry's disease
  - Storage disorder
- Amino acid transporter defects
  - Cystinosis
  - Lysinuric protein intolerance

Methylmalonic acidemia is well known to cause renal damage that eventually necessitates transplant. The mechanism is not well understood, but it may be oxidative damage from mitochondrial electron transport chain dysfunction and not necessarily methylmalonic acid concentration itself that ultimately leads to renal failure [7, 8]. In tyrosinemia type 1 (the hepatorenal form), renal parenchyma is damaged by high levels of succinylacetone. This may be mitigated with nitisinone (NTBC) therapy, even after liver transplant [9]. Glycogen storage disease (specifically type Ia) may lead to long-term impairment of renal function and result in hyperfiltration. If the disorder is treated by liver transplant, this risk is diminished, but not eliminated, and early medical intervention may prevent or slow renal damage [10]. Chronic complications in treated GSD1a patients include risk for primary renal tumors. Finally, Fabry's disease is one of the few lysosomal storage disorders to affect the kidney; proteinuria is one of the earliest signs of renal involvement, but over the years this can progress to renal failure due to deposition of Gb3 glycolipids.

4.2.4 The Brain

The brain is a high-energy-requiring organ, and so it is particularly susceptible to disorders of energy metabolism, as in mitochondrial disorders and disorders of fatty acid oxidation. It is also one of the major organs damaged in disorders of intoxication both acutely (in urea cycle disorders, organic acidemias, maple syrup urine disease, and glutaric acidemia type 1), subacutely (in X-linked adrenoleukodystrophy and severe lysosomal disorders), and chronically (in phenylketonuria as well as most intoxication disorders). Functionally, damage to the brain can lead to loss of vision, hearing, motor coordination and movement inhibition (leading to movement disorders and abnormal posturing), and seizures. Clinical signs and symptoms of inborn errors affecting the brain depend largely on the nature and location of insult (Box 4.7).

Box 4.7: Inborn Errors of Metabolism Affecting the Brain

Examples include:

- Phenylketonuria
  - Abnormal myelination
  - Neurotransmitter deficiency
- Fatty acid oxidation disorders
  - Hypoglycemia without ketones leading to seizures and brain injury
- Maple syrup urine disease
  - Cerebral edema
  - Abnormal myelination
- Urea cycle defects
  - Cerebral edema
- Organic acid disorders
  - Metabolic stroke (particularly in the basal ganglia)

Mitochondrial diseases are a broad group of disorders affecting the function of the electron transport chain, proliferation of mitochondria, or transport of molecules into mitochondria to enable function. There are over 200 specific disorders involving over 1,000 genes, both in the nuclear DNA and the circular mitochondrial DNA (mtDNA) found in multiple copies within

the mitochondrial matrix. A common final pathophysiology in many of these disorders is Leigh disease. This phenotype, marked by characteristic MRI findings including T2 hyperintensity of the basal ganglia, deep white matter, and brain stem, is found in a large number of specific mitochondrial disorders. The most common genetic causes include SURF1-associated complex IV deficiency and ATP6-associated NARP mutation T8993C; however, there are at least 26 known genetic causes of Leigh including mtDNA deletions and duplications, point mutations, and mitochondrial DNA depletion (one of the most common of which is POLG1 deficiency) [11, 12]. The clinical course typically involves severe hypotonia and muscle weakness leading to respiratory failure. Damage to the basal ganglia (here and in several other disorders below) can lead to severe dystonia as well.

Brain atrophy and nonspecific demyelination may be phenotypes in mitochondrial disease, thought to result from a combination of energy depletion and oxidative stress. Symptoms include cognitive decline, motor disabilities, and/or seizures. A less common presentation of mitochondrial disease in the brain is metabolic stroke, as typified by the condition mitochondrial encephalopathy, lactic acidosis, and stroke (MELAS). The most common cause of MELAS is the well-known A3243G mtDNA mutation affecting the mitochondrial $tRNA^{Leu}$. The mechanism is thought to be a combination of mitochondrial energy depletion, oxidative stress, lactate production, and angiopathy with poor nitrous oxide responsiveness [13]. The eye is often considered an extension of the brain and therefore is also susceptible to mitochondrial dysfunction. POLG1, the only mitochondrial DNA polymerase, as well as several other mitochondrial depletion-associated genes can cause specific paralysis of the extraocular muscles. Mitochondrial disorders also result in pigmented retinopathy.

Other energy depletion disorders include disorders of fatty acid oxidation, ketone disorders (both synthesis and ketolysis), and disorders of glucose metabolism. These often present with a global neurologic phenotype resulting in altered mental status and/or seizures. Often these are in the setting of fasting, vomiting, or generalized illness. Damage is not chronic or progressive, unless there are multiple and/or severe metabolic crises.

Acute disorders of intoxication that affect the brain include urea cycle disorders, some amino acidopathies, and organic acidemias. Urea cycle disorders and organic acidemias result in hyperammonemia, which directly causes cerebral edema and damage to neurons. Here too, multiple repeated events can lead to a more global, chronic damage. Maple syrup urine disease, resulting from a defect in branched-chain keto acid dehydrogenase, results in the buildup of branched-chain amino acids and their associated alpha-keto acids. The most damaging of these molecules is leucine, which causes acute cerebral edema and neuronal damage [14]. Oxidative damage may also play a role [15]. In the absence of hyperammonemia, organic acidemias including propionic acidemia and methylmalonic acidemia can also result in chronic damage to the white matter and basal ganglia. This is thought to occur even in the setting of well-controlled disease [16]. The underlying mechanism is not known.

The organic acidemia, glutaric acidemia type 1 (GA-1), is confined to the brain alone. It is set apart from other organic acidemias by its natural history. The primary lesion in GA1 is acute and permanent necrosis of the basal ganglia associated with catabolism and fevers. Children are at highest risk between 6 months and 2 years of age. After the age of 6 years, acute cerebral events are extremely rare [17]. Some adults may present with headaches and white matter changes [18], and some (including those in families with known, severe, symptomatic disease) are completely unaffected. Classic MRI findings include lesions in the basal ganglia, macrocephaly, subdural bleeding, and frontotemporal atrophy.

A second condition, in which pathophysiology is primarily manifested in the brain, is nonketotic hyperglycinemia (NKH), a disorder of glycine metabolism. As mentioned above, the glycine cleavage complex resides in cells of the liver and brain only. While the liver is unaffected in this condition, increased amounts of glycine in

the brain are associated with severe neonatal seizure activity thought to be caused by glycine's excitatory effects on the NMDA receptor [19].

Subacute to chronic damage in the white matter (demyelination) and poor development of the white matter (hypomyelination) are associated with storage diseases. Hypomyelinating disorders include Tay-Sachs, Salla, and some forms of Niemann-Pick and Gaucher disease. Peroxisomal disorders (specifically those involved in peroxisomal biogenesis) result in Zellweger-like phenotypes in which children are affected with severe hypotonia, vision and hearing loss, and difficult to control seizures. Phenotypically similar, but biochemically unrelated, disorders include the neuronal ceroid lipofuscinoses (NCL). NCL tends to be more rapidly progressive with a marked deterioration of neuronal function over the course of several years. Lysosomal disease including Krabbe disease and metachromatic leukodystrophy presents with both hypo- and demyelination [20]. Demyelination, or leukodystrophy, is likely a result of innate immune activation [21]. One of the more severe and rapidly progressive demyelinating disorders is X-linked adrenoleukodystrophy. Here, very long-chain fatty acids (VLCFA) cannot enter the peroxisome. Through mechanisms not yet defined, this buildup of VLCFA [22] results in a rapidly progressive loss of myelination, typically in the midline occipital region moving distally and anteriorly. This is characterized by a "leading edge" of enhancement indicating inflammation on gadolinium contrast MRI. Treatment for this is bone marrow transplant before symptoms progress.

In most of these disorders, white matter damage is patchy, and the basal ganglia are not affected. Treatments to protect the brain are few. In some disorders enzyme replacement and substrate reduction are possible, but the efficacy in the brain, an organ "protected" by the blood-brain barrier, is often poor.

Finally, chronic damage may occur in intoxicating disorders. Besides the organic acidemias mentioned above, the most classic example of this is phenylketonuria (PKU). Untreated, PKU results in severe cognitive impairment, anxiety, motor impairment, spasticity, and seizures. Damage is thought to result from phenylalanine toxicity directly, oxidative damage to neuronal tissue, and decreased dopamine, norepinephrine, and serotonin production. With the institution of dietary therapy after detection by the newborn screen, most of these issues can be ameliorated or avoided. Other therapies including large neutral amino acids, PEGylated phenylalanine ammonia lyase (Peg-PAL) (BioMarin, Novato, CA), and sapropterin dihydrochloride (Kuvan®, BioMarin, Novato, CA) are also being investigated as adjuncts to dietary therapy for improvement of neurologic outcome.

Understanding the underlying pathophysiology has made it possible to effectively manage these inborn errors with the goal of preserving quality of life and preventing mortality. The organ(s) affected by each disorder determines the target of therapy and the impact a disorder has on the body as a whole.

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Metabolic Intoxication Syndrome in a Newborn

5

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Contents

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Core Messages

- Metabolic disorders should be considered alongside other diagnoses in all neonates with unclear, severe, or progressive illness.
- The course of a metabolic disorder presenting with intoxication syndrome is often very sudden and severe.
- Without timely and proper diagnosis and treatment, metabolic intoxication syndrome can often lead to irreversible organ damage or death.
- Prevention of accumulation of metabolic toxins and promotion of anabolism are the most important steps in treatment in metabolic intoxication.

5.1 Background

The term inborn errors of metabolism (IEM) was introduced over a hundred years ago and, still today, these diseases are responsible for many diagnostic and therapeutic dilemmas [4]. Metabolic disorders are often undiagnosed due to the erroneous belief that they are very rare. While particular metabolic disorders occur infrequently, when combined, inborn errors of metabolism become a large group of diagnoses, with a worldwide incidence of approximately 1:1,000

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live births [2, 5, 6]. Inborn errors of metabolism can result from all types of genetic inheritance, including mitochondrial, with most diseases having an autosomal recessive type of inheritance [1, 6, 7].

5.2 Classification

There are many ways to categorize inborn errors of metabolism. When considering the effectiveness of therapeutic procedures in acute illness, metabolic disorders can be classified into five groups: (1) disorders presenting with intoxication syndrome, (2) disorders of reduced tolerance to fasting, (3) disorders of mitochondrial energy metabolism, (4) disorders of neurotransmission, and (5) disorders with limited therapeutic options in illness [8].

5.2.1 Disorders Presenting with Intoxication Syndrome

Intoxication disorders include urea cycle disorders, organic acidurias, aminoacidopathies, fatty acid oxidation disorders, and carbohydrate disorders such as galactosemia or hereditary fructose intolerance. In these disorders, a partial or complete lack of enzymatic activity causes the accumulation of substances proximal to the metabolic block in tissues and body fluids, where they act as toxins (Fig. 5.1). Treatment is based on limiting the substances that are the source of the toxic metabolites and introducing alternatives (e.g., drugs, procedures) that speed the elimination of those toxic metabolites.

5.2.2 Disorders of Reduced Tolerance to Fasting

Some inborn errors of metabolism present with hypoglycemia, occurring after periods of extended fasting. Disorders of reduced tolerance to fasting include glucose homeostasis disorders such as glycogen storage disorders, gluconeogenesis disturbances, and inborn hyperinsulinism. Mitochondrial fatty acid oxidation disorders are also considered part of these diseases; however, due to the accumulation of toxic acylcarnitines, the traits that typically present with intoxication syndromes may also manifest. The main treatment is to supply glucose and stop fat oxidation.

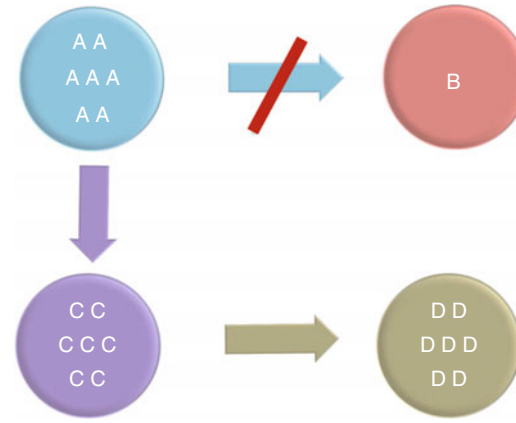

Fig. 5.1 The acute and progressive intoxication from a metabolic block, leading to accumulation of compound A, C, and D while providing insufficient quantities of B

5.2.3 Disorders of Mitochondrial Energy Metabolism

Pyruvate dehydrogenase complex (PDHC) deficiency and electron transport chain disorders are examples of mitochondrial energy disorders. The primary goal of treatment is to minimize acidosis. In prevention of lactic acidosis (especially in PDHC deficiency) glucose supply has to be limited.

5.2.4 Disorders of Neurotransmission

These disorders, such as pyridoxine dependent epilepsy, present with seizures that can be treated with vitamin B_6 and folic acid.

5.2.5 Disorders with Limited Therapeutic Options in Acute Illness

For some diseases (nonketotic hyperglycemia, molybdenum cofactor deficiency, sulfite oxidase deficiency), progressive encephalopathy is the main presenting symptom. In others (peroxisomal diseases, glycosylation disorders), aggravation of the chronic disease is caused by illness (e.g., infections).

5.3 Suspicion of an Inborn Error of Metabolism in a Neonate

During pregnancy, the placenta provides a protective environment for fetal development. Both mother and child are generally unaffected by most inborn errors of metabolism that present in a child after birth. There are, however, certain disorders that disturb the energy metabolism of the affected fetus, including intracerebral metabolism, with secondary penetration of inappropriate metabolites from brain tissue into body fluids (e.g., nonketotic hyperglycemia). An inborn error of metabolism should be also suspected in the case of non-immunological fetal edema or if there are any signs that fetal metabolism is negatively influencing the mother. Noteworthy examples include HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) and acute fatty liver of pregnancy (AFLP) in the mother. Both are suggestive of an infant affected with a fatty acid oxidation disorder. The neonate born from such a pregnancy should be treated as potentially affected, until inherited mitochondrial fatty acid metabolism disorders are excluded [9, 10].

Immediately after birth and during the first days of life, infants with an inborn error of metabolism usually appear asymptomatic and normal. However, the presence of dysmorphic features often indicates a metabolic disorder (e.g., coarse facial features in some lysosomal storage disorders). Characteristic facial features of children born with hyperinsulinism may be indicative of a metabolic disorder, whereas microcephaly may be present in the offspring of mothers with phenylketonuria [2, 3, 5, 11] (Fig. 5.2).

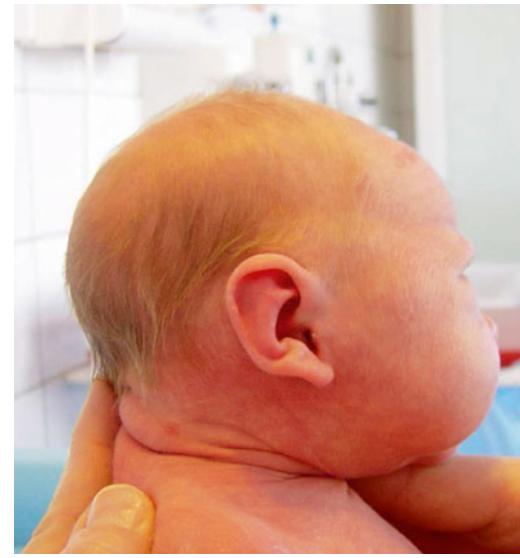

Fig. 5.2 Microcephaly in a newborn with maternal PKU syndrome (MPKU) born from a mother with classical phenylketonuria treated since 33 weeks gestation (Photo courtesy of Dr. Maria Giżewska)

Seizures are another frequent clinical manifestation of inborn errors of metabolism in the neonate. This may be a leading or progressive symptom (especially in disorders with intoxication syndrome) appearing after child is comatose or together with other progressive neurological changes.

Muscle hypotonia is a trait demonstrated by many children born with an inherited metabolic disease. “Floppy baby syndrome,” typical for such disorders as Prader-Willi syndrome and severe motor neuron diseases, may be also present in inborn lactic acidosis, some respiratory chain disorders, nonketotic hyperglycemia, molybdenum cofactor deficiency, sulfite oxidase deficiency, peroxisomal disorders, glycosylation disorders, and Pompe disease [1, 2].

Inborn errors of metabolism should be considered in children with cardiovascular pathology as well. Cardiac insufficiency in child with cardiomyopathy and/or arrhythmia, acute life-threatening episodes (ALTE), or sudden infant

death (SIDS) can be indicative of fatty acid oxidation defects, respiratory chain disorders, glycosylation disorders (frequently with pericardial exudation), or Pompe disease [1–3, 5].

It is important to remember that a small child will respond to illness in similar ways regardless of whether the cause is acquired or genetic. Therefore, inherited metabolic disorders should be suspected in all sick children, especially neonates (Box 5.1). Placing a metabolic disorder at the very end of the list of differential diagnostic possibilities can be a mistake. They should be considered along with other, more frequent causes of sudden health deterioration of the neonatal or pediatric patient, especially if the child presents with one or a combination of signs such as encephalopathy, liver damage, or cardiomyopathy [1–3, 6, 12]. Only intensive, multidirectional diagnostic and therapeutic processes will ensure that we "… do not miss a treatable disorder" (Professor JM Saudubray) [2, 12].

Box 5.1: Consideration of an IEM in a Neonate

- The neonate has a limited repertoire of responses to a severe illness, regardless of whether the disease has an infectious, genetic, or traumatic background as the cause.
- In the first days of life, an infant with an undiagnosed inborn error of metabolism may not show any symptoms of the metabolic disorder.
- An inherited metabolic disorder should be considered in all neonates with an unexplained, overwhelming, or progressive disease, particularly after a normal pregnancy and delivery [3].
- The time between the first symptoms of metabolic intoxication and the initiation of effective treatment is often associated with the infant's prognosis and/or survival.
- Suspect that any neonatal death, particularly those who have been attributed to sepsis, may have been due to an inborn error of metabolism [2].

5.4 Presentation of a Newborn with Intoxication Syndrome

A disorder presenting with intoxication syndrome is often very sudden and severe in its course. Without timely and proper diagnosis and treatment, it can lead to irreversible organ damage or death. On the other hand, if diagnosed and treated properly and urgently, the short- and long-term consequences of the intoxication syndrome may be prevented or ameliorated [2, 12].

There are many metabolic toxins that accumulate due to enzymatic dysfunction (Box 5.2).

Box 5.2: Examples of Metabolic Toxins

- Ammonia in urea cycle disorders
- Leucine and its branched chain ketoacids, such as 2-oxoisocaproic acid, in maple syrup urine disease
- Isovaleryl-CoA and its metabolites in isovaleric aciduria
- Galactose-1-phosphate in galactosemia
- Acylcarnitines in fatty acid oxidation diseases

While inborn errors of metabolism with intoxication syndrome can also occur in a preterm neonate, they are most frequently diagnosed in term delivery neonates born from uneventful pregnancies and uncomplicated births. Patients typically have normal birth weight, a high Apgar score, and no dysmorphic features. During the first days, or even weeks of life, they are considered healthy. However, if the patient is of a specific ethnic group or has a history of parental consanguinity, a family history may reveal unexplained deaths in young children or similar illness in a sibling or other blood relatives.

After an asymptomatic period, typical signs of intoxication or "poisoning" as a result of the accumulation of harmful metabolites begin. The length of time of the apparent health can vary across the spectrum of disorders and is shorter if the accumulating metabolite is particularly toxic. For example, ammonia can accumulate to toxic concentrations within hours in cases of severe urea cycle disorders or can manifest over a few days in organic acidurias. At times, the relationship between feeding (breast milk or infant

formula) and onset of the first symptoms can be noted. Catabolism occurs as part of the normal adaption to living outside the womb and causes the child's condition to deteriorate even prior to the introduction of oral feeding [5].

The symptoms of acute intoxication may be very similar to that of other diseases (Box 5.3), may often lead to a misdiagnosis and, at times, death. On the other hand, some metabolic disorders can predispose a neonate to frequent neonatal period complications such as infections like *E. coli* sepsis in children with galactosemia or hematological complications such as central nervous system hemorrhage in hyperammonemia or thrombocytopenia due to bone marrow suppresion in some aminoacidurias [2, 7].

Box 5.3: Disorders in Infants That Present with Symptoms Similar to IEM

- Generalized infection
- Birth trauma
- Respiratory distress syndrome
- Congenital cardiac disorders
- Endocrine diseases (e.g., congenital adrenal hyperplasia or neonatal diabetes)

The first symptoms that typically appear in an infant with an inborn error of metabolism with intoxication syndrome are similar to other illnesses yet often change dramatically in severity within a short period of time (Box 5.4).

Box 5.4: Clinical Presentation of Neonates with Metabolic Intoxication [1, 2]

- Poor sucking reflex resulting in feeding difficulties and poor oral intake
- Vomiting leading to dehydration and weight loss
- Muscle tone abnormalities
- Involuntary movements (boxing or pedaling)
- Seizures
- Increasing somnolence, progressing to stupor and coma, ultimately leading to death

Table 5.1 Characteristic odor detected in patients with selected inborn errors of metabolism [3, 13]

Inborn errors of metabolism	Odor
Isovaleric acidemia Glutaric acidemia type II 3-Hydroxy-3-methylglutaric aciduria	Sweaty feet
Maple syrup urine disease (MSUD)	Maple syrup, burnt sugar
Phenylketonuria (PKU)	Musty, mousey
Tyrosinemia type I	Cabbage-like
3-Methylcrotonylglycinuria Multiple carboxylase deficiency	Cat's urine
Hypermethioninemia	Rancid butter, rotten cabbage
Trimethylaminuria	Fishy
Cystinuria	Sulfurous

Neonates presenting with intoxication syndromes and who are comatose often have neurovegetative symptoms including breathing disorders with apnea, hiccups, bradycardia, and hypothermia. Some patients may emit a characteristic scent, which can be detected in the presence of the child and during an examination of a urine, blood, stool, cerumen, or cerebrospinal fluid sample [2, 3] (Table 5.1).

Neonates may also present with symptoms of hepatic failure including jaundice, elevated transaminases, hypoalbuminemia with ascites, and clotting disturbances. This presentation, often with accompanying tubulopathy, may suggest the diagnosis of tyrosinemia type 1, or in the presence of hypoglycemia, *E. coli* infection, kidney enlargement, and glaucoma may be suggestive of the diagnosis of galactosemia.

5.4.1 Biochemical Diagnostics

When suspecting an inborn error of metabolism, especially those with metabolic intoxication, specific laboratory testing should be conducted simultaneously while excluding other causes of a sudden or progressive deterioration of a neonate. This should include four basic biochemical blood tests: electrolytes, ketones, lactic acid, and ammonia [2]. An anion gap should also be considered when accessing biochemical test results. It is calculated using the formula

[Na^+ mmol/l] – ([Cl^- mmol/l] + [HCO_3^- mmol/l]) (Chap. 8). In a healthy, full-term baby, the anion gap should not exceed 15 mmol/L. A value higher than 16 mmol/L suggests a metabolic disorder, most frequently an organic aciduria.

A careful analysis of the medical history including family background, pregnancy, first days of life, history of present illness, and clinical condition of the patient, backed up with the interpretation of basic biochemical test results, often allows for establishing the reasonable initial suspicion of an inborn error of metabolism and directs further diagnostic and therapeutic actions [13–15] (Table 5.2).

It is crucial to provide proper conditions for collecting samples of blood and other biological materials to obtain reliable tests results. It is especially important when measuring ammonia and lactic acid, both of which require free flowing blood (no tourniquet), and transporting on ice to the lab for immediate analysis. Other conditions causing elevated lactic acid (hypoxia, infection, trauma, or stress when obtaining sample from the child) should be excluded [2, 3, 5]. When possible, blood samples should be secured prior to beginning a specific treatment or before ceasing oral feeding to preclude false-negative results.

Elevated ammonia, resulting from the increased production and/or disturbed detoxification of waste nitrogen, warrants particularly urgent identification and action. Ammonia is highly toxic to the brain, and hyperammonemia is considered to be a medical emergency with a high risk of irreversible neurological damage or death. Primary hyperammonemia is caused by the defect of urea cycle enzymes, an ornithine transporter, or an aspartate/glutamate transporter defect. Secondary elevation of ammonia can be observed in other metabolic disorders such as organic acidurias, fatty acid oxidation disorders, disturbances of some respiratory chain disorders, inborn hyperinsulinism, and conditions causing liver damage

Table 5.2 Basic biochemical tests performed in sick neonates and examples of possible interpretation in the direction of inborn errors of metabolism [2, 13–15]

Tests	Example of possible clinical interpretation
Blood cell count with blood smear	Pancytopenia-thrombocytopenia-leukopenia in organic acidurias; hemolytic anemia in galactosemia, congenital erythropoietic porphyria, glycolytic and pentose-phosphate enzymes deficiencies; macrocytic anemia in inborn errors of cobalamin and folate metabolism
Blood gases pH, pCO_2, HCO_3, pO_2	Metabolic acidosis in organic acidurias (anion gap); respiratory alkalosis in hyperammonemias
Glucose	↓ in organic acidurias (possible transient hyperglycemia, which together with ketones in the urine, can lead to wrong diagnosis of diabetes mellitus type 1), fatty acid oxidation disorders, galactosemia, tyrosinemia type 1, hereditary fructose intolerance, glycogen storage disease type 1, hyperinsulinism
Electrolytes	Hypocalcemia in organic acidurias
Urea	↓ in urea cycle disorders, lysinuric protein intolerance ↑ in malonyl-coA decarboxylase deficiency, cystinosis, hyperoxaluria type 1
Creatinine	↓ in creatinine biosynthesis defects
Ammonia	↑ in urea cycle disorders, organic acidurias, fatty acid oxidation disorders, maple syrup urine, biotinidase deficiency, hyperinsulinism + hyperammonemia syndrome
Lactate	↑ in respiratory chain disorders, pyruvate dehydrogenase and carboxylase deficiency, fatty acid oxidation disorders, glycogen storage disorder type 1, sometimes in organic acidurias and urea cycle defects, biotinidase deficiency
Uric acid	↓ in molybdenum cofactor deficiency In glycogen storage disease type 1
Transaminases (and other liver tests)	↑ in urea cycles disorders, fatty acid oxidation disorders, galactosemia, tyrosinemia type 1, hereditary fructose intolerance, alpha-1-antitripsin deficit, peroxisomal disorders, congenital disorders of glycosylation
Creatine kinase	↑ in fatty acid oxidation disorders
Cholesterol	↓ in Smith-Lemli-Opitz syndrome, 3-methylglutatonic aciduria, methylmalonic aciduria
Ketones in urine	*Absent* together with hypoglycemia in fatty acid oxidation disorders *Present* with metabolic acidosis (ketoacidosis) in organic acidurias

and/or infections. Temporary hyperammonemia can occur in preterm newborns as transient hyperammonemia (*THAN*), as a result of maintained blood flow through ductus venosus when the hepatic portal vein (and detoxification in liver) is being bypassed [2, 14].

The symptoms of hyperammonemia are nonspecific, in most cases, and the leading manifestation is a neurological presentation with fast progressing encephalopathy. Ammonia should be assessed in every neonate suspected of septicemia, especially with neurological symptoms and respiratory alkalosis and in children with a loss of appetite and vomiting that suffer from a loss of consciousness [14]. In healthy neonates, the ammonia concentration should not exceed 110 μmol/L; however, a sick neonate can have values as high as 180 μmol/L. Higher values, especially ones greater than 200 μmol/L, as well as ammonia values that rise rapidly have a high likelihood of a diagnosis of an inborn error of metabolism. About 50 % of children who have ammonia concentrations higher than 200 μmol/L suffer from a metabolic disorder [3, 15] (Fig. 5.3).

Newborn screening results with analysis of amino acid and carnitine esters using tandem mass spectrometry (MS/MS) are helpful in determining if a sick neonate has an inborn error of metabolism and should be performed in any infant who has not been tested. Children with intoxication syndromes may already be ill by the time the newborn screening results are available. The urine organic acid analysis with gas chromatography-mass spectrometry (GC-MS) and analysis of the amino acid profile in plasma and cerebrospinal fluid should be also considered for additional testing. Therefore, while obtaining a sample of cerebrospinal fluid in a child diagnosed with a probable central nervous infection, but with suspicion of a metabolic disorder, an additional 1–2 mL of fluid should be taken and frozen for later diagnostic testing should it be needed. Enzymatic activity in various tissues or molecular examinations may also be necessary in advanced stages of diagnostic testing.

Sometimes, despite the best efforts, it is impossible to save ill neonates and they pass before a final diagnosis is made. To ensure proper genetic counseling, detailed information for the parents regarding the causes of their child's death, and for further family planning, it is helpful to collect biological samples *perimortem*.

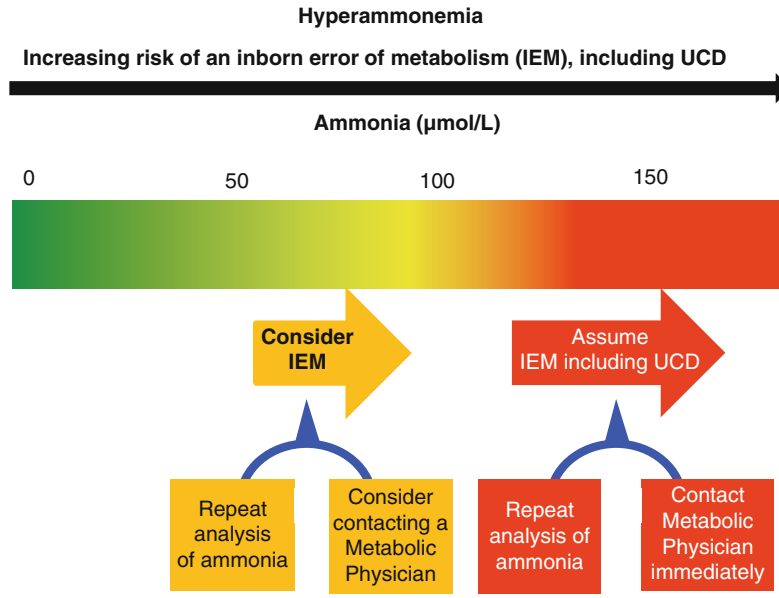

Fig. 5.3 Increasing consideration of an inborn error of metabolism as ammonia concentrations increase

5.5 Treating a Neonate with Intoxication Syndrome

Every severely ill neonate should be treated immediately, even before a final diagnosis is made. The first stages of treatment are nonspecific and are aimed at ensuring the patient's survival and preventing irreversible damage [7, 8, 16] (Box 5.5).

Box 5.5: Goals of Emergency Treatment in a Neonate Suspected to Have an Inborn Error of Metabolism

- Ensure basic life functions (ventilator and circulatory support)
- Provide sufficient energy supply
- Prevent accumulation of toxic metabolites
- Correct acid-base balance
- Introduce supporting therapies, such as:
  - Treating infections
  - Managing seizures
  - Supply cofactors (even empirically)

The successful treatment of neonate with severe metabolic intoxication is not possible without securing a vascular catheter (often central venous catheter) that is essential for: (1) administering glucose in high concentrations, (2) providing intensive hydration and managing biochemical disturbances, (3) total or partial parenteral feeding, and (4) toxin elimination.

The section below describes steps that are often introduced in infants when the presentation, course of the disease, and biochemical testing results suggest an inborn error of metabolism, especially with intoxication syndrome. It should be always kept in mind that clinical scenarios differ and treatment decisions are based on the clinical judgment of the metabolic team and should be always considered on an individual basis. Many therapeutic procedures are conducted simultaneously.

5.5.1 Stage 1: Provision of Glucose, Cessation of Feedings

When taking care of a neonate suspected of having an inborn error of metabolism, especially if the patient suffers from encephalopathy or severe liver dysfunction, administration of any substance that may act as a toxin must be ceased. The first step is the immediate discontinuation of oral feeding as well as protein and fat supplementation. However, an energy-deprived neonate will become catabolic, leading to degradation of tissues, resulting in intoxication from the metabolites coming from endogenous proteins and fat. This makes it critical to provide adequate energy in the form of glucose. This should be done as quickly as possible, but not before obtaining samples for laboratory testing.

At first, a 10 % glucose solution in amount of 150 mL/kg/24 h can be administered, using peripheral vascular catheters. This solution in a volume of 10 mg/kg/min delivers about 60 kcal/kg every 24 h. This may be sufficient temporarily in some diseases where the patient has a lower tolerance to fasting (e.g., fatty acid oxidation disorders) but may be too low in children suffering from intoxication syndrome. Patients with intoxication syndrome require higher doses of glucose (12–15 mg/kg/min) not only to prevent hypoglycemia but also to stop catabolism and promote anabolism. Excessive fluid administration is avoided when providing a high-glucose concentration solution by administering the glucose via a central venous catheter. If hyperglycemia occurs, with blood glucose concentrations above 150–160 mg/dL, it should be corrected with insulin provided intravenously in doses of 0.05–0.1 units/kg/h. The dose should be modified according to the blood glucose results [2, 8].

Due to the risk of increased lactic acidosis, the administration of glucose can be potentially dangerous in some energy metabolism disorders, particularly in PDHC deficiency. However, PDHC deficiency is very rare with a very poor prognosis, except in those patients who respond to thiamin supplementation. Considering the number of metabolic disorders in which glucose supplementation can improve a patient's clinical condition, such a course of action is justified. However, the aforementioned procedure should be accompanied by regular monitoring of arterial blood gas. In the case of severe lactic acidosis, the glucose infusion should be limited to 3–5 mg/kg/min.

After the exclusion of fatty acid oxidation disorders, intravenous lipid solution should be introduced [8] (Appendix C).

5.5.2 Stage 2: Medical Management

Correct the acid-based balance. If blood pH <7.0–7.2, provide a slow administration of IV 8.4 % $NaHC0_3$ in a dose of 0.25–0.5 mEq/kg/h (up to 1–2 mEq/kg/h) keeping in mind the potential risk of hyponatremia, brain edema, and hemorrhage into the central nervous system. In the case of severe lactic acidosis hypernatremia with sodium concentrations above 160 mmol/L can occur that limits the use of sodium bicarbonate ($NaHC0_3$). Trometamol (THAM) and/or dialysis should be considered as a solution.

Ensure proper hydration. Hydration is important not only to correct dehydration that often accompanies metabolic intoxication but also to provide a way to eliminate toxins. Recommended amounts of fluids are 150/mL/kg/day, higher doses may require forced diuresis to avoid cerebral edema. Proper hydration status should not be corrected suddenly and, depending on the level of dehydration, should be planned out over 24–48 h [17].

The correction of electrolytes, blood sugar, and hydration in the neonate is based on recent biochemical test results, performed as frequently as every 1–2 h during the initial illness. It is also important to monitor diuresis and weight changes. The concentrations of potassium should range above 3.5 mmol/L and sodium from 135 to 140 mmol/L.

5.5.3 Stage 3: Detoxification

Hyperammonemia (especially in neonates) is a condition requiring prompt attention. After ceasing protein intake, supplying high concentrations of IV glucose, and restoring proper hydration status while being mindful of the risk of cerebral edema, the next step is to reduce ammonia concentrations. Certain drugs help to eliminate ammonia through different modes of action. Arginine and citrulline increase the elimination of ammonia by the urea cycle. Nitrogen scavenging drugs, such as sodium benzoate and sodium or glycerol phenylacetate, bind with glycine and glutamine to create hippurate and phenylacetylglutamine that can be excreted with urine (Chap. 15). N-Carbamyglutamate is similar in its structure to N-acetylglutamate – natural activator of cofactor for carbamoyl phosphate, the first enzyme of urea cycle and normalizes ammonia levels. It is especially helpful in patients with N-acetylglutamate synthase (NAGS) but it is also used in secondary hyperammonemias associated with several organic acidurias, making the drug useful even when the final diagnosis has not yet been determined [3, 14, 15].

Sodium benzoate and sodium phenylbutyrate/phenylacetate can be toxic, especially with a concentration exceeding 2 and 4 mmol/L, respectively. There is also a possibility of increased sodium and decreased potassium especially when sodium phenylbutyrate and sodium benzoate are administrated together; therefore, electrolytes should be closely monitored.

In the case of rapidly increasing hyperammonemia, with ammonia values exceeding 400–500 μmol/L or if there is no significant decreasing of ammonia values (after 4 h of treatment or, if after 12–24 h of treatment, the ammonia concentration still exceeds 200 μmol/L), a swift decision should be made to eliminate ammonia with extracorporeal methods. The limitation of peritoneal dialysis or blood transfusion is that these procedures are not so effective and induce catabolism. Hemofiltration or hemodialysis should be started, and the best method to use depends upon the patient's body mass and experience of the medical staff. If there is no possibility of performing hemodialysis, the patient should be immediately transferred to another center. If a transfer is not possible, peritoneal dialysis can be considered as a relatively simple method of extracorporeal filtration [8, 14, 17].

5.5.4 Stage 4: Promotion of Anabolism

Proper caloric intake is crucial from the first moments of treating a neonate with an intoxication syndrome. Aside from glucose, lipids are an

Table 5.3 Examples of cofactor responsive inborn errors of metabolism

Cofactors use in inborn errors of metabolism with metabolic intoxication			
Disorder	Cofactor	Therapeutic dose	Frequency of responsive variants
Biotinidase deficiency	Biotin	5–10 mg/day	All cases
Folinic acid-responsive seizures	Folinic acid	5–15 mg/day	All cases
Glutaric aciduria type 1	Riboflavin	20–40 mg/day	Rare
Homocystinuria	Pyridoxine	50–500 mg/day	~50 %
Hyperphenylalaninemia due to disorders of biopterin	Tetrahydrobiopterin	5–20 mg/day	All, but no improvement in CNS neurotransmitter levels
Methylmalonic aciduria	Vitamin B_{12}	1 mg Im/day	Some
Maple syrup urine disease (MSUD)	Thiamin	10–15 mg/day	Rare
Multiple carboxylase deficiency	Biotin	10–40 mg/day	Most
OAT	Pyridoxine	20–600 mg/day	~30 %
Propionic aciduria	Biotin	5–10 mg/day	Possible never
Pyridoxine-responsive seizures	Pyridoxine	50–100 mg/day	All cases

Adapted from Walter and Wraith [19]

important energy source when promoting anabolism in a patient with hyperammonemia, organic aciduria, and aminoacidopathy. Lipids can be administered only after fatty acid oxidation disorders are ruled out. Recommended lipids dosing is from 1.0 to 3.0 g/kg/day or higher [8, 14].

After 24–48 h of the initial cessation of oral feeding and stopping parenteral protein and lipids, protein should be reintroduced into the diet starting with 25–50 % of daily requirement and gradually increasing over the course of next few days. If protein is eliminated for longer than 24–48 h, endogenous protein turnover begins and the synthesis of toxic metabolites increases. The administration of amino acid substitutes using a gastrostomy tube may become necessary. In many cases, partial breast-feeding is possible.

5.5.5 Stage 5: Other Supportive Treatment

Treating of infections will reduce one potential promoter of catabolism and prevent further episodes of decompensation. Treatment should be administered with an effective antipyretic and seizure management and, in some cases, antiemetic drugs, such as ondansetron. When treating seizures, avoid drugs that may inhibit mitochondrial function such as valproic acid or chloral hydrate [17].

L-carnitine is given in many metabolic disorders as a supplement or to correct a carnitine deficiency. The dose of carnitine can vary between 50 and 100 mg/kg/day, and in some organic acidurias, as much as 200–300 mg/kg/24 days may be necessary. In some of the long-chain fatty acid oxidation disorders, use of carnitine is controversial, and in the view of potential adverse effects (formation of cardiotoxic acylcarnitines), supplementation at time of metabolic decompensation should be avoided [18].

In propionic and methylmalonic aciduria, metronidazole, given orally, inhibits the production of propionic acid by gut bacteria. In isovaleric aciduria and methylcrotonyl-CoA carboxylase deficiency, glycine accompanied by carnitine supplementation increases the elimination of toxic metabolites. In many severe conditions, empiric administration of substances that act as cofactors proves to be helpful, and this treatment option should not be neglected (Table 5.3) [19].

5.6 Summary

Individual inborn errors of metabolism are very rare, but as a group, they represent a quite common cause of acute deterioration in newborns. In the first days to weeks of life, neonate with inborn errors of metabolism and metabolic intoxication may be asymptomatic or present with symptoms similar to the more common manifestation of disorders of early infancy, including generalized

infection, birth trauma, respiratory distress syndrome, and others. A careful analysis of medical history of present illness and clinical condition of the patient, backed up with the interpretation of basic biochemical test results, often allows for establishing a reasonable suspicion of an inborn error of metabolism and directs further diagnostic and therapeutic actions. Late effects of the treatment depend on the time between the first symptoms of metabolic intoxication occurred and the initiation of the effective treatment. Prevention of the accumulation of metabolic toxins and promotion of anabolism are the most important steps in the treatment of metabolic intoxication.

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Anabolism: Practical Strategies

6

Johan L.K. Van Hove

Contents

Core Messages

- Anabolism is a metabolic state of protein synthesis.
- In the immediate postprandial metabolism, carbohydrate is the preferred source of energy. After 12–15 h of fasting, fat is used as the energy source.
- For maintenance of anabolism, not only energy should be provided but also essential amino acids.

6.1 Background

Anabolism is a metabolic state in which the body produces synthetic reactions, in particular protein synthesis. It is the opposite of catabolism, a metabolic state where proteins are broken down. Since many metabolic diseases involve the breakdown of metabolites, in particular protein-derived metabolites, avoiding catabolism is an important aspect of therapy. This is particularly important during illnesses where adequate intake of food is often compromised. For anabolism, both energy and constituent components are needed. These include sufficient essential amino acids, but also vitamins, minerals, and essential fatty acids. Provision of adequate energy is a first consideration. Energy can be derived from carbohydrates or from fat. Obtaining energy from protein is limited by the nitrogen load.

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During illness, carbohydrates can be provided as small frequent meals, as drip-feeding, and sometimes as continuous drip-feeding. Using alpha-dextrin maltose preparations has the added advantage of low osmolality and easy digestion.

6.2 Fasting and Postprandial Metabolism

In the immediate postprandial period, human metabolism prefers the use of carbohydrates. After a meal, first, there is absorption of food that usually takes between 3 and 4 h (see chapter 26).

Thereafter, energy will be provided by glycogenolysis (breakdown of glycogen) and gradually by gluconeogenesis (synthesis of glucose from noncarbohydrate sources, such as amino acids and glycerol). After 12–15 h of fasting, lipolysis will start and metabolism will gradually switch to the use of fat as the primary energy source. Disorders that affect metabolism of fat or protein will become more problematic when fasting time extends to 12–15 h. Free fatty acids increase after 12 h, and ketones start to increase after 15 h fasting [1]. Indeed, the earliest times recorded of hypoglycemia in a fatty acid oxidation disorder such as medium-chain acyl-CoA dehydrogenase (MCAD) deficiency occurred at 12 h fasting time [2]. Thus, for these disorders, fasting longer than 15 h should be avoided.

Thus, families should be taught to think in terms of hours fasting since the last good meal. During the night when the child sleeps, there is a natural fasting time, often of 10–12 h. If the child experiences a problem either with the evening feeding or with the morning feeding, this natural fasting time will be extended into a duration that is not acceptable. Thus, practical strategies for such times should be prepared. If the evening meal is not taken due to poor appetite or vomiting with illness, then parents should keep the time since the last meal, often lunch, into account. The maximum fasting duration of 15 h will likely occur during the night. Thus, the child should be awakened at night to attempt to feed and if unsuccessful should be brought to the hospital for care. If the problem occurs in the morning, the child has already been fasting overnight, and the maximum fasting duration is only a few hours off. Often, frequent small meals are better tolerated than a single large amount. If not tolerated, a high concentrated carbohydrate drink can be offered.

Concentrated solutions of alpha-dextrin maltose can be used [3]. They have a low osmolality and are easily digested. The concentration that can be tolerated increases with age: 15 % in infants less than 1 year, 20 % at 1–3 years, 25 % 3–6 years, and 30 % from age 6 years on. The amount can be calculated from Table 6.1.

For most patients, taking 60 mL each hour results in adequate intake of energy. This solution is rapidly absorbed, often within 30–60 min. Giving a tablespoon of 15 mL every 15 min is usually tolerated the best. Caution should be taken during diarrheal illnesses as the alpha-dextrin maltose can cause osmotic diarrhea and result in hyponatremia. Alpha-dextrin maltose solution is incomplete nutrition and should not be used for long duration (24–36 h maximum). The solution can also be used to shorten the duration of fasting surrounding medical procedures such as anesthesia. Stomach clearing in 2 h after an alpha-dextrin maltose solution has been documented [4].

Table 6.1 Glucose polymer guidelines for illness

Glucose solution (%)	Age for use	Grams of glucose polymer per 100 mL	Amount of glucose polymer in 4 fluid ounces	Energy per 100 mL	Energy per ounce
15	0–12 months	15	8 tsp	60	16.8
20	1–3 years	20	11 tsp	80	22.4
25	3–6 years	25	4.5 tbsp	100	28
30	6+ years	30	5.5 tbsp	120	33.6

Adapted from Van Hove et al. [3]

6.3 Acute Episodes and Hospitalization

During acute illness, the need for energy is often 100–120 % of normal. Caloric needs can be estimated from published tables [5]. It is usually safe to start provision of calories with intravenous glucose, but this often does not provide sufficient calories. Not only should one consider the amount of glucose to prevent hypoglycemia but also the total calories provided. To fulfill energy needs, usually a combination of intravenous glucose and fat (as IntraLipid®, Baxter International Inc., Deerfield, IL) should be provided. Optimal energy is provided by a mixture of carbohydrates and lipids in infants [6].

For maintenance of anabolism, not only energy should be provided but also essential amino acids. For patients who have a restriction of specific amino acids, such as in propionic acidemia, early introduction of a metabolic formula with gastric drip-feeding can be used. For disorders with limitation of general protein intake, such as urea cycle defects, protein should be restricted. The requirement for essential amino acids should still be fulfilled. Otherwise, this will induce catabolism with more severe protein catabolism. A first practical approach can be to provide a medical food containing essential amino acids via gastric feeding or drip-feeding. If all enteral feedings are not possible, then intravenous protein should be given without delay to provide the essential amino acids. In some cases, specialized parenteral nutrition without specific amino acids is needed, such as BCAA-free PN in maple syrup urine disease. The amount of essential amino acids needed can be estimated from published sources [5]. Plasma amino acid concentrations should be monitored regularly to ensure that sufficient amounts of essential amino acids are provided. Many patients already are deficient in essential amino acids upon admission [7]. Provision of essential amino acids should commence immediately rather than waiting for a decrease in ammonia or other toxin.

Finally, sufficient amounts of vitamins must be provided. Many patients often have had poor nutrition intake before their admission. This creates risk of thiamin deficiency. Thiamin deficiency exacerbates the energy deficit, increasing the risk for neurological damage. Thus, extra thiamin should be provided to avoid neurological problems when large quantities of carbohydrates are given during early phases of treatment.

6.4 Summary

The promotion of anabolism, the state in which the body builds new components, is key in the treatment and prevention of illness in many inherited metabolic diseases. Many metabolic disorders affect catabolic processes, such as disorders of the breakdown of fatty acids, amino acids, or glycogen. Anabolism can also decrease the accumulation of toxic substances by decreasing production and promoting excretion when switching from a catabolic state to one of synthesis. When a patient is anabolic, there is reduced generation of ammonia, free amino acids, and subsequent metabolites, such as organic acids. During times of acute illness in metabolic patients, resting energy expenditure can be increased as well as protein synthesis for anabolism. Certain patients in intensive care setting may have variable resting energy expenditure, which optimally should be measured by indirect calorimetry. Filling energy needs with carbohydrates can be done with multiple small meals or with gastric drip-feeding to minimize metabolic decompensation. An easily digested carbohydrate source is alpha-dextrin maltose, which can be used during acute illness. Uncooked cornstarch may be used in older patients that require a slow release of energy. The most important component needed for anabolism is calories. Synthetic processes are energy dependent, and a positive caloric situation is needed to provide for an environment conducive to anabolism.

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Protein Requirements in Inherited Metabolic Diseases

7

Steven Yannicelli

Contents

Core Messages

- Protein is a critical part of the diet in individuals with inherited metabolic diseases (IMD).
- Current Dietary Reference Intakes may underestimate protein needs for individuals with IMD.
- Low "whole" protein diets without the use of amino acid-based medical foods may not contain sufficient protein for majority of individuals with an inherited metabolic disease.
- Distributing protein intake throughout the day facilitates anabolism.
- Protein requirements for catch-up growth should be calculated as adjusted weight for age and not actual weight.

7.1 Background

Protein is found in all cells and has multiple functions in the human body including structural, hormonal, enzymatic, immunologic, and regulation of acid-base balance. Protein contains 16 % nitrogen. There are 20 amino acids used for protein function in humans. These are divided into three categories: indispensable amino acids (also called essential amino acids) that cannot be synthesized in the body and must be supplied from the diet, dispensable (also called nonessential

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Table 7.1 Classification of amino acids in the human diet

Indispensable, dispensable, and conditionally indispensable amino acids in the human diet		
Indispensable (essential)	Dispensable (nonessential)	Conditionally indispensable
Histidine	Alanine	Arginine
Isoleucine	Aspartic acid	Cysteine
Leucine	Asparagine	Glutamine
Lysine	Glutamic acid	Glycine
Methionine	Serine	Proline
Phenylalanine		Tyrosine
Threonine		
Tryptophan		
Valine		

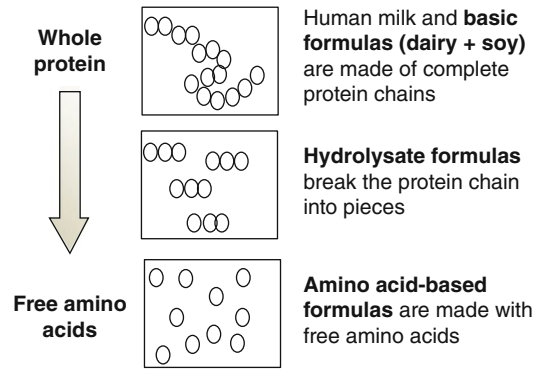

Fig. 7.1 Complexity of protein in human milk/standard formulas, hydrolysate formulas, and amino acid-based formulas

amino acids) that are synthesized endogenously, and conditionally indispensable amino acids (Table 7.1). An example of a conditionally indispensable amino acid is in phenylketonuria (PKU) where tyrosine is not sufficiently hydrolyzed from phenylalanine, making tyrosine an indispensable amino acid.

7.2 Biological Value and Digestibility of Protein Composition

Protein composition and quality influence the rate of digestion, absorption, and ability to provide sufficient nitrogen and amino acids for growth. Protein quality is determined by digestibility and amino acid composition, with indispensable amino acids of high importance. An imbalance of indispensable amino acids or an insufficient amount of a single indispensable amino acid ("limiting amino acid") will negatively affect protein synthesis and turnover. For this reason, all indispensable amino acids must be provided in sufficient quantities to meet requirements and drive protein synthesis.

Protein digestibility and amino acid composition differ among the various forms of protein. Standard infant formulas and breast milk contain whole protein where all amino acids form a complex bond or polypeptide. Other infant formulas contain protein hydrolysates where protein is broken into specific chain lengths or dipeptides and tripeptides, whereas other formulas contain free amino acids (Fig. 7.1). Medical foods for the nutrition management of inherited metabolic diseases are commonly in a free L-amino acid form. An exception is glycomacropeptide (GMP), a protein hydrolysate naturally low in phenylalanine and used in some medical foods for individuals with PKU. Compared to whole protein, free amino acids in metabolic medical foods have increased rates of absorption and oxidation [1–3].

7.3 Protein Turnover

Protein turnover is the process by which the body contributes to the free amino acid pool through a balance of synthesis and degradation. This constant turnover, or resynthesizing of endogenous protein, occurs in the body's cells. The majority of protein turnover is in the liver and intestines, with less occurring in the skeletal muscle [4, 5]. Rates of protein turnover and deposition differ through the lifespan, with infants having about four times greater daily protein turnover rate as adults [6, 7]. Consequently, protein requirements are highest in infants. When protein synthesis is equal to degradation, the body is in "balance" (Fig. 7.2).

Borsheim and colleagues [9] reported that protein synthesis is driven by indispensable

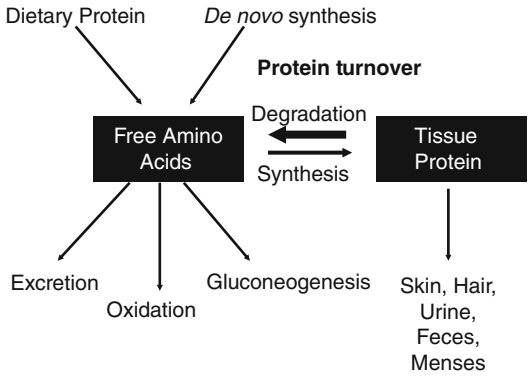

Fig. 7.2 Dietary and de novo protein synthesis (Adapted from Institute of Medicine (U.S.) et al. [8])

amino acid content and not dispensable amino acid content of diet [9]. Increasing the concentration of extracellular indispensable amino acids, particularly leucine, was reported to initiate protein synthesis regardless of absolute value of the concentration [10]. Of all the indispensable amino acids, leucine is an important protein synthesis agonist [11].

During catabolic crisis, protein degradation is more active than synthesis, resulting in negative protein balance. In patients with severe metabolic disorders, this is a concern and creates a challenge to clinicians to reduce *de novo* protein degradation. During acute metabolic crises, the goal is to maintain an adequate amino acid pool by ensuring that protein/amino acids are not eliminated from the diet for prolonged periods and adequate calories are provided.

Many studies have reported that the protein source affects nitrogen retention and whole body nitrogen [12, 13]. Dangin and colleagues [14] reported that protein digestion rates regulate protein retention in fast-acting (whey) and slow-acting (casein) proteins [14]. Whey is rapidly digested and results in a quick rise in plasma amino acids stimulating protein synthesis [12]. One study in rats compared casein with free amino acids [15]. Results showed increased weight gain and decreased renal nitrogen excretion in rats fed casein compared to free amino acids, indicating improved whole body nitrogen homeostasis. Monchi and associates also reported that rats fed a casein hydrolysate compared to those fed free amino acids had statistically significant higher body nitrogen, weight gain, and net protein utilization [16]. Children with PKU fed free amino acids compared to age-matched controls fed whole protein showed statistically poorer growth and lower total body nitrogen, despite consuming the same amount of total dietary protein [17].

7.4 Other Factors Influencing Protein Utilization

Dietary amino acid adequacy is markedly influenced by energy balance. Sufficient energy intake must be provided in diets for patients with inherited metabolic diseases to preserve protein for synthesis and adequate growth. Throughout the lifespan sufficient energy must be supplied for weight maintenance.

Protein, as well as nonprotein energy (i.e., carbohydrates and fats), must be provided in sufficient amounts to drive protein synthesis and prevent protein-energy deficiency [18]. However, too many nonprotein calories will increase weight but not lean body mass, which may be the case in certain patients with inherited metabolic disorders on low protein, high caloric intakes. The type of nonprotein energy can make a difference on protein status. Studies have indicated that carbohydrate (CHO), and not fat, can reduce postprandial protein degradation [19, 20]. Net protein utilization improved by 5 % and nitrogen retention by 14 % when carbohydrate was offered. Excess CHO without protein stimulates postabsorptive proteolysis and protein synthesis [21].

Very early studies in infants and children on free amino acid-based diets reported 20–25 % additional nonprotein energy was required to support nitrogen balance [22, 23]. However, in patients with metabolic disorders who have limited mobility or are nonambulatory, fewer total calories often suffice in maintaining growth and weight maintenance [24].

Distributing protein throughout the day positively influences protein synthesis [25–27] in patients on an unrestricted diet. Optimal protein distribution is shown in Fig. 7.3.

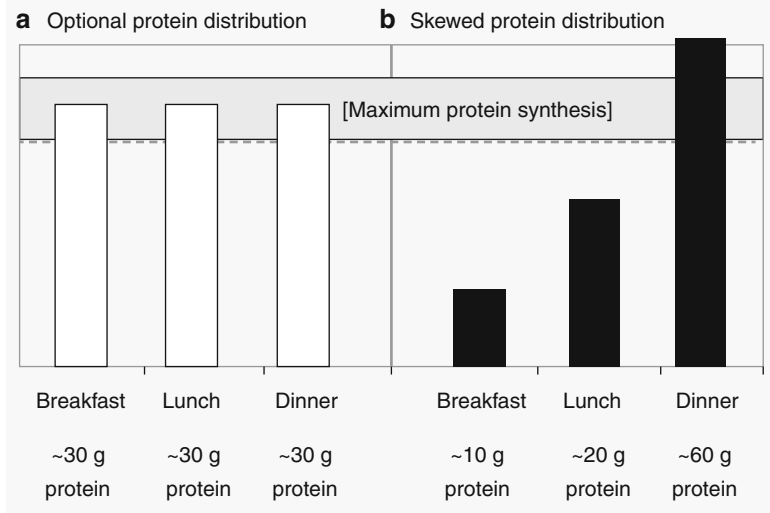

Fig. 7.3 Optimal distribution of daily protein intake (Adapted from Paddon-Jones and Rasmussen [28])

Paddon-Jones et al. reported optimal protein synthesis when dietary protein was provided three times per day compared with the same total amount of protein given in various amounts [26, 29]. In patients with PKU, providing amino acid-based medical food throughout the day compared to a single dose of similar protein equivalents had a positive effect on protein synthesis [30]. A positive effect on plasma phenylalanine concentrations was also reported [30]. For patients with a metabolic disorder, providing medical food along with limited whole protein foods is most beneficial to optimize synthesis.

7.5 Protein Requirements for the General Population

Protein requirements for both the general populace and for patients with a metabolic disease need to be used as benchmarks and not clinical dogma. Recommended Dietary Allowances (RDA) and Dietary Reference Intakes (DRI) for age are determined by a number of factors, including minimum requirements for age. The Estimated Average Requirement (EAR) is "the average daily nutrient intake estimated to be necessary to meet the requirements of half of the healthy individuals in a group" [18]. The RDA is the "average daily dietary intake sufficient to meet the nutrient requirements of nearly all (97–98 %) healthy individuals in a group."

Protein requirements have been determined primarily by nitrogen balance studies wherein calculation of intake versus excretion of nitrogen reflects either synthesis or catabolism. The Estimated Average Requirement (approx. 0.66 g/kg/day) is derived from the amount of nitrogen necessary for nitrogen balance to be zero in balance studies. The RDA for protein is based on the EAR plus a safety factor resulting in a protein recommendation of 0.8 g/kg/day for adults [18, 31]. No tolerable upper limits have been set for either protein or indispensable amino acids due to insufficient data.

Another method of determining protein requirements is the indicator amino acid oxidation technique. This method utilizes a carbon-labeled isotope (L-[1-^{13}C]) tracer that is ingested orally, and oxidation of this labeled carbon is measured in expired breath as $^{13}CO_2$. This method is based on the assumption that if one indispensable amino acid is deficient, all other amino acids will be oxidized until that particular indispensable amino acid is available in adequate amounts, at which point oxidation of the amino acid pool, including the tracer, will be the lowest [32]. Using the indicator amino acid technique, researchers reported that protein recommendations are as much as 30 % higher than the WHO recommendations that are based on nitrogen balance studies [33]. The indicator amino acid oxidation method may help researchers reevaluate current protein requirements [34, 35].

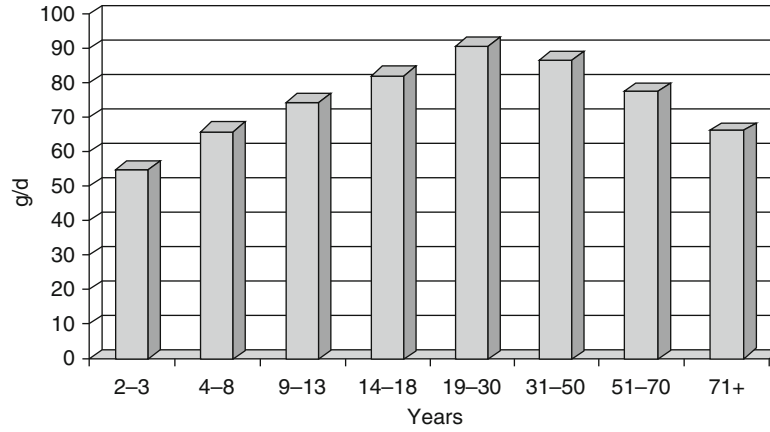

Fig. 7.4 Protein intake (g/d) by age – NHANES, 2003–2004 [36]

The differences in protein recommendations among different countries and within countries at different time points indicate that recommendations continue to evolve based on research and understanding of needs [36–38] (Appendix D). Figure 7.4 shows that actual protein intake in the United States is above the recommended intakes.

7.6 Protein Requirements in Inherited Metabolic Diseases

For patients with metabolic disorders requiring amino acid-based metabolic formulas, the RDA may not be the best indicator for protein adequacy because nitrogen balance studies that determine requirements are based on healthy individuals. The optimal amount of protein to provide to patients with metabolic disorders is not well established. Comparing the World Health Organization (WHO) protein recommendations to a standard protocol often used by metabolic dietitians [39], a significant variance can be observed in patients with phenylketonuria (PKU) [40]. For example, the current WHO recommendation is 0.9 g of protein per kilogram of body weight versus the updated guidelines for PKU that recommend an intake of 2.5–3.5 g of protein per kilogram of body weight [41, 42]. WHO recommendations, like the US RDA, reflect the consumption of high biologic value proteins and not elemental formulas.

Infants with PKU were fed two amino acid-based formulas, one with slightly lower protein equivalents (2.74 g/100 kcal) than the other formula (3.12 g/100 kcal). Both formulas were significantly higher than the Infant Formula Act guidelines of 1.7 g/100 kcal. After 6 months, the infants receiving the higher protein formula showed significantly greater weight, length, and head circumference and improved tolerance to restricted dietary phenylalanine (38 %) [43].

Normal growth acceleration and protein status indices were reported in infants and toddlers with organic acidemias treated for 6 months with an amino acid-based formula [44]. Subjects with linear growth consumed nearly 120 % of the FAO/WHO protein requirements and slightly less than 100 % of recommended energy. Despite normal linear growth and protein status indices, plasma isoleucine and valine remained significantly below normal reference values. When comparing protein and energy intake in patients with propionic acidemia who were growing adequately versus those who suffered from poor growth, it was shown that higher protein intakes from both whole protein and amino acid-based formulas were beneficial [44, 45].

In patients with metabolic disorders, adequate protein and indispensable amino acid intakes are needed to promote anabolism, prevent protein and amino acid insufficiency, and promote normal growth and development. Risk of protein over-restriction is a serious concern and can lead to protein-energy malnutrition and poor growth

Table 7.2 GMDI/SERC recommended intakes of phenylalanine, tyrosine, and protein for individuals with PKU [41, 42]

Age	Phenylalanine (mg/day)	Tyrosine (mg/day)	Protein[a] (g/kg)
Infants to <4 years[b]			
0 to <3 months[b,c]	130–430	1,100–1,300	3–3.5
3 to <6 months[b]	135–400	1,400–2,100	3–3.5
6 to <9 months[b]	145–370	2,500–3,000	2.5–3
9 to <12 months[b]	135–330	2,500–3,000	2.5–3
1 to <4 years[b,d]	200–320	2,800–3,500	≥30
>4 years to adults[e]	200–1,100	4,000–6,000	120–140 % RDA for age[f]

[a]Protein recommendations for individuals consuming phenylalanine-free amino acid-based medical foods as part of their protein source

[b]Recommended intakes for infants and children <4 years of age are adapted from [39] and are for individuals with the classical form of phenylketonuria treated with a phenylalanine-restricted diet alone

[c]Phenylalanine requirements for premature infants with phenylketonuria may be higher

[d]Tolerance is usually stable by 2–5 years of age as phenylalanine requirements are based on a combination of size (increasing with age) and rate of growth (decreasing with age). For any individual, phenylalanine intake is adjusted based on frequent blood phenylalanine monitoring

[e]Adapted from van Spronsen et al. [51]. Range of phenylalanine intake is for the entire spectrum of phenylketonuria (mild to classical)

[f]Recommended protein intake from elemental-protein medical food is greater than the RDA and necessary to support growth in individuals with phenylketonuria

[45–50]. In contrast, too much protein may be contraindicated and result in metabolic decomposition and worse.

Traditionally in the United States, the recommended protein intakes for infants with PKU and other inborn errors of metabolism range from 3.0 to 3.5 g/kg body weight or higher [40, 41] which is significantly greater than the DRIs for age [39]. The higher recommended intakes may not be fully supported by some practicing metabolic clinicians. Van Rijn et al. measured whole body protein metabolism in healthy adults with PKU compared to healthy adult controls. Using primed-continuous infusion methods with [1-^{13}C] valine, these authors showed that whole body protein metabolism in PKU adults was not significantly different than healthy controls when given RDA for protein [35]. The nutrition guidelines for PKU developed by Genetic Metabolic Dietitians International (GMDI) (www.gmdi.org) and the Southeast Regional Newborn Screening and Genetics Collaborative (SERC) recommend for adults protein intakes closer to the RDA with an added safety factor of 120–140 % [42]. For infants, the new recommended intakes are more in line with Dutch guidelines (2009, unpublished data) of 2.5 g protein/kg body weight [41, 42] (Table 7.2).

In inherited metabolic disorders, not only is total protein a major consideration, but also the balance of individual amino acids. Excessive or imbalanced plasma amino acid concentrations negatively affect absorption, protein synthesis, and brain concentrations of indispensable amino acids. In PKU, high blood phenylalanine concentrations cause high phenylalanine concentrations on the brain [52, 53]. In organic acidemias and maple syrup urine disease, imbalances in several or more indispensable amino acids can significantly affect protein synthesis (Fig. 7.5) (Chap. 11).

Providing sufficient protein and energy in patients with severe metabolic disorders, such as organic acidemias, can be a challenge in maintaining optimal nutrition status. Failure to thrive, anorexia, compromised immune functions, vomiting/diarrhea, and metabolic decompensation are not uncommon [46, 54]. Severe feeding difficulties are also common [54]. In patients with organic acidemias and urea cycle disorders, protein from both whole protein and amino acid-based formulas must be carefully balanced. Inadequate and excess dietary protein can exacerbate metabolic crisis. The foundation of nutrition management is a moderate protein, moderate energy (mostly in non- or limited-ambulatory patients) to promote

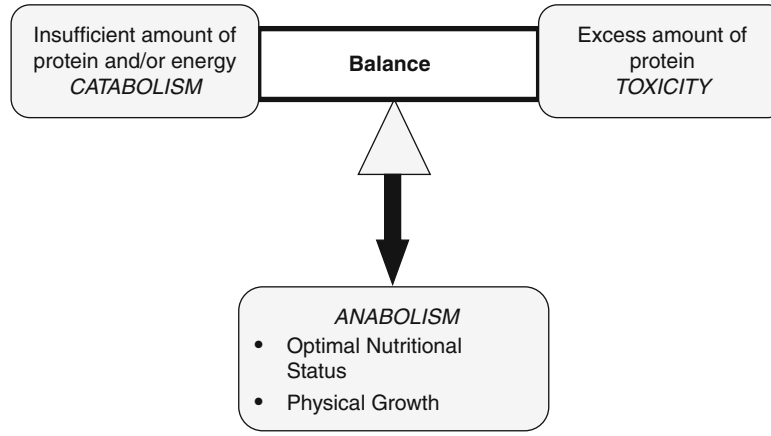

Fig. 7.5 Protein balance is obtained by providing sufficient protein and/or energy for anabolism while not in excess to cause toxicity

Table 7.3 Potential nutritional consequences of an imbalance or inadequacy of protein and/or energy intake [38]

Protein and energy intake	Possible effects
Adequate protein	Adequate growth [55]
Adequate energy	
Adequate protein	Dietary protein used for energy [56]
Inadequate energy	Adults: loss of body protein [57–59]
	Children: reduced growth [60]
	No value in increasing protein without increase in energy
Inadequate protein	Reduced body protein stores and weight loss [61, 62]
Adequate energy	Children: reduced growth [60, 63]
	Potential increased body fat [60, 64–66]
Inadequate protein	Weight loss or poor growth if too low [67]
Inadequate energy	May "adapt" and result in decreased energy expenditure to conserve stores
Excessive protein	May impact bone health if too disproportionate [68]
Adequate or inadequate energy	Infants: risk of metabolic and renal stress [64]
	High protein/low CHO diets have benefits during weight loss [59, 69–71]
Excessive protein	Overweight and obesity [56, 60]
Excessive energy	

anabolism. The exact amount of restricted indispensable amino acids and whole protein is determined by age, disease severity, blood analytes, and growth rate [46] (Table 7.3).

In patients with organic acidemias and urea cycle disorders, approximately 50 % of total protein is consumed in amino acid-based formulas. The amount of total protein and percentage of amino acid-based formulas is variable [44, 72]. Reported clinical outcomes based on dosage of amino acid-based medical foods are variable. Touati et al. [72] reported near-normal growth velocity in patients with propionic acidemia in total protein intakes lower than recommended [72]. The amount of whole protein consumed per age was 0.92 g/kg (age 3), 0.78 g/kg (age 6), and 0.77 g/kg at 11 years of nutrition management. After age 3 most patients received some amino acid-based medical foods. Most patients suffered from feeding disorders, and many were given nocturnal feedings.

7.7 Assessing Protein Status

Protein status can be assessed by several biomarkers, including plasma amino acid profiles, albumin, and transthyretin (prealbumin) [42]. No marker alone is sufficient in determining status (Box 7.1).

Box 7.1: Assessing Protein Sufficiency

- Biochemical
  - Hepatic transport proteins

Total protein
Albumin (20 day half-life)
Prealbumin (48 h half-life)
Retinol-binding protein (24 h half-life)

  - Plasma amino acids
  - Blood urea nitrogen
- Anthropometrics (length, height, weight, head circumference)
- Nutrition-focused physical findings
  - Hair (loss of pigment, alopecia, dull, dry, brittle)
  - Muscle wasting (interosseous muscle, clavicle, biceps/triceps)
  - Poor healing (e.g., severe diaper rash in infants)

Plasma amino acid profiles measure present dietary protein intakes and may be difficult to assess alone without other biomarkers [73]. Serum albumin is a commonly used biomarker reflecting overall protein nutrition. However, transthyretin (prealbumin) is a more sensitive protein marker reflecting short-term changes in protein status. In critically ill patients, prealbumin can be an important screening tool for protein-calorie malnutrition [74]. In PKU, prealbumin has been reported as an important biomarker for evaluating protein status [75]. Low concentrations of prealbumin have been reported in treated patients with PKU [75] and may be inversely related to linear growth in protein-insufficient patients with PKU [76]. For patients with inborn errors of metabolism, plasma amino acid profiles are important biomarkers necessary to prevent toxicity or deficiency of any indispensable amino acid. Routine assessment is recommended, with more frequent measurement recommended during times of metabolic crisis or rapid periods of growth.

Protein insufficiency may result in poor growth, hair loss, muscle wasting, and bone demineralization. Many of these signs and symptoms have been reported in treated patients with metabolic disorders, especially in those with severe genotypes [45, 77]. In certain patients with inherited metabolic disorders, the increased risk of infections, anorexia, and frequent acute metabolic crises makes it difficult to provide sufficient nutrients to promote growth and support good nutritional status.

7.8 Summary

In summary, in inherited metabolic disorders, to achieve optimal growth and development, total protein recommendations are higher than DRIs when the majority of protein is provided by amino acid-based medical foods. To maintain balanced amino acid profiles and nitrogen retention for growth, patients who are dependent on amino acid-based medical foods should consume greater amounts of protein and calories than currently recommended, and protein synthesis is optimized if distributed evenly throughout the day [18, 31, 55]. The number of studies reported on reduced nitrogen retention, protein synthesis, and increased digestion rate and oxidation of free amino acids compared to higher-order proteins provides evidence that total protein intakes greater than recommended values for the normal populace are clinically indicated in metabolic disorders. Attention to assessing protein status and supplying adequate protein is paramount in supporting growth and reducing comorbidities.

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Laboratory Evaluations in Inherited Metabolic Diseases

8

Curtis R. Coughlin II

Contents

Core Messages

- Routine laboratory tests are commonly available and include electrolytes, ammonia, lactate, ketones, and carnitine; these are helpful in evaluating whether a patient may have a metabolic disorder.
- Metabolic laboratory tests are specialized tests that are reviewed by a biochemical geneticist and include plasma amino acids and acylcarnitines, urine organic acids, and acylglycines that are helpful for pinpointing a metabolic diagnosis and/or monitoring treatment.
- Evaluation of laboratory findings should always include consideration of the patient's clinical status, such as presence of illness and length of fasting.

8.1 Background

Inherited metabolic diseases (IMD) are a heterogeneous group of disorders, and the clinical phenotype within a disease can be variable which often results in difficulty in establishing a diagnosis. An acute event consistent with a disorder of intoxication may be suggestive of a metabolic disease, although often a patient's presentation and clinical findings are nonspecific [1]. As a result, the ability to obtain various laboratory

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tests is crucial to establishing the diagnosis of an inherited metabolic disease.

In this chapter the laboratory evaluations are separated into "routine laboratories," which refer to those tests available in most clinical laboratories, and "metabolic laboratories," which refer to those tests typically performed under the purview of a biochemical geneticist. This is an artificial separation for the benefit of the reader as often multiple laboratories are utilized for both the diagnosis and management of inherited metabolic diseases. The reader should use the information in this chapter, and the reference material within, to gain an understanding of the laboratories integral to working within the field of metabolism. The chapter will focus on laboratory tests used in the diagnosis of inherited metabolic disorders; many of these are also used for monitoring nutrition management and will be discussed in disease-specific nutrition management chapters.

8.2 Routine Laboratories

The routine laboratories covered in this section are not unique to inherited metabolic diseases and are performed in most major medical centers. These laboratory studies provide valuable information to the clinician and can strongly suggest a diagnosis of an inherited metabolic disease (Table 8.1). Also the results of these laboratory tests may be available at the bedside or within a few hours and may guide the clinician in treating an acute episode before a specific diagnosis can be confirmed.

8.2.1 Acidosis

An acidosis refers to a disturbance of the patient's acid-base balance and infers that the pH is below that of blood's physiologic pH (7.4): with a pH <7.35 indicating acidemia and a pH >7.45 indicating alkalemia. When a metabolic acidosis is identified, an anion gap should be calculated to determine if an unmeasured anion (or acid) is present. Laboratories may also provide an anion gap when metabolites are available. The calculated anion gap is based upon an assumption that the value of the major cation (sodium) is relatively equal to that of the major anions (bicarbonate and chloride) [2]. Although other cations (potassium, calcium, magnesium) exist in blood, it is convenient to group these together and assume the variation among this unmeasured group is minimal. Therefore the anion gap can be calculated by subtracting the major anions (Cl^- + HCO_3^-) from the major cation (NA^+) (Box 8.1). A normal anion gap should equal to 12 ± 4 mEq/L.

A *non-anion gap acidosis* (hyperchloremic acidosis; anion gap <16 mEq/L) is characterized by an acidosis where the anion gap is unchanged from the patient's baseline. This occurs as the decrease in serum bicarbonate is equaled by the rise in serum chloride [3]. Bicarbonate is typically lost from the gastrointestinal tract (i.e., diarrhea) or through the kidneys (i.e., renal tubular acidosis) [4]. Although a few metabolic disorders result in a non-anion gap acidosis (i.e., Fanconi-Bickel syndrome, OMIM# 227810), a non-anion gap acidosis is typically not the result of an inborn error of metabolism.

Table 8.1 Routine laboratory studies and inherited metabolic diseases

Disorder	pH	Ammonia	Glucose	Ketonuria	Lactate	Other
Urea cycle disorders	↑	↑↑↑	Normal	Normal	Normal	Increased glutamine, orotic acid in some UDC
Organic acidemias	↓↓↓	↑↑	↓Normal	↑↑↑	↑↑	Anion gap, neutropenia, thrombocytopenia
MSUD	Normal	Normal	Normal	↓ Normal	Normal	
FOD	↓	↑	↓↓↓	↓↓↓	↑	Elevated CK, transaminases
GSD	↓	Normal	↓↓↓	Normal	↑↑	Elevated triglyceride, uric acid, and ALT
Mito disorders	↓↓	Normal	Normal	Normal	↑↑↑	Increased lactate, pyruvate, alanine
Tyrosinemia	↓ Normal	Normal	↓ Normal	Normal	Normal	Liver failure, increases AFP, renal Fanconi syndrome

An *anion gap acidosis* (anion gap >16 mEq/L) suggests an increase in unmeasured anion(s), and a significantly elevated anion gap (>20 mEq/L) should always be evaluated further as there are no physiologic processes to generate unmeasured anions [2]. An anion gap acidosis is highly suggestive of an inherited metabolic disease, although an anion gap acidosis may also be iatrogenic or the result of an ingestion, i.e., overdose of salicylic acid (aspirin). Whenever an acidosis is identified, it is important to determine the presence or absence of an anion gap. After establishing the anion gap, relatively few tests are indicated in order to identify the cause of the acidosis (Fig. 8.1).

Box 8.1: Calculating an Anion Gap

Anion gap = Na^+ - (Cl^- + HCO_3^-)		
Normal anion gap = 12–16 mEq/L		
	Normal anion gap	Elevated anion gap
Na^+	140	140
Cl^-	16	8
HCO_3^-	110	110
	140–(16+110)=*14*	140–(8+110)=*22*
	Unmeasured anions (acids) are normal	Unmeasured anions (acids) are elevated
		Elevated acid results in a loss of bicarbonate

8.2.2 Ammonia

An elevated ammonia concentration can be the result of primary or secondary defect of the urea cycle. Ammonia is mainly a by-product of amino acid metabolism, although it is also produced by intestinal urease-positive bacteria. The urea cycle converts ammonia (or ammonium, NH_4^+) to urea, which is excreted by the renal system in order to keep the serum concentration of ammonia low. An impairment of the urea cycle results in

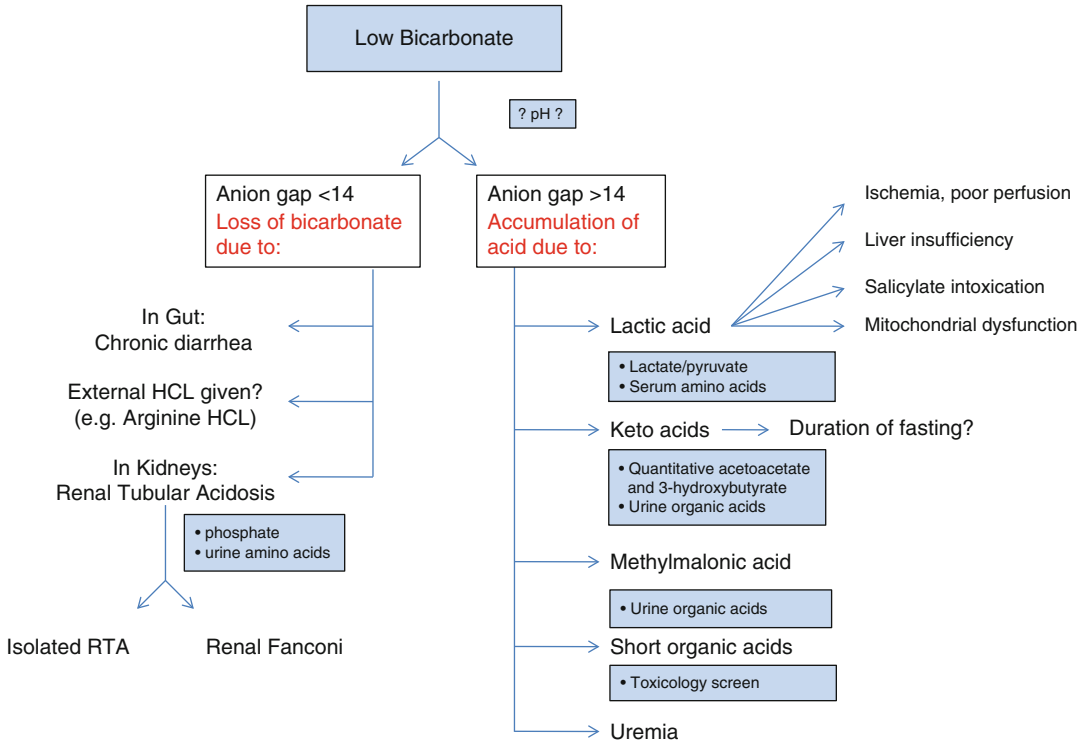

Fig. 8.1 Flow diagram for the evaluation of a patient with an acidosis due to low pH or an apparent acidosis due to low bicarbonate depending on the absence or presence of an anion gap

decreased excretion of urea and increased retention of ammonia. Hyperammonemia appears to be toxic to the central nervous system (CNS), and hyperammonemia may result in cognitive disabilities, seizures, cerebral palsy, and irreversible brain damage [5, 6]. The pathophysiology of hyperammonemia resulting in CNS damage is unclear. Glutamine accumulation, resulting in impaired cerebral osmoregulation or excitotoxic injury, or energy failure may play a major role in the resultant cognitive impairments, although ammonia remains an important surrogate for both disease control and prognosis [7].

Primary urea cycle defects are caused by a deficiency of any of the six urea cycle enzymes (Chap. 15) and result in insufficient disposal of waste nitrogen. As a result, nitrogen accumulates in the form of ammonia and as its precursors, as glutamine and glycine. Primary defects in an enzyme of the urea cycle *typically* result in higher ammonia levels than secondary impairments of the urea cycle, although exceptions occur.

In secondary defects of the urea cycle, such as in disorders of organic acid and fatty acid metabolism, the buildup of toxic metabolites impairs the function of the urea cycle [8]. For example, propionyl-CoA, which accumulates in patients with propionic acidemia and methylmalonic acidemia, is hypothesized to competitively inhibit N-acetylglutamate synthetase resulting in decreased activity of the urea cycle [9]. Hyperammonemia due to secondary defects is usually less severe than in primary defects and is often resolved by treating the underlying disorder and may not require the use of ammonia scavenger medications or dialysis, which are standard therapies in primary urea cycle defects. Hyperammonemia may be an underappreciated finding in fatty acid oxidation disorders and is the predominant biochemical finding in patients during an episode of metabolic decompensation [10].

It is important for the clinician to discern whether hyperammonemia is a result of a primary or secondary urea cycle defect in order to provide appropriate treatment. For example, the use of standard treatments for urea cycle disorders, such as IntraLipid®, could be fatal if a patient had a fatty acid oxidation disorder. The clinical presentation and other laboratory studies may aid the clinician in discerning between various possible inherited metabolic diseases when elevated ammonia is identified (Appendix E).

Hyperammonemia may also result from congenital or acquired causes that are not related to inherited metabolic diseases. Examples of congenital causes include malformations such as portosystemic shunts, extrahepatic portal vein obstructions, and cirrhosis with portal hypertension. Transient hyperammonemia of the newborn (THAN) is typically identified in premature infants and does not appear to have a neurologic effect on those asymptomatic preterm infants [11]. Liver failure may also result in fulminant hyperammonemia. In severe liver failure, all of the enzymes expressed in the liver are deficient, resulting in complete impairment of the urea cycle as well as a deficiency of other important liver-specific enzymes such as the glycine cleavage enzyme.

8.2.3 Glucose and Ketones

Hypoglycemia is the hallmark of disorders of energy metabolism, such as fatty acid oxidations disorders. Hypoglycemia is typically defined by blood glucose concentration below 55 mg/dL (3 mmol/L) in children and is often associated with clinical symptoms, such as shakiness, pale skin, sweating, confusion and, in extreme cases, seizures and coma. Normally, as glycogen stores are depleted, beta-oxidation of fatty acids produces both glucose and ketones, with ketones being utilized preferentially over glucose in some tissues, including the brain [12].

Defects of *fatty acid oxidation* interfere with the production of ketones as a result of impaired beta-oxidation. Hypoglycemia results from excessive use of glucose by peripheral tissues and the inability to synthesize ketone bodies which can be used as alternative fuels [12]. Patients with fatty acid oxidation defects often have significant hypoketotic hypoglycemia, although it is important to note that there may be mild ketone production and, in rare circumstances, significant

ketonuria. Patients with fatty acid oxidation disorders, even those with significant residual enzyme activity, can experience severe, even fatal, hypoglycemia in the setting of extreme fasting or a significant illness [13].

Glycogen storage diseases also present with hypoglycemia, but in contrast to disorders of fatty acid oxidation, relatively normal ketone production is noted. In general, hypoglycemia in these disorders is due to either the defects in the synthase of liver glycogen (glycogen synthase deficiency) or defects in the metabolism of liver glycogen (glycogen storage).

Hypoglycemia is also a hallmark of inborn errors of *ketogenesis and ketone body utilization*. These disorders are typically characterized by metabolic decompensation with ketoacidosis [14]. Quantification of ketone bodies, such as acetoacetic acid and beta-hydroxybutyric acid, is critical to understanding the etiology of hypoglycemia. Idiopathic ketotic hypoglycemia is a relatively common "diagnosis" in children with hypoglycemia and normal ketone production. Idiopathic ketotic hypoglycemia should only be considered when known causes of hypoglycemia have been reasonably excluded. Ketone production is a normal physiologic process that occurs when blood glucose is low. Obtaining a detailed history including the length of fasting [15, 16] as well as a physical exam to determine if there is hepatomegaly or cardiac involvement can help in understanding the cause of hypoglycemia (Fig. 8.2).

8.2.4 Lactate

Lactate exists as two stereoisomers, L-lactate and D-lactate, although L-lactate is the predominant physiologic anion and mainly discussed in the information below. The majority of plasma lactate is derived from glucose metabolism (65 %) and amino acid metabolism through the degradation of alanine (15–20 %) [17]. Lactic acidemia refers to blood lactate levels that are above those typically seen in blood (approximately <2.0 mM).

A lactic acidosis may result from increased lactate production, which is a by-product of compensatory mechanisms in disorders of energy

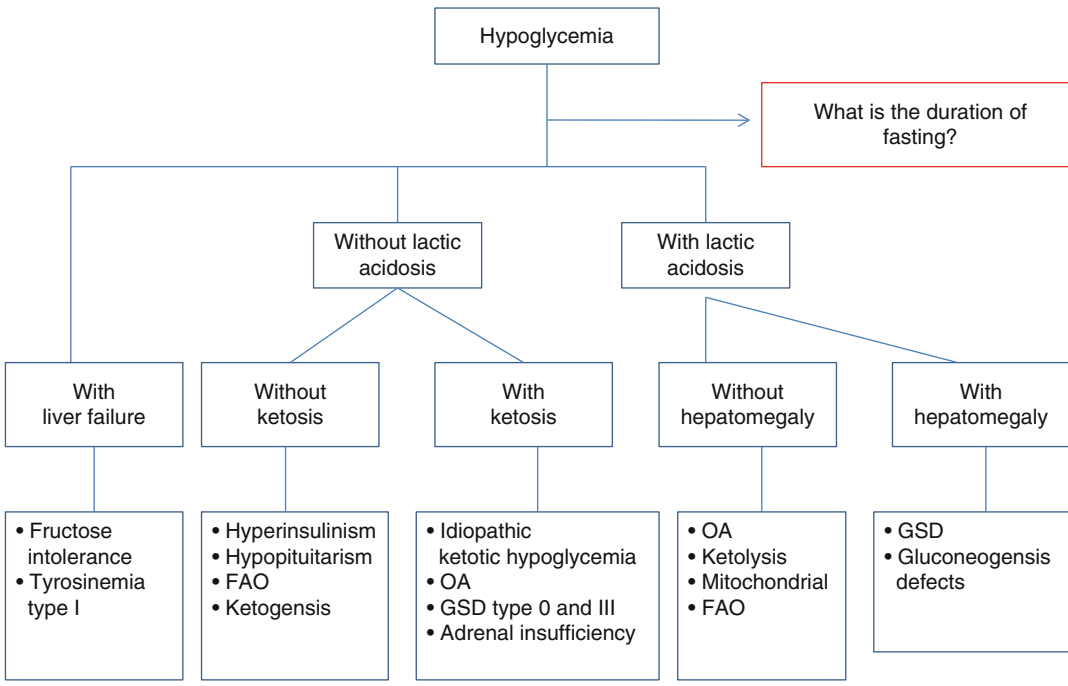

Fig. 8.2 Flow diagram for the evaluation of a patient with hypoglycemia depending on the absence or presence of lactic acid. *FAO* fatty acid oxidation, *OA* organic acidemia, *GSD* glycogen storage disease

metabolism. In defects of pyruvate metabolism, such as pyruvate dehydrogenase deficiency, glucose cannot enter the tricarboxylic acid (TCA) cycle and is diverted to glycolysis [18]. Similarly, in disorders of the mitochondrial respiratory chain, ATP generation is impaired and cells become dependent on glycolysis. Glycolysis rapidly generates ATP, although the by-product of this reaction is the accumulation of lactate. Lactate acidemia may also be a result of defects in lactate removal. The gluconeogenesis pathway is the major pathway for lactate clearance, and defects in the enzymes involved in this pathway (pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-bisphosphatase, and glucose 6-phosphatase) will result in a lactic acidosis [19].

Lactic acidemia is also reported in a variety of conditions including sepsis, chronic liver disease, and tissue hypoxia. It is also important to note that mild lactate evaluations may be due to inappropriate sample collection such as the stress (struggle) of the patient during the blood draw. Also, significant lactate elevations in healthy individuals have been documented after anaerobic exercise or prolonged exercise of fast-twitch muscle [20].

In conclusion, it should be evident that there is nothing "routine" about the above laboratories. These laboratory studies provide valuable and timely information which is especially important in the acute setting. The astute clinician can utilize the results of these laboratory studies and often prioritize the diagnosis of an inherited metabolic disease into a specific category (i.e., a fatty acid oxidation disorder). Although a suspicion of a metabolic disease can be discerned with routine laboratory studies, it is often difficult to establish a specific diagnosis, such as very long-chain acyl-CoA dehydrogenase deficiency as opposed to long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. The metabolic laboratories discussed below are useful in establishing a specific diagnosis.

8.3 Metabolic Laboratories

The tests discussed within this section refer to those studies that typically occur within a metabolic laboratory and are reviewed by a biochemical geneticist. The information stated below is general, and the techniques, platforms, cutoffs, and interpretation of these tests will vary among laboratories. It is always important to interpret a laboratory test within the context of a patient's history, and these laboratory studies are highly influenced by diet, timing of the laboratory study with the last meal, and current medications. Also, a single metabolite does not always correlate with a specific disease, which is why interpretation of these studies by a trained biochemical geneticist is integral to the testing process.

8.3.1 Carnitine Profile

Carnitine is a hydrophilic molecule that plays a pivotal role in both normal physiologic process of beta-oxidation and the elimination of abnormal organic acids [21]. The majority of carnitine (approximately 75 %) is derived from dietary sources such as meat and dairy products, with the reminder of carnitine requirements provided through endogenous synthesis [22]. Carnitine is highly conserved through renal tubular reabsorption. Therefore, renal tubular loss of carnitine can result in significant, and pathologic, loss of carnitine.

The carnitine profile quantifies the amount of carnitine present (total carnitine) as well as the carnitine that is free or bound by an ester link to acyl-CoA (acyl or esterified carnitine) (Box 8.2).

Primary carnitine deficiency results from a defect in the carnitine transporter within the plasma membrane resulting in the inability to reabsorb carnitine and in significant loss of urinary carnitine. As a result, extremely low serum

Box 8.2: Carnitine Profile

Total carnitine	All carnitine species – both free carnitine and carnitine bound to acyl-CoA
Free carnitine	Carnitine species that are *not* bound to acyl-CoA
Acyl (or esterified) Acyl carnitine	Carnitine species that *are* bound to acyl-CoA

carnitine concentrations are present and are reflected with very low free carnitine concentrations (<5 μM compared to normal of 25–50 μM) [21]. Carnitine plays an important role in aiding the transfer of long-chain fatty acids into the mitochondria and in binding to acyl residuals to aid in the elimination of abnormal organic acids. If a significant amount of carnitine is bound to an acyl-CoA, the percentage of bound carnitine (acylcarnitine) will be high, suggesting a disorder of fatty acid or organic acid metabolism. Also, if a significant percentage of carnitine is bound to an acyl-CoA, a secondary deficiency of free carnitine may occur (i.e., low free carnitine). Except when a primary carnitine disorder is suspected, an abnormal carnitine panel should prompt the clinician to request an acylcarnitine profile and/or urine organic acids.

8.3.2 Acylcarnitine Profile

Carnitine is esterified to a fatty acid molecule to form an acylcarnitine, which can then be transported across the mitochondrial membrane to provide a substrate for beta-oxidation. Carnitine can also esterify large organic molecules within the mitochondria due to a defect in fatty acid or organic acid metabolism. In defects of fatty acid oxidation, a fatty acyl-CoA molecule will be metabolized through beta-oxidation until it reaches the enzymatic defect. At the metabolic block, the acyl-CoA will continue to accumulate within the mitochondria. Organic acids are also conjugated to coenzyme A and abnormal organic acids will also accumulate due to a metabolic block. These accumulating acyl-CoAs can esterify carnitine into acylcarnitines that are transported out of intracellular compartments into the blood where they can be easily detected [23].

Acylcarnitine analysis can be performed by various methods, such as gas chromatography-mass spectrometry (GC/MS) and capillary electrophoresis, although the introduction of tandem mass spectrometry (MS/MS) revolutionized the use of acylcarnitine analysis for inherited metabolic diseases [24, 25]. Acylcarnitine analysis is able to identify the accumulation of acyl-CoA esters having from 2 to 18 carbons (C2-C-18) and, when compared to internal standards, the specific pattern of elevated carnitine esters can be diagnostic of an inherited metabolic disease (Fig. 8.3).

It is important to emphasize that an informative result is typically characterized by a specific pattern of acylcarnitine species as opposed to a single abnormal metabolite [26]. Although both the overall pattern of the profile and the interpretation by a biochemical geneticist are extremely important, the metabolic provider should still be familiar with the associations of specific metabolites and inherited metabolic diseases (Table 8.2).

8.3.3 Amino Acid Analysis

In many ways, the modern field of metabolism can be traced back to the identification and quantification of a single amino acid, most notably through the identification of phenylketonuria (PKU) and the development of an accurate and cost-effective analysis for phenylalanine [27, 28]. The single amino acid tests eventually were replaced by various methods that quantify all of the amino acids, such as high-performance liquid chromatography, gas chromatography-mass spectrometry (GC/MS), and tandem mass spectrometry (MS/MS). Regardless of the method used, amino acid analysis is typically diagnostic for all amino acidopathies, such as phenylketonuria, maple syrup urine disease, and tyrosinemia. An abnormal amino acid profile can also aid in the diagnosis of non-amino acidopathies such as urea cycle disorders and pyruvate carboxylase deficiency.

Blood specimens are recommended for investigation of aminoacidopathies because amino acid concentrations are fairly stable in blood, urine amino acids analysis, on the other hand, is appropriate for disorders of amino acid renal transport such as cystinuria. Amino acid analysis in cerebral spinal fluid may be appropriate to aid in diagnosis (i.e., nonketotic hyperglycinemia) and management (i.e., cerebral amino acid disorders) of various IMD. The composition of an

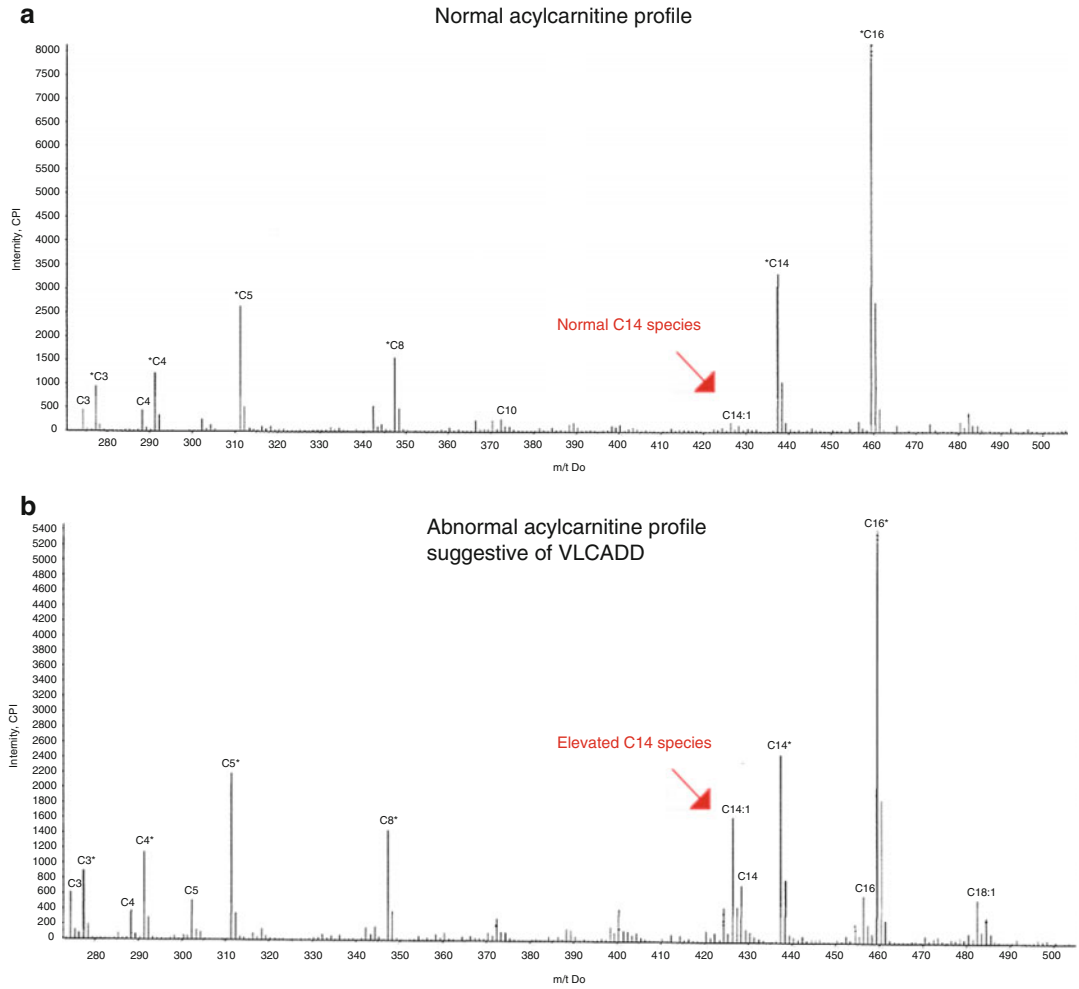

Fig. 8.3 Acylcarnitine profiles with examples of a normal acylcarnitine profile (**a**) and an acylcarnitine profile with elevated C14 esters suggestive of VLCAD deficiency (**b**) (Acylcarnitine profiles are courtesy of the Goodman Biochemical Genetics Laboratory)

Table 8.2 Clinical presentation associated with abnormal amino acids

Catabolism	↑ Leucine, isoleucine, valine
Protein restriction	↓ Leucine, isoleucine, valine
Lactic acidemia	↑ Alanine
Hyperammonemia	↑ Glutamine
Seizure medication	↑ Glycine

amino acid profile is highly dependent on the nutritional intake of essential and nonessential amino acids [29]. Other clinical conditions are associated with abnormal amino acid profiles such as significant protein restriction and certain medications (Table 8.2).

8.3.4 Organic Acid Profile

Disorders of organic acidemia (or aciduria) are characterized by excretion of organic acids, or acids that do not contain an amino group. As a result, organic acids historically could not be analyzed by the same techniques that were performed for amino acid analysis [30], but organic acids bound to carnitine can be identified by MS/MS. Organic acid profiles are similar to other metabolic laboratory tests where an elevated metabolite is suggestive of an inherited metabolic disease (Fig. 8.4), although examining the pattern of organic acid excretion is still paramount.

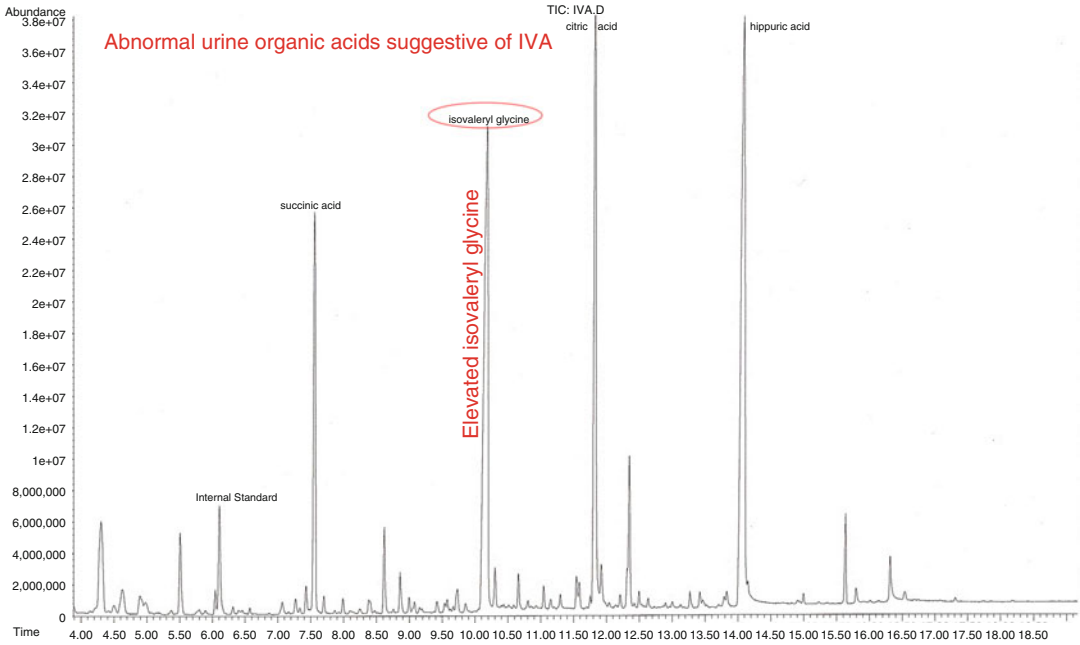

Fig. 8.4 An abnormal urine organic acid profile indicating abnormal accumulation of isovalerylglycine (indicated with a *red circle*). This is suggestive of isovaleric acidemia (and organic acid disorder of leucine metabolism) (Organic acid profile courtesy of the Goodman Biochemical Genetics Laboratory)

Organic acids do accumulate in the serum, although they are poorly reabsorbed by the kidney, resulting in much higher concentrations of organic acids in urine than serum [30]. Similar to the other testing already discussed, causes other than a metabolic disorder, such as medications or physiologic ketosis, can result in an abnormal organic acid profile.

8.3.5 Interpretation of Metabolic Laboratories

As mentioned throughout this chapter, various factors may impact the interpretation of metabolic laboratories. For example, a patient who is well may have normal metabolic laboratories but may have significantly abnormal metabolic laboratories when catabolic [12]. Conversely, metabolism may be compromised when a patient is severely ill, such as in severe heart failure [31], which can make discerning a primary inborn error of metabolism from a secondary impairment of metabolism difficult. As a result, a patient may require metabolic laboratory studies in both the well and catabolic state. Nutritional intake and medications can significantly alter metabolic laboratory profiles. As a result, a regimen may be initiated prior to obtaining a sample especially when following laboratory values for treatment. This may require the laboratories to be following a significant fast or at a prescribed time after a meal. The timing of the sample draw may differ among metabolic clinics, although consistent timing for sample collection may allow the clinician to follow laboratory trends over time.

8.4 Confirmatory Testing

Confirmatory testing is often pursued after a probable diagnosis is made based upon the above basic and metabolic laboratories. Confirmatory testing is typically consistent of genetic or enzymatic analysis. Each of these testing modalities has limitations. For example, complete genetic analysis may require multiple tests (i.e., sequencing and copy number variation analysis). Also in

certain diseases genetic testing may only identify a subset of affected individuals. For example, in ornithine transcarbamylase deficiency (OTCD), a proximal urea cycle disorder, genetic testing is only informative in 80 % of affected individuals [32, 33]. Enzyme testing is considered the gold standard of confirmatory testing. Enzyme testing also has limitations as it may require a cell line requiring a skin biopsy, or may require a specific tissue where the enzyme is expressed. For example, OTCD enzyme analysis requires a liver biopsy for measurement of the hepatic enzyme activity. Enzyme activity is often a continuum and there may be an overlap between the enzyme activity in milder patients and heterozygotes (carriers) of a metabolic disease. This can lead to difficulty with interpretation of the enzyme result. Many of these limitations will be discussed in disease-specific nutrition management chapters.

The clinical presentations of inherited metabolic diseases are often nonspecific, and various laboratory tests provide the information necessary to establish a diagnosis. Often multiple routine and metabolic laboratory studies are needed, and these laboratory studies are complementary. The astute clinician can use the combination of these laboratory studies to initiate emergency treatment in an acute setting, to make a probable diagnosis, and to evaluate the long-term treatment of patients with an inborn error of metabolism.

Case Example

History of present illness: A 3-day-old infant was seen by the pediatrician due to decreasing oral intake after being discharged from the hospital at 48 h of life. The pediatrician assessed that the infant was lethargic and referred him immediately to the emergency department (ED). At presentation, an astute ED resident elected to check ammonia, which was elevated at 445 mmol/L (ref. range <100 mmol/L). She is concerned that the patient has a urea cycle disorder and calls the metabolic department as well as orders routine laboratory studies (Table 8.1).

Laboratory studies: Laboratory studies noted a acidosis with a pH of 7.08. The resident calculated an anion gap of 32, which confirmed an anion gap acidosis as the bicarbonate was 6 mmol/L, sodium was 145 mmol/L, and chloride was 107 mmol/L (Box 8.1). Due to the anion gap acidosis, the resident requests both a lactate (which was relatively normal at 2.2 mmol/L) and urine organic acids (Fig. 8.1).

Diagnosis: The metabolic attending physician discussed the case with the resident and is concerned that the patient has an organic acidemia and not a urea cycle disorder. She also recommends an acylcarnitine profile and plasma amino acids to the already ordered urine organic acids. While the laboratories are pending, the metabolic physician recommends immediate treatment for the probable organic acidemia, including discontinuation of protein feeds and administration of IV glucose at 1.5 times maintenance. The next day urine organic acids noted elevated 3-hydroxypropionic and methylcitric, acylcarnitine profile noted elevated C3 (propionylcarnitine), and plasma amino acids noted an elevated glycine of 569 (ref. range 232–540 nmol/mL). These metabolic laboratories confirmed the diagnosis of propionic acidemia (PROP). Although treatment was already initiated due to the results of the routine laboratory studies, the diagnosis of PROP allowed the clinical team to give more specific treatment recommendations and to provide detailed genetic counseling for the family.

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Part II

Aminoacidopathies

Phenylketonuria: Phenylalanine Neurotoxicity

9

Maria Giżewska

Contents

Core Messages

- Phenylketonuria (PKU) was the first inherited metabolic disease identified by newborn screening and treated with diet to prevent the development of intellectual disability.
- Classification of the severity of phenylketonuria is based on the type of the genetic mutations in phenylalanie hydroxylase (PAH) gene, dietary phenylalanine tolerance, and pretreatment blood phenylalanine concentrations.
- The etiology of brain damage in PKU has not been fully elucidated; however, high blood phenylalanine concentrations are associated with changes in brain morphology (gray and white matter) and decreased neurotransmitter synthesis.

9.1 Background

Phenylketonuria (PKU) is an inherited autosomal recessive metabolic disease characterized by characterized by decrease activity of enzyme phenylalanine hydroxylase (PAH) [1]. The Norwegian biochemist and physician Asbjorn Folling discovered PKU in 1934 by detecting phenylketones in the urine of siblings with mental retardation, with subsequent identification of altered

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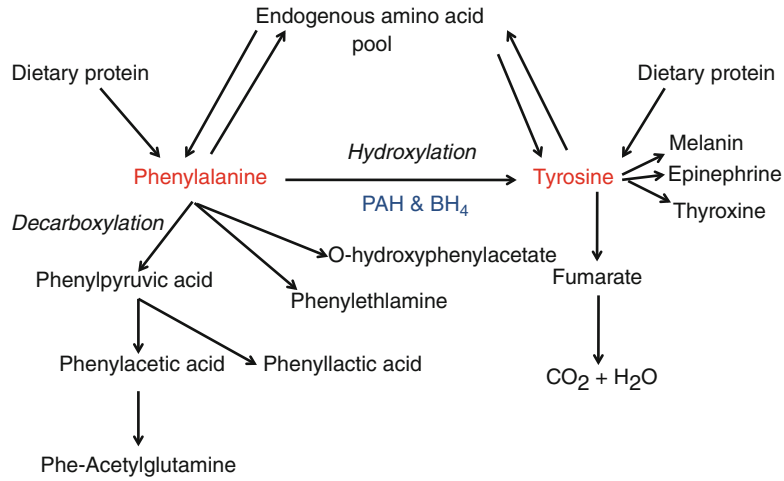

Fig. 9.1 Phenylalanine hydroxylase (*PAH*) and tetrahydrobiopterin (*BH4*) in the presence of molecular oxygen (*O2*) convert phenylalanine to tyrosine. The alternate pathway of phenylalanine metabolism results in the accumulation of phenylalanine as well as phenylpyruvic acid and other phenylketones that are excreted in the urine (Adapted from Scriver and Kaufman [4] and Acosta [1])

phenylalanine metabolism as the cause of this disease [2, 3]. Phenylalanine hydroxylase is the enzyme that converts phenylalanine to tyrosine in the presence of the cofactor tetrahydrobiopterin (BH_4) and molecular oxygen (Fig. 9.1). Loss of PAH activity results in elevated blood phenylalanine concentrations and is referred to as hyperphenylalaninemia (HPA) or phenylketonuria (PKU).

Phenylketonuria is the exemplar of the effectiveness of newborn screening as it was the first inherited metabolic disease in which individuals were identified by newborn screening and treated with diet before the development of intellectual disability associated with untreated PKU [5–7]. If left untreated or ineffectively managed, PKU can cause severe intellectual disability, as well as complex neurological and behavioral disorders. Severely affected patients are unable to live independently, often requiring specialized and continuous supervised care. Conversely, early and continuously treated patients typically have normal or nearly normal cognitive development.

9.2 Biochemistry

Phenylalanine is an indispensible amino acid that cannot be synthesized by the human body (Chap. 7). It comprises 3–7 % of all dietary protein. After protein ingestion and digestion, phenylalanine is absorbed from the gastrointestinal tract to the liver via the portal vein. Phenylalanine is either hydroxylated into tyrosine via PAH in the liver or is incorporated into new proteins in tissues [4]. Hyperphenylalaninemia due to decreased activity of PAH manifests as spectrum of disorders (severe, moderate, or mild PKU and non-PKU hyperphenylalaninemia). Deficiencies in the activity of PAH cofactor - tetrahydrobiopterin (BH_4) represent a group of metabolic disorders that result not only in hyperphenylalaninemia but also in alterations in tyrosine (Tyr) and tryptophan (Trp) metabolism. BH_4 is also a cofactor for tyrosine hydroxylase and tryptophan hydroxylase, as well as three isoforms of nitric oxide synthase. Therefore, proper functioning of BH_4 is essential for the synthesis of dopamine, catecholamines, serotonin, melanin, and nitric oxide [8] (Fig. 9.1).

Phenylalanine can also be transaminated to phenylpyruvic acid as an alternative to hydroxylation by phenylalanine hydroxylase. Phenylpyruvic acid, along with other ketones, is excreted in the urine as phenylacetic acid, phenylacetylglutamine and phenyllactic acid. This pathway of phenylalanine metabolism is much less effective than hydroxylation [1, 4].

The enzyme PAH has a complicated structure consisting of three domains: regulatory, catalytic, and C-terminal domain. The regulatory domain contains a serine residue that is involved in activation by phosphorylation. The catalytic domain is responsible for cofactor and ferric iron binding, while the C-terminal domain is associated with inter-subunit binding [9]. The liver is the primary

Table 9.1 Classification of phenylketonuria, hyperphenylalaninemia, and tetrahydrobiopterin diagnoses

Classification of phenylketonuria	Pretreatment blood phenylalanine concentrations	Percentage of residual PAH activity
Unaffected	50–100 μmol/L (0.50–1.8 mg/dL)	Not applicable
Tetrahydrobiopterin deficiencies	120–2,120 μmol/L (2–35 mg/dL)[a]	Varies
Mild hyperphenylalaninemia[b]	120–360 μmol/L (2–6 mg/dL)	>5 %
Mild phenylketonuria	360–900 μmol/L (6–15 mg/dL)	1–5 %
Moderate phenylketonuria	900–1,200 μmol/L (6–20 mg/dL)	1–5 %
Severe (classical) phenylketonuria	>1,200 μmol/L (>20 mg/dL)	<1 %

Adapted from Camp et al. [20]
[a]Some concentrations may be normal
[b]Initiation of dietary treatment depends on baseline phenylalanine concentrations, and disagreement exists regarding the need for treatment

site of phenylalanine hydroxylase activity, but it is also synthesized in the kidneys, pancreas, and brain.

9.3 Genetics

Phenylketonuria is an autosomal recessive disorder. The majority (98 %) of genetic mutations associated with PKU occur at the phenylalanine hydroxylase locus [4], on the long arm of chromosome 12, in the region of q22-q24.1. More than 850 mutations in the phenylalanine hydroxylase locus have been described thus far, with 60 % being missense mutations [10]. The incidence of PKU varies widely among ethnic groups and geographical regions (Box 9.1).

Box 9.1: Global Incidence of PKU

Region/country	Incidence
Europe [11]	1:10,000
United States [12, 13]	1:13,000
Turkey [14]	1:4,200
China [15, 16]	1:11,000–1:25,000
Finland [17]	1:100,000
Thailand [18]	1:200,000
Global [11]	1:12,000

The sub-Saharan African populations experience a very low incidence of all types of this disorder [19]. Globally, the incidence in screened populations is estimated at 1:12,000 with a carrier frequency of 1:55 [11].

Although the correlation between genotypes and biochemical phenotype, pretreatment phenylalanine concentrations, and phenylalanine tolerance is well established, the correlation between clinical phenotype including neurological, intellectual, and behavior outcomes is weak [11].

9.4 Diagnosis

In most developed countries, PKU is identified by newborn screening by the presence of elevated phenylalanine and/or phenylalanine to tyrosine (Phe:Tyr) ratio in the dry blood spot collected in the first days to week of life (Chap. 2). Tandem mass spectrometry (MS/MS) is the method of choice in analyzing the blood spots; however, other methods such as the Guthrie microbiological inhibition test, enzymatic techniques, or high-pressure liquid chromatography (HPLC) are also used in some laboratories. After a positive newborn screening result, the patient is evaluated at a metabolic center for confirmatory testing and to rule out BH_4 deficiency by the analysis of pterin, as well as dihydropteridine (DHPR) activity, in the blood. In Europe, a BH_4 loading test is often performed and allows for identification of patients with BH_4-responsive variants of PKU, as well as BH_4 deficiencies caused by the disturbance in the production and/or recycling of BH_4 [8] (Table 9.1).

9.5 Clinical Presentation

Untreated, late-treated, or poorly controlled patients have chronically elevated blood phenylalanine concentrations that lead to progressive

and irreversible neurological, psychological, behavioral, as well as physical impairments that significantly impact quality of life. The degree of impairment depends on the blood concentration of phenylalanine with the most severe symptoms observed in untreated patients with the classical form of the disease. Although severe intellectual disability (with IQ scores often below 50) is the most typical presentation, untreated patients may demonstrate many other symptoms of persistent hyperphenylalaninemia (Box 9.2).

Box 9.2: Symptoms of Untreated Classical PKU

- "Musty" odor (urine and body)
- Hypopigmentation of the skin, hair, and iris
- Eczema
- Intellectual disability
- Neurological (seizures, tremor)
- Behavioral (hyperactivity, self-injury)
- Psychological (depression, anxiety, agoraphobia)

The outcome of early detected and treated PKU is generally favorable; however, even with good metabolic control, some individuals may demonstrate a higher prevalence of neuropsychological complications, including decreased executive functioning, internalizing disorders, and low self-esteem [21, 22].

9.6 Dietary Treatment

The cornerstone of dietary management in PKU is the limiting of the consumption of the offending amino acid, phenylalanine. In general, the therapeutic diet is restricted in all high-protein foods, such as meats, fish, eggs, nuts, dairy, legumes, bread, and pasta [23]. The amount of phenylalanine a patient can consume daily depends on the residual activity of PAH and other factors including the patient's age and growth rate [24]. The concept of limiting dietary phenylalanine was first demonstrated in the early 1950s by Bickel et al. as they showed positive effects on behavior in a young patient with PKU [5]. The development of formulas that were low in phenylalanine but contained other amino acids made the dietary treatment of PKU possible. During the early years of PKU treatment, it was generally believed that a low-phenylalanine diet could be discontinued around 6 years of age with no adverse effects [25–27]; however more recent studies indicate that for the best outcome, a "diet for life" is the optimal mode of treatment [28–31]. According to the recent National Institute of Health in 2012 recommendations treatment should be started in all patients with hyperphenylalaninemia with blood phenylalanine concentrations greater than or equal to 360 μmol/L [13]. Current management practices in Europe vary widely, but most centers also use the value of ≥360 μmol/L as an indication for dietary treatment [32]. Target phenylalanine concentrations used for the long-term follow-up in many European centers are age specific, while in the United States the goal is to maintain plasma phenylalanine concentrations below 360 μmol/L [20] across all age groups.

9.7 Phenylalanine Neurotoxicity

Eighty years after the discovery of PKU, the pathogenesis of brain dysfunction and the exact mechanisms of phenylalanine neurotoxicity have yet to be elucidated. Although there is a common agreement about the relationship between blood phenylalanine concentration and cognitive outcome in PKU, the concentration of phenylalanine

Box 9.3: Theories of the Pathogenesis of PKU

- Impairment of large neutral amino acid (LNAA) transport across the blood-brain barrier (BBB) with disturbances in neurotransmitter metabolism [33, 37]
- Impairment in cholesterol synthesis and disturbances in myelin metabolism [33]
- Altered brain protein synthesis [34, 37]
- Interference with the glutamatergic system directly involved in brain development [35, 37–39]
- Altered glycolysis via inhibition of pyruvate kinase and other enzymes involved in brain energy metabolism [36]

and a deficiency of other large neutral amino acids in the brain are believed to be the main factors causing neurotoxicity. The impact of elevated blood phenylalanine concentrations, which is especially harmful during early infancy, is complex and multidirectional [33–36] (Box 9.3).

The typical symptoms of untreated individuals with PKU are the manifestation of the neurotoxic effect of phenylalanine on the central nervous system. A morphological change in the brain in patients with PKU affects both white and gray matter. Microcephaly, where the brain mass can be 80 % of that of a healthy individual, is characteristic feature for many untreated PKU patients [37]. This symptom is caused by myelin structure anomalies that result in a loss of myelin volume, disturbances in cortical neuronal development, diffuse cortical atrophy, and general abnormalities in protein synthesis [37, 39, 40].

Phenylalanine neurotoxicity affects the brain and related structures during critical windows of growth and development. Periods of particularly rapid growth make neuronal cells especially vulnerable to excessive amounts of toxic factors (e.g., phenylalanine) or a lack of substances needed for an optimal development [41].

In PKU, similarly as in the other inherited disorders of amino acid metabolism, the fast growing brain of the fetus is protected by the mother's enzymatic activity. The disturbances appear after birth, and the central nervous system is at risk of damage until the brain is fully developed and matured [42]. Despite the fact that the increase in brain mass and the creation of synaptic connections occurs mainly during the first year of life, the full development of some areas (e.g., prefrontal cortex or white matter myelination) is not complete until adulthood (Box 9.4).

Box 9.4: Brain Development

- The decrease in brain mass and the creation of synaptic connections occurs mainly during the first year of life.
- Full development of some areas (e.g., prefrontal cortex and white matter myelination) is not complete until adulthood.

The last region to mature in the prefrontal cortex is the dorsolateral area responsible for cognitive functions [43]. During the first few years of life, patients with PKU that are inappropriately treated and have poorly controlled blood phenylalanine concentrations suffer from inhibited growth of the cortex and a disrupted myelination process. Of note, the neurotoxic influence of phenylalanine is present throughout life; therefore, all patients with PKU require lifelong, multidisciplinary care and maintaining blood phenylalanine concentrations within the treatment range. The risk of progressive neuropsychiatric manifestations of PKU in adulthood is higher in patients with poor metabolic control in infancy and early childhood [44].

High concentrations of blood phenylalanine result in increased uptake of phenylalanine into the brain and concomitant decrease in the uptake of other large neutral amino acids (LNAA). Phenylalanine is transported into the brain by one of the LNAA carriers, the L-amino acid transporter 1 (LAT-1) [45–48]. This transporter also selectively transports the amino acids valine, isoleucine, methionine, threonine, tryptophan, tyrosine, and histidine. The binding of the LNAA to the LAT-1 transporter is a competitive process; the rate of transport is proportionate to the blood concentration of all the transported amino acids [49]. This system has the highest affinity for phenylalanine, which in case of its high concentration in the blood, significantly decreases the transport of other LNAA and more phenylalanine is transported into the brain. By influence on the activity of tyrosine and tryptophan hydroxylases, elevated brain phenylalanine concentrations also negatively impact the synthesis of catecholamines and serotonin in the brain due to the altered metabolism of tyrosine and tryptophan [4].

The distribution of dopamine synthesis significantly alters activity of dopaminergic neurons of prefrontal cortex, especially dorsolateral area which receives a large dopamine projection and is characterize by very high dopamine turnover [46]. It has been established that dopamine deficiency and disturbances in neurotransmitter balance may be responsible for cognitive and executive functions deficits, as well as emotional problems even in patients that were treated earlier. The intensity of these dysfunctions is related to the degree of

hyperphenylalaninemia. Those observations form basic assumptions for the tyrosine-dopamine theory, which explains the complex abnormalities of neuropsychological functions, resulting from the intracerebral decrease of dopamine, secondary to tyrosine deficiency [41, 50, 51].

9.8 White Matter Pathology

PKU is associated with a diffuse brain pathology, including white matter changes which can be observed even in early and continuously treated patients [42]. As an integral part of the neuronal network, white matter takes a crucial role in brain functioning. It is fundamental for proper motor and sensory functions, as well as sensory organ activity. Damage done to the white matter causes complex neurobehavioral syndromes, even if cortical and subcortical regions of the gray matter remain intact [52]. The brains of individuals exposed to high blood phenylalanine concentrations from the early childhood present with hypomyelination and astrocytic gliosis [42]. In addition, foci of segmental demyelination and areas of status spongiosus may occur [37, 40, 46]. In histopathological research done in mice by Malamud et al., the abovementioned phenomenon was described as diffuse vacuole formation occurring alongside the nerve fibers or in proximity of oligodendrocytes and stratifying myelin layers [53]. Complex disturbances of myelin metabolism in patients with PKU were coined with the term: dysmyelination [54, 55]. The main function of the myelin sheath surrounding an axon is to facilitate the rapid conduction of action potentials along the axons for signal transmission and neurotransmitter synthesis [50]. The myelin takes part in axon maturation, and therefore damage to it causes disturbances in nervous system function. This means that alterations in myelin synthesis itself as a primary cause can lead to secondary neuronal dysfunction and neurotransmitter synthesis abnormalities, including disturbances in the synthesis of dopamine (*myelin-dopamine theory*). Myelin-induced maturation of axons is also necessary for proper branching of dendrites during brain development, which is essential for the formation of the brain network [38, 50, 56].

Elevated brain phenylalanine concentrations influence the functioning of oligodendrocytes (glial cells responsible for myelin production) and thus proper axon functioning. Two types of oligodendrocytes are present in the central nervous system. The first type, phenylalanine-sensitive oligodendrocytes, is found in close proximity to neuronal networks that are myelinated after birth. With the exception of the cerebellum, these pathways are localized in the frontal brain structures (optic tract, corpus callosum, subcortical white matter and periventricular white matter). This group of oligodendrocytes is sensitive to phenylalanine concentrations, even in early treated patients, and therefore when the brain is exposed to high phenylalanine concentrations, myelin synthesis is disrupted. This leads to axons lacking proper myelin sheathing, further lowering the number of dendritic connections, decreasing the nervous conductivity and neurotransmitter production in presynaptic areas. The second type of oligodendrocyte cells is phenylalanine nonsensitive oligodendrocytes. These cells myelinate the axon before birth and are situated primarily in the hindbrain structures (internal capsule and brainstem) and in the spinal cord [50, 57].

Phenylalanine and related metabolites inhibit activity of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase (Fig. 9.2). This enzyme is critical for proper synthesis of cholesterol in phenylalanine-sensitive oligodendrocytes located in the frontal brain, especially in the prefrontal cortex. Locally synthesized cholesterol makes up approximately 30 % of all myelin lipids of the brain tissue. The function of cholesterol is not only structural but is also required for proper neuronal signal transmission [50]. Inhibition of HMG-CoA reductase by phenylalanine is partially reversible in some individuals. This explains the improvement in myelination observed in MRI scans of poorly controlled patients who have returned to diet and have lowered their blood phenylalanine concentrations. The reduction in phenylalanine allows for proper myelin production in the phenylalanine-sensitive oligodendrocyte population [50, 57, 58] (Fig. 9.3).

Phenylalanine neurotoxicity can be also discussed with regard to the impact of elevated phenylalanine concentrations on the oligodendroglial enzyme, Phe-sensitive ATP-sulfurylase. This enzyme is engaged in the synthesis of cerebrosul-

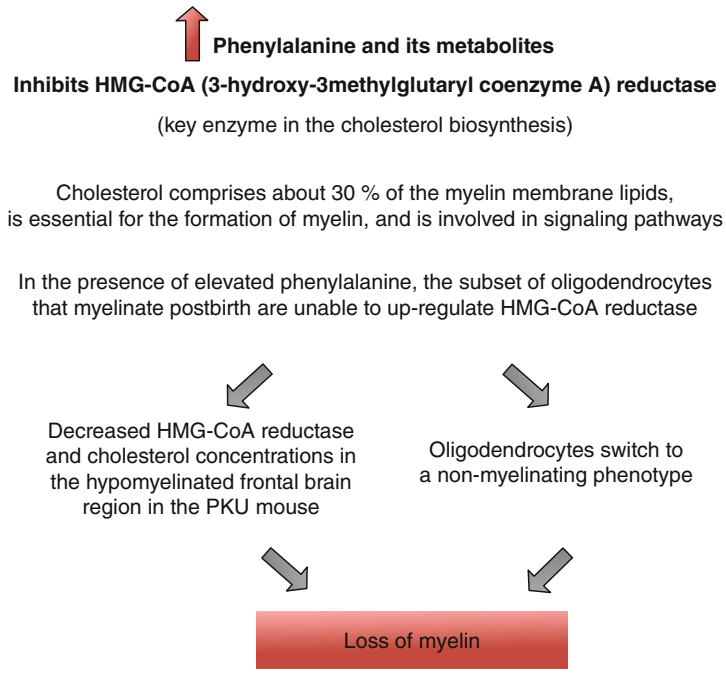

Fig. 9.2 Inhibition of HMG-CoA reductase resulting in the loss of myelin formation due to elevated phenylalanine concentrations in the brain [50]

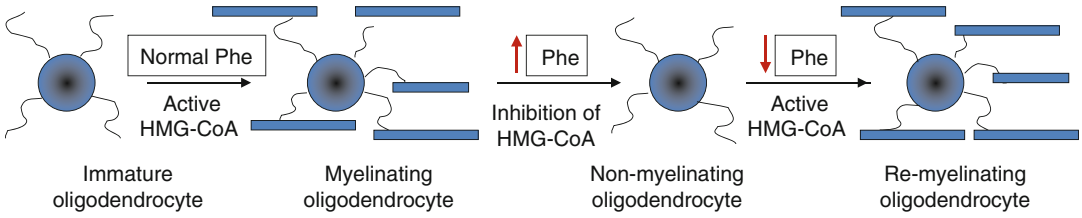

Fig. 9.3 Hypothesized effect of elevated phenylalanine concentrations on Phe-sensitive oligodendrocyte phenotypes in the forebrain [50]

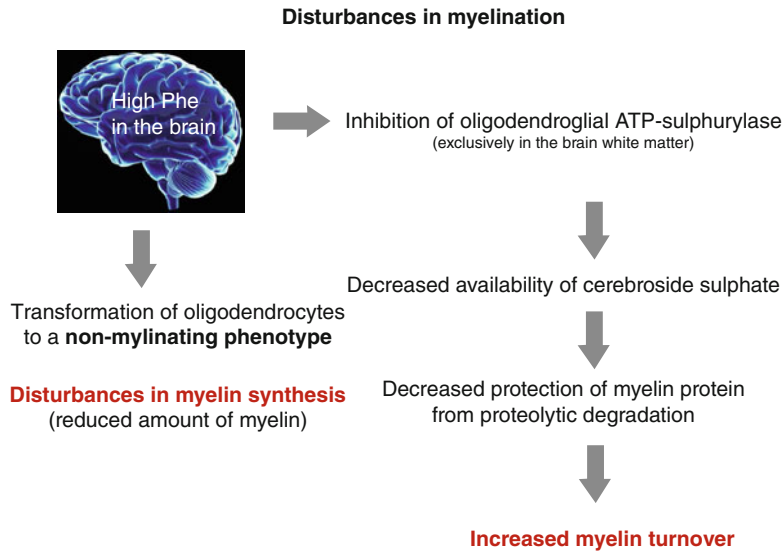

Fig. 9.4 Disturbances in myelination in the brain of patients with PKU [46, 53, 59–61]

fatides that protect the myelin base protein responsible for preventing myelin degradation. A lack of cerebrosulfatides results in an increase in the process of myelin degradation and if not compensated for by proper synthesis, consequently leads to complex dysmyelination changes [40, 46] (Fig. 9.4).

According to Dyer et al., white matter pathology in untreated PKU is a developmental process, whereby elevated phenylalanine concentrations arrest the myelination causing reduced myelin formation and hypomyelination. In early treated patients, myelin lesions reflect demyelination or dysmyelination and represent loss or impairment of previously assembled myelin [50] (Box 9.5).

Box 9.5: Dysmyelination Changes in PKU [38, 42, 54]

White matter abnormalities are a result of:

- *Demyelination* (loss of formed myelin) in treated individuals
- *Hypomyelination* (lack of myelin formation) in untreated individuals

The diffuse character of white matter pathology in PKU may compromised multiple pathways resulting in different deficits in motor skills, coordination, visual functioning, processing speed, language, memory and learning as well as attention and executive functioning [42].

White matter abnormalities (WMA) detected in patients with PKU by conventional magnetic resonance imaging (MRI) were first reported at the end of 1990s [62, 63].

Structural manifestations of phenylalanine neurotoxicity include dysmyelination in the white matter of the brain, revealed with MRI as intense lesions and cortico-subcortical atrophy on T2-weighted images with specifically high-signal intensity in periventricular white matter. White matter abnormalities may be explained by cytotoxic edema and dysmyelination changes with increase in free water trapped in myelin sheaths [44]. The size and distribution of WMA vary between patients with localization in the white matter of temporal and occipital lobes as the most common areas affected [42] (Figs. 9.5 and 9.6).

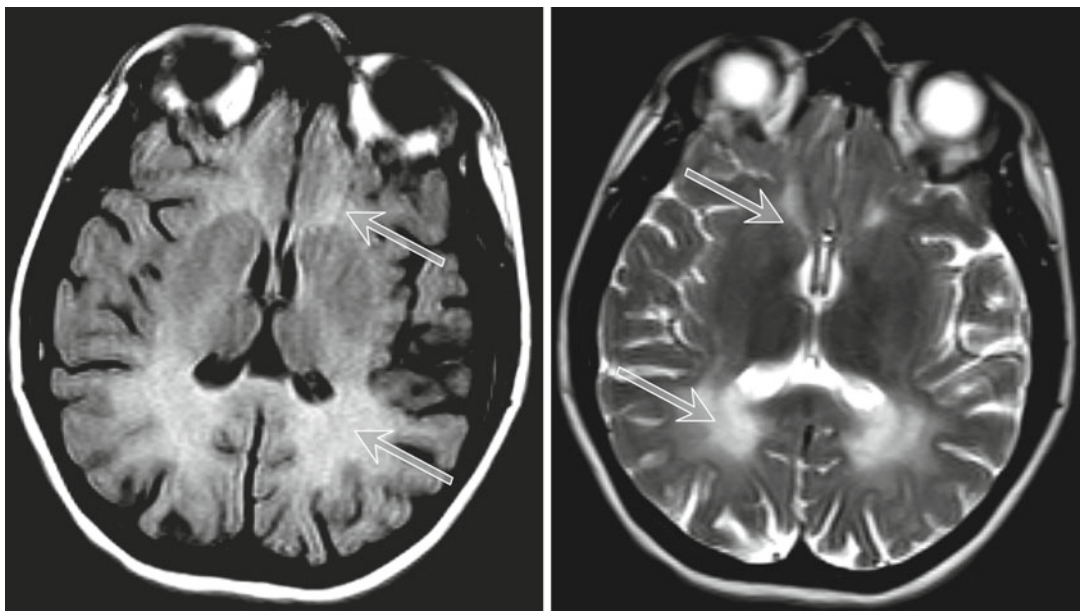

Fig. 9.5 Magnetic resonance imaging of the brain: T2-weighted images using FLAIR (*fluid attenuated inversion recovery*) and FSE (*fast spin echo*) reveal enhanced signal intensity representing white matter abnormalities (WMA) in all lobes (*arrows*) – female (MM) aged 27 years on low-phenylalanine diet from 3 to 8 years of age, blood phenylalanine concentration at MRI was 1,571 µmol/l, DQ 32

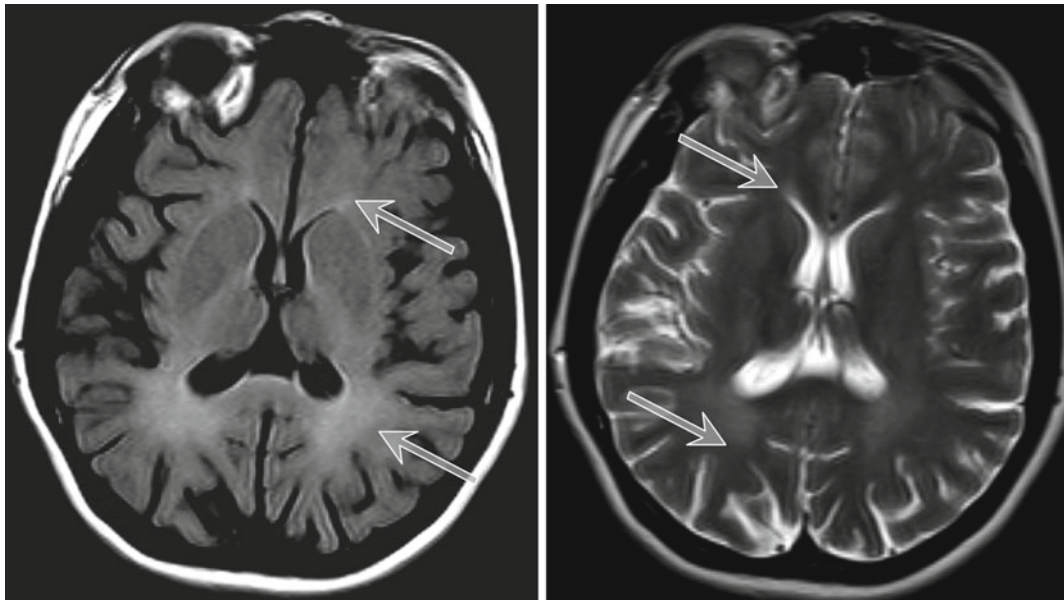

Fig. 9.6 Regression of hyperintense lesions in white matter (WMA) of all lobes (*arrows*) during low-phenylalanine diet. Magnetic resonance imaging of the head (T2-weighted images, FLAIR, and FSE). Same patient (Fig. 9.5) after 7 months of treatment. Mean blood phenylalanine concentration was 724 μmol/L; blood phenylalanine concentration at MRI was 690 μmol/L

9.9 Gray Matter Pathology

Phenylalanine also influences the gray matter, with the greatest effect being observed in the neocortex. A state of chronic hyperphenylalaninemia, especially at birth, profoundly affects the neocortex on multiple levels (Box 9.6).

Box 9.6: Effects of Hyperphenylalaninemia on Gray Matter [37, 39]

- Inhibition of growth process of the pyramidal pathways
- Disrupted dendritic growth resulting in formation of fewer connections
- Increased cell density of prefrontal cortex
- Inadequate synaptogenesis resulting in decreased synaptic density

It is especially crucial to take into consideration that humans, unique from all other organisms, have the most developed prefrontal cortex that allows for our most sophisticated intellectual and emotional abilities.

9.10 Summary

If untreated or poorly controlled, especially in early childhood, PKU can result in severe intellectual disability, neurological deficits, and/or psychological/psychiatric manifestations. Early diagnosis with early and continuous effective treatment allows patients with PKU to achieve normal intellectual development. The pathogenesis of phenylalanine neurotoxicity in PKU is very complex and still far from being fully understood. It consists of both white and gray matter pathology related to high brain phenylalanine concentrations. One of the main mechanisms of neurotoxicity is the impairment of brain neurotransmitter metabolism, especially dopamine, which disrupted synthesis may affect proper functioning of the brain, especially the prefrontal cortex. It is difficult to predict the late outcome of

the discontinuation of treatment in adults with early treated PKU; however, due to the effects of high phenylalanine concentration on the brain, it is recommended that patients with PKU be monitored and remain in metabolic control for life.

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Phenylketonuria: The Diet Basics

10

Sandy van Calcar

Contents

Core Messages

- The goal of nutrition management of phenylketonuria (PKU) is to maintain blood phenylalanine concentrations between 120 and 360 μmol/L.
- The diet for PKU includes medical foods low in or devoid of phenylalanine and limited quantities of phenylalanine from intact protein sources.
- Frequent monitoring of blood phenylalanine concentrations is key to successful diet management.
- Frequent adjustments in the diet are needed to achieve desired blood phenylalanine concentrations as well as to promote normal growth and development.
- A variety of PKU medical foods and modified low-protein foods are available to accommodate different nutrient needs and taste preferences throughout the life span.
- Maintaining the diet is challenging for many patients with PKU; alternative therapies are available, but most still require some degree of diet modification.

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10.1 Background

Phenylketonuria (PKU) is an inborn error in phenylalanine metabolism caused by a deficiency of the phenylalanine hydroxylase (PAH) enzyme (Fig. 10.1). The cofactor for PAH is tetrahydrobiopterin (BH_4). In PKU, blood concentrations of phenylalanine accumulate, affecting myelin and neurotransmitter production (Box 10.1) [1, 2].

With the defect in PAH, phenylalanine is not converted to tyrosine; thus, tyrosine becomes a conditionally essential amino acid and must be supplied in the diet. The incidence of PKU in those of Northern European descent is approximately 1 in 10,000 births with varying incidences in other populations [3]. PKU is inherited in an autosomal recessive pattern. Both parents carry the gene for PKU but do not show any signs of the disorder. With each pregnancy there is a 25 % chance of having a child affected by PKU. Over 500 mutations have been described in the PAH gene [3].

The untreated PKU phenotype was first described in 1934 by Asbjorn Fölling in two siblings with severe mental retardation [4]. Other signs and symptoms of untreated PKU may include seizures and autistic-like behavior. Eczema with light hair and light complexion can also be seen and is caused by the deficiency of tyrosine [5], a precursor in melanin production. The screening test for PKU was developed by Robert Guthrie, and newborn screening for PKU first started in Massachusetts in 1961 [6]. This

Box 10.1: Principles of Nutrition Management for Phenylketonuria
Restrict: Phenylalanine
Supplement: Tyrosine
Toxic metabolite: Phenylalanine

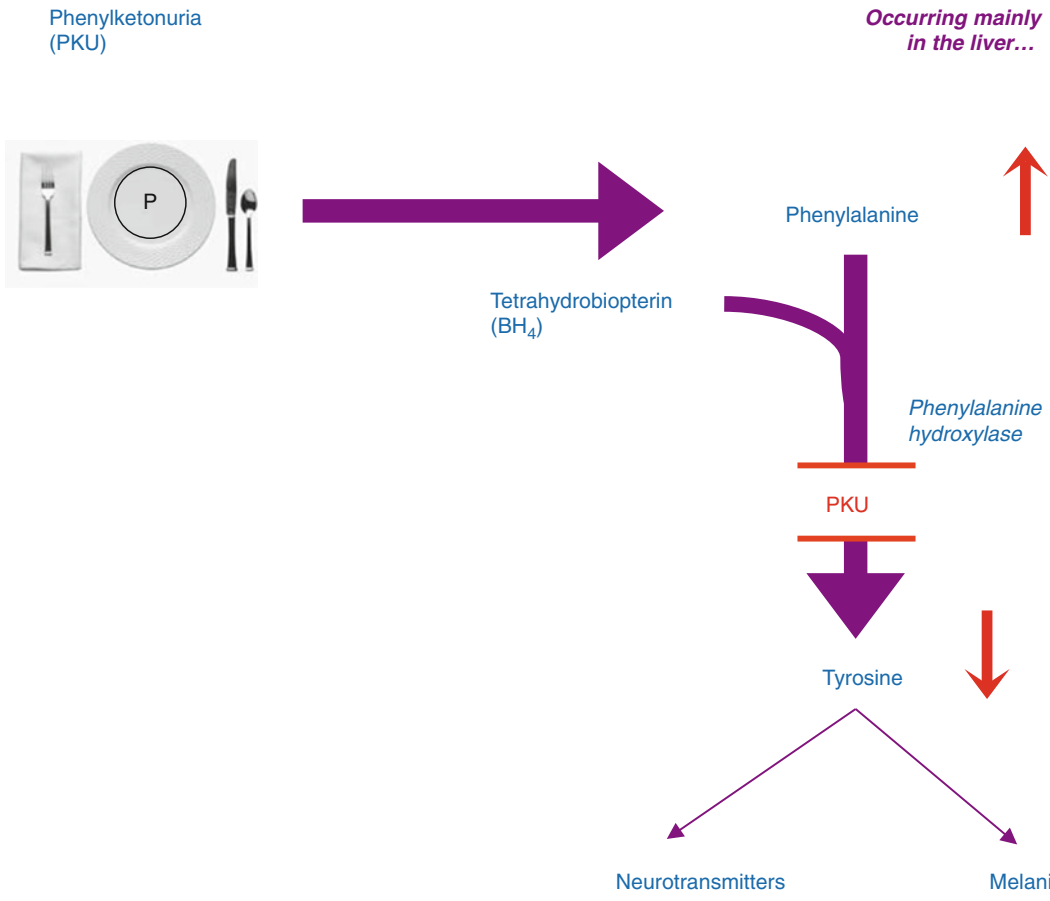

Fig. 10.1 Metabolic pathway of the conversion of phenylalanine to tyrosine

public health initiative has allowed for early diagnosis and initiation of diet treatment and has ameliorated the untreated phenotype (Chap. 2).

10.2 Nutrition Management

10.2.1 Chronic Nutrition Management

Diet treatment for PKU includes a diet restricted in phenylalanine and an amino acid-based medical food devoid of phenylalanine. Medical foods for PKU provide all other indispensable amino acids, tyrosine, fat, carbohydrate, and micronutrients. The amino acids in medical foods provide the majority of protein for patients with PKU. The amount of intact protein required to meet phenylalanine needs is often so limited that, without use of a medical food, protein deficiency would develop [7]. Medical foods also supply the majority of energy, especially in the diets of infants and toddlers.

Tetrahydrobiopterin (BH_4) is the cofactor for the enzyme PAH. Nearly 50 % of individuals with PKU are "responders" to the prescription form of BH_4 (Kuvan®, Biomarin Pharmaceutical Inc., Novato, CA) allowing a reduction and/or stability in blood phenylalanine concentrations with increased phenylalanine tolerance in some individuals. The therapeutic use of BH_4 is addressed in Chap. 12.

When an infant is referred to a metabolic clinic with a positive newborn screen for PKU, and the diagnosis is confirmed, the first step is to reduce the blood phenylalanine concentration into the treatment range of 120–360 μmol/L (2–6 mg/dL) [8]. Once the blood phenylalanine concentration trends down toward treatment range, a source of intact protein is added to provide the infant's phenylalanine needs. The goal is to provide sufficient phenylalanine to promote adequate growth and protein nutriture, but prevent excessive phenylalanine intake that will increase the blood concentration above the treatment range. The amount of phenylalanine needed from the diet depends on the severity of the disease as well as other factors, such as growth rate. Frequent monitoring is crucial for the management of blood phenylalanine concentrations.

There are several ways to initiate the diet depending on the initial blood phenylalanine concentration (Fig. 10.2). Complete removal of dietary phenylalanine by offering only a phenylalanine-free medical food for a prescribed amount of time can quickly reduce the blood phenylalanine concentration; this is often referred to as a "washout" period. The higher the initial blood phenylalanine concentration, the longer the time required to reduce the concentration of phenylalanine into treatment range (Box 10.2). The time it takes to obtain blood phenylalanine results from a laboratory can influence the decision to remove phenylalanine entirely from the diet. If results will not be available before the phenylalanine concentration is expected to decrease into the recommended range, a source of phenylalanine should be added to the formula to prevent excessively low blood concentrations. For those with lower initial phenylalanine concentrations, it may

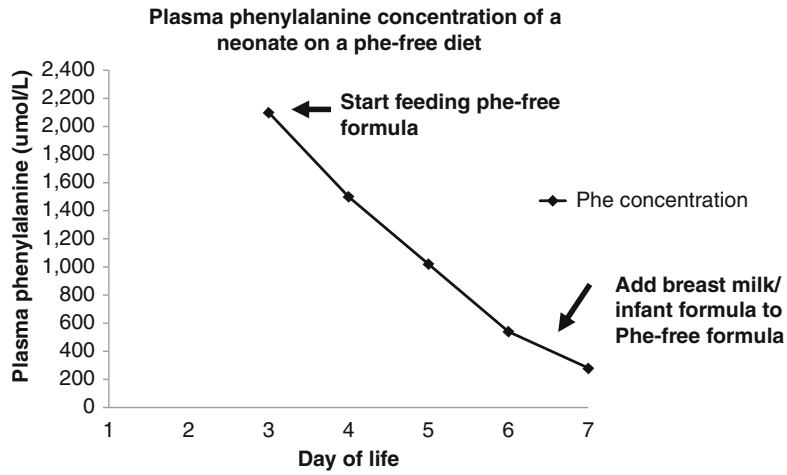

Fig. 10.2 Example of decreasing blood phenylalanine concentration in a neonate started on a Phe-free diet at day of life 3

Box 10.2: Suggested Time Frame for Initial Removal of Phenylalanine from the Diet

Diagnostic Phe concentration	Remove dietary Phe for (h)
360–600 µmol/L (6–10 mg/dL)	24
600–1,200 µmol/L (10–20 mg/dL)	48
1,200–2,400 µmol/L (20–40 mg/dL)	72
>2,400 µmol/L (>40 mg/dL)	96

be prudent to initially prescribe 25–50 % of estimated phenylalanine needs to avoid decreasing the blood concentration below the treatment range.

Once the blood phenylalanine concentration is close to or within the treatment range, the next step is to add a calculated amount of standard proprietary infant formula or breast milk to the phenylalanine PKU medical food to provide the estimated phenylalanine needs of the infant. This diet calculation is somewhat different depending on whether infant formula or breast milk is used as the source of intact protein (Boxes 10.3 and 10.4).

The range of dietary phenylalanine required by an infant is 130–430 mg/day [8]. Exactly how much phenylalanine to prescribe after the initial "washout period" is a matter of judgment – often those with higher initial blood phenylalanine concentrations require less phenylalanine introduced into the diet. For example, an infant with an initial blood phenylalanine concentration of 1,600 µmol/L may be prescribed 45 mg of phenylalanine per kilogram after the recommended 72-h washout period whereas an infant with an initial blood phenylalanine concentration of 900 µmol/L would be prescribed 55 mg of phenylalanine per kilogram after the suggested washout period of 48 h. The following table can serve as a guideline to establish the amount of dietary phenylalanine to initially introduce (Table 10.1).

However, as the results from newborn screening tests are becoming available more quickly than ever, very high blood phenylalanine concentrations are not seen as often as in the past, and it may be difficult to determine how much dietary phenylalanine to initially prescribe. In this case, it is appropriate to estimate phenylalanine needs in the middle of the recommended range (40–50 mg/kg/day) as a starting point for calculation.

Infants with PKU consume a similar volume of formula or breast milk as any typically developing infant. Thus, the caloric density of the initial medical food prescription should

Box 10.3: Initiating Nutrition Management of an Infant with PKU (Using Standard *Infant Formula* as the Source of Phenylalanine)

Goal: Reduce plasma phenylalanine concentrations to between 120 and 360 µmol/L.

Step by step:

1. Establish intake goals based on the infant's diagnostic blood phenylalanine, clinical status, and laboratory values.
2. Determine amount of standard infant formula required to provide the amount of phenylalanine required to meet the infant's needs. Determine the amount of protein and energy that will be provided by this amount of formula.
3. Subtract the protein provided by the standard infant formula from the infant's total protein needs. Calculate amount of phenylalanine-free medical food required to meet the remaining protein needs.
4. Determine the number of calories provided by both the infant formula and phenylalanine-free medical food. Provide the remaining calories from a phenylalanine-free medical food.
5. Calculate amount of tyrosine provided by both the infant formula and phenylalanine-free medical food.
6. Determine the amount of fluid required to provide a caloric density of 20–25 kcal/oz.
7. Divide this volume of medical food into feedings for a 24-h period.

(Diet calculation examples are provided at the end of this chapter)

Box 10.4: Initiating Nutrition Management of an Infant with PKU (Using *Breast Milk* as the Source of Phenylalanine)

Goal: Reduce plasma phenylalanine concentrations to between 120 and 360 μmol/L.

Step by step:

1. Establish intake goals based on the infant's diagnostic blood phenylalanine, clinical status, and laboratory values.

   Note: (In breast-fed infants, the lower end of the protein goal is usually sufficient, since breast milk contains less protein than infant formula, but it is of high biological value)
2. Determine amount of breast milk required to provide the infant's estimated phenylalanine needs. Determine the amount of protein and energy that will be provided by this volume of breast milk.
3. Subtract the calories provided by the breast milk from the infant's total energy needs.
4. Subtract the protein provided by the breast milk from the infant's total protein needs. Calculate amount of phenylalanine-free medical food required to meet the remaining protein requirement.
5. Determine the number of calories provided by both the breast milk and phenylalanine-free medical food. Provide the remaining calories from additional phenylalanine-free medical food.
6. Calculate amount of tyrosine provided by both the breast milk and phenylalanine-free medical food.
7. Determine the amount of fluid required to provide a 20 kcal/oz formula.

be approximately 20 kcal/oz unless other factors necessitate a higher caloric concentration. Osmolarity and renal solute load should be determined if the formula concentration is greater than 24 kcal/oz. Caregivers need to be instructed to feed ad lib as the infant should be able to self-regulate the frequency of feeding and volume of formula consumed. Ask the parents to record how often and how much of the formula the infant is consuming for several days. If the amount of formula consumed is above the amount prescribed, then adjust the formula prescription to include more PKU medical food. If the amount of formula that the infant is consuming is less than the amount prescribed, then adjust the formula prescription by reducing the amount of PKU medical food that is added to the formula. The goal is to make sure that the infant consumes the entire volume of the regular infant formula over a 24-h period to meet his/her phenylalanine prescription.

If a mother is breast-feeding her infant, it is possible to design a PKU diet to allow her to continue feeding from the breast. Mature breast milk contains less protein, and thus less phenylalanine, than an equivalent amount of regular infant formula (Table 10.2). The goal is to provide the appropriate amount of breast milk

Table 10.1 Suggested guidelines to establish the amount of dietary phenylalanine to introduce into the diet prescription after the removal of phenylalanine from the diet ("washout period")

Nutrient	Diagnostic blood phenylalanine concentration	Amount of Phe to prescribe after "washout" period (mg/kg)
Phenylalanine	<600 μmol/L (<10 mg/dL)	70
	600–1,200 μmol/L (10–20 mg/dL)	55
	1,200–1,800 μmol/L (20–30 mg/dL)	45
	1,800–2,400 μmol/L (30–40 mg/dL)	35

*If levels are below 600 μmol/L, prescribe a diet with phenylalanine in the upper range of estimated needs (25–70 mg/kg/day).

Table 10.2 Comparison of nutrients in a typical standard infant formula and mature breast milk

In 100 ml (standard dilution)	Infant formula	Breast milk – mature
Phenylalanine	60 mg	46 mg
Tyrosine	58 mg	53 mg
Protein	1.4 g	1.05 g
Energy	68 kcal	70 kcal

to supply the phenylalanine requirement of the infant in order to maintain blood phenylalanine concentrations within treatment range. This is accomplished by providing a combination of breast milk and PKU medical food.

There are several approaches to designing a diet that uses breast milk as the source of phenylalanine. The approach of Greve [9] is based on the estimated caloric needs of the infant.

When feeding the infant, it is best if the medical food is provided by itself and not in combination with breast-feeding. Divide the prescribed medical food in appropriate volumes throughout the 24-h period, then allow ad lib breastfeeding for all other feedings. Using this method allows the mother to empty her breast during a feeding. Often the feeding schedule can allow for alternating breast and PKU medical food feedings or a breast-feeding followed by two feedings of medical food. Alternatively, the volume of phenylalanine-free medical food can be distributed in smaller volumes (e.g., 1–2 oz) over more feedings throughout a 24-h period. After consuming the medical food, the infant is allowed to breast-feed until he/she is full. This method may not work if a relatively large volume of medical food is required as the mother may find it more difficult to maintain her breast milk supply. For a mother electing to breast-feed her infant, it is important that she expresses or pumps breast milk to maintain adequate milk production.

The only way to know if a diet prescription needs to be adjusted is to measure blood or plasma phenylalanine concentrations. The recommended frequency of monitoring is provided in guidelines developed by Genetic Metabolic Dietitians International [10]. Figure 10.3 shows the diet adjustments that will need to be made whether the infant is receiving phenylalanine from infant formula or from breast-feeding.

Expect to adjust the diet prescription frequently, especially during the first two months of life. Having a record of the time and volume of feedings will be helpful. How much to change a phenylalanine prescription depends on the blood phenylalanine concentration. Recommended intakes for patients with PKU are provided in Table 10.3. Increasing or decreasing phenylalanine intake in 10 % increments is typical, but the percent change can be greater if concentrations are <60 μmol/L (<1 mg/dL) or greater than 600 μmol/L (10 mg/dL). However, adjustments are individualized for each patient.

The American Academy of Pediatrics recommends that infants start solid foods between 4 and 6 months of age [11]. At this time, the phenylalanine provided by the infant formula or

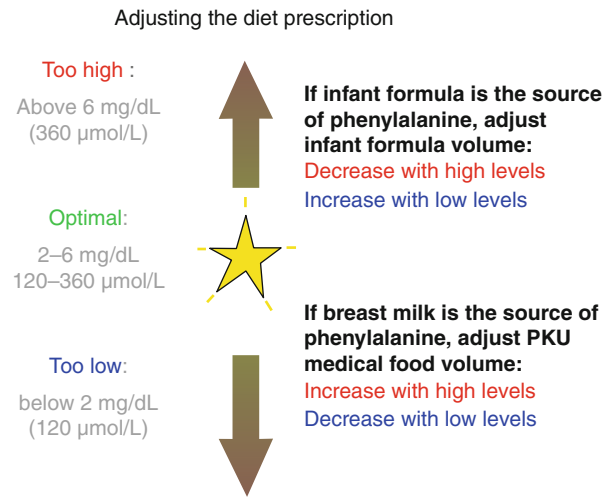

Fig. 10.3 Blood phenylalanine concentrations and recommended diet adjustments

Table 10.3 Recommended intake for a patient with PKU [8, 10, 18]

Age	Protein (g/kg)	Phenylalanine (mg/kg)	Phenylalanine (mg/day)	Tyrosine (mg/kg)	Tyrosine (mg/day)
Birth to 3 months	3.0–3.5	25–70	130–430	300–350	1,100–1,300
3 to <6 months	3.0–3.5	20–45	135–400	300–350	1,400–2,100
6 to <9 months	2.5–3.0	15–35	145–370	250–300	2,500–3,000
9 to <12 months	2.5–3.0	10–35	135–330	250–300	2,500–3,000
1 to 7 years	≥30 g	–	200–400	–	2,800–3,500

Energy, vitamin, and mineral intakes should meet the DRI and normal fluid requirements [19, 20] (Appendix G.). These are average ranges
Adjustments should be made based on growth, laboratory findings, and health status

breast milk will be decreased and replaced with phenylalanine from solids. One approach is to start with rice cereal and then introduce fruits and vegetables. Before solid foods are introduced, parents are taught to count phenylalanine from foods either as milligrams of phenylalanine or as exchanges (1 exchange = 15 mg phenylalanine) [12]. A transition plan is developed with the reduction in either the volume of regular infant formula or the number of breast milk feedings over a 24-h period. For mothers who are breast-feeding, it may become more difficult to maintain an adequate milk supply as solids are introduced. When transitioning from a bottle to a cup, only medical food or water should be provided. Initially, juice or other sweetened beverages should not be offered from the cup.

After infancy, the PKU medical food continues to be the mainstay of the diet. Medical food provides almost all of the protein requirement and often a majority of energy needs.

A wide variety of medical foods are available for those over age 2. Most clinics transition from a complete medical food designed for infants to one designed for toddlers and children. These products provide carbohydrate, fat, and micronutrients, in addition to phenylalanine-free amino acids. However, some medical foods designed for older age groups contain little or no fat, and others are concentrated in protein with little fat or carbohydrate. These medical foods can meet protein needs with a smaller volume and are often used for those requiring a lower energy formula. However, use of these lower energy options can also lead to excessive phenylalanine intake from foods. Various convenience forms of medical food such as bars, tablets, and ready-to-drink products can be offered to older children. Products made with glycomacropeptide (GMP), a protein source that is low in phenylalanine and supplemented with limiting amino acids, are also available. The phenylalanine in GMP often needs to be considered in the daily phenylalanine prescription [13]. Table 10.4 provides an overview of some of the medical foods available to patients with PKU.

To meet the phenylalanine needs, the diet includes naturally low-protein foods such as fruits and most vegetables, and foods with a moderate amount of protein such as starchy vegetables and, depending on phenylalanine tolerance, regular grain products. It is often easy for those with PKU to overconsume foods with a moderate amount of protein since small quantities can provide a significant amount of phenylalanine. These foods need to be weighed or measured to maintain good metabolic control [10].

The PKU diet may also include modified low-protein foods, such as low-protein pasta, breads, and baking mixes that are made from wheat or other starch, thus reducing the phenylalanine content. These products usually are ordered from specialty food companies. The benefit of using these products is that they increase the energy content and the variety of foods in the diet, yet most are very low in phenylalanine. There are

Table 10.4 Selected medical foods for the treatment of PKU

Infant/toddler (complete)[a]	Older child/adult (complete)[a]	Older child/adult (incomplete)[b]
Periflex® Early Years[c]	Glytactin BetterMilk[f,h]	Camino Pro drinks[f]
Periflex® Junior Plus[c]	Periflex® Advance[c]	Glytactin RESTORE[f,h]
Phenex-1[d]	Phenex-2[d]	Glytactin Complete Bars[f,h]
Phenyl-Free 1[e]	Phenyl-Free 2[e]	Glytactin RTD[f,h]
	Phenyl-Free HP[e]	Glytactin SWIRL[f,h]
	PhenylAde Essential[c]	Lanaflex[c,i]
		Lophlex LQ[c]
		Lophlex powder[c]
		Periflex® LQ[c]
		PhenylAde 40/60[c]
		Phlexy-10 Drink Mix[c]
		Phlexy-10 tablets/capsules[c]
		PhenylAde AA Blend[c]
		PhenylAde MTE[c]
		PhenylAde PheBLOC[c,i]
		PhenylAde RTD[c]
		PKU 2/PKU 3[c]
		PKU Coolers 10, 15, 20[g]
		PKU Express[g]
		PKU Gel[g]
		XPhe Maxamaid[c]

[a]Contains L-amino acids (minus phenylalanine) as protein source unless noted, as well as fat, carbohydrate, vitamins, and minerals
[b]Contains L-amino acids (minus phenylalanine) as protein source unless noted. Low in or devoid of fat, carbohydrate, vitamins, and/or minerals. See company websites for specific nutrient composition
[c]Nutricia North America (Rockville MD; nutricia-na.com)
[d]Abbott Nutrition (Columbus OH; abbottnutrition.com)
[e]Mead Johnson Nutrition (Evansville IN; meadjohnson.com)
[f]Cambrooke Therapeutics (Ayer, MA; cambrookefoods.com)
[g]Vitaflo USA (Alexandria, VA; vitaflousa.com)
[h]Contains glycomacropeptide as the protein source
[i]Contains large neutral amino acids as the protein source

several resources for low-protein cooking that can help families learn to use modified low-protein foods. The diet can also include "free foods," which are carbohydrate- and/or fat-based foods with little or no phenylalanine. Aside from adding extra energy to the diet, these foods are often of poor nutritional value and need to be used in moderation or in combination with healthier choices. Aspartame (NutraSweet™/Equal™) is made from the amino acids aspartate and phenylalanine. Patients with PKU should avoid products containing aspartame.

Like other chronic disorders, maintaining diet treatment is often difficult for adolescents and adults with PKU. However, maintenance of good metabolic control is correlated to improved long-term cognitive outcomes [14]. Studies have also suggested that the variability of phenylalanine concentrations over time may also be a significant predictor of long-term outcome [15]. Thus, efforts need to be made to support and motivate individuals in these age groups to continue diet therapy. One strategy to simplify the PKU diet is to count grams of protein rather than milligrams of phenylalanine. In this approach, fruits and most vegetables are "free" and do not need to be counted. A protein prescription is then provided for higher phenylalanine foods, such as starchy vegetables and grain products [16].

Adults with PKU who were previously treated but are not currently following the PKU

Box 10.5: Returning to Diet

Recommended Steps to Restart the PKU Diet:

1. Introduce medical food.
2. Remove any high-protein foods from the diet.
3. Consider BH_4 supplementation.
4. Reintroduce counting/limiting medium-protein foods.
5. Reintroduce low-protein products.
6. Establish connections with other adults on diet.

(See National PKU Alliance (NPKUA) website, adultswithpku.org, for more information)

diet strictly often consume little or no medical food, while avoiding most high-protein foods and diet soda containing aspartame. Adolescents and adults who have discontinued diet may return to their metabolic clinics to restart the diet. This requires several steps, outlined above (Box 10.5).

10.2.2 Acute Management

During illness or other catabolic stress (surgery, fractures, etc.), patients with PKU often have elevated blood phenylalanine concentrations. Unlike other inherited metabolic diseases, such as organic acidemias or urea cycle disorders requiring a decreased protein intake during illness, in patients with PKU, the diet is generally not modified during illness. Patients are encouraged to follow medical advice in treating the illness. Some medications contain aspartame; these should be avoided if an equivalent medication without aspartame is available, but if not, treating the illness takes priority over avoiding the phenylalanine in the medication.

10.3 Nutritional Monitoring

Monitoring is key to successful management of the patient with PKU (Box 10.6). Particularly important is the frequent monitoring of blood phenylalanine and tyrosine. These results are needed to adjust the diet to ensure that blood phenylalanine remains in the treatment range of 120–360 μmol/dL. In many clinics, blood phenylalanine is monitored in between clinic visits by analysis of blood that has been collected at home on a filter paper card. These specimens are

Box 10.6: Nutrition Monitoring of a Patient with Phenylketonuria[a]

- Routine assessments including anthropometrics, dietary intake, physical findings (Appendix N)
- Laboratory monitoring
  - Diagnosis specific
    - Plasma or blood spot amino acids
      - Phenylalanine
      - Tyrosine
  - Nutrition-related laboratory monitoring of patients on protein-restricted diets may include markers of:
    - Protein sufficiency[b] (plasma amino acids, prealbumin)
    - Nutritional anemia (hemoglobin, hematocrit, MCV, serum vitamin B_{12} and/or methylmalonic acid, total homocysteine, ferritin, iron, folate, total iron-binding capacity)
    - Vitamin and mineral status (Total 25-hydroxyvitamin D, zinc, trace minerals)
    - Essential fatty acid sufficiency: plasma or erythrocyte fatty acids
    - Others as clinically indicated

[a]For suggested frequency of monitoring see GMDI/SERC PKU Nutrition Guideline (www.southeastgenetics.org/ngp) or ACMG guideline (Appendix P)

[b]Further described in Chap. 7.

often tested by the newborn screening laboratory. There can be variability between the blood phenylalanine results obtained from filter paper blood spots compared to those in plasma measured on an amino acid analyzer. One study found that levels measured in filter paper blood spots were, on average, 26.1 % lower than the amino acid analyzer [17]. Anthropometric measurements, nutritional intake, biochemical data, and neurocognitive development should be assessed periodically [8].

10.4 Summary

Early diagnosis and treatment of PKU is a well-known example of the success of newborn screening and diet treatment in preventing intellectual disability associated with the untreated disorder. The goal of nutrition management of PKU is to maintain blood phenylalanine concentrations of 120–360 μmol/L throughout the life span while providing adequate nutrition. The diet is based on medical foods that are low in or devoid of phenylalanine, as well as limited quantities of intact protein from foods. The diet is highly individualized based on the patient's phenylalanine tolerance and food preferences, frequent monitoring of blood phenylalanine and tyrosine, as well as assessment of growth and biochemical parameters to ensure nutrient adequacy. The diet is challenging to follow, and alternative therapies increase dietary phenylalanine tolerance in some patients with PKU.

10.5 Diet Calculation Examples for an Infant with PKU

Example 1:
Infant with PKU using standard infant formula as the source of whole protein.

Patient information	Nutrient intake goals (per day)
Ten (10) day old infant male weighing 3.8 kg who was diagnosed with PKU based on elevated blood phenylalanine concentration of 25.2 mg/dL[a] (1,500 μmol/L) on day 6 of life	Recommended initial phenylalanine prescription: 45 mg/kg **Protein:** 2.5–3.5 g/kg **Tyrosine:** 300–350 mg/kg **Energy:** 95–135 kcal/kg **Fluid:** 150 mL/kg Recommended caloric density of formula: 20–25 kcal/oz

[a]For the online diet calculation example, please use mg/dL

Steps in diet calculation: This child will be placed on PKU Periflex® Early Years powdered formula. An interactive step-by-step guide of this diet calculation is available at www.imd-nutrition-management.com.

Example 2:
Infant with PKU using standard infant formula as the source of whole protein.

Patient History
A newborn infant tested positive for PKU upon newborn screening. The initial blood phenylalanine concentration was 1,800 μmol/L (30 mg/dL). Based on this result, all phenylalanine was removed from the diet for 72 h ("wash-out" period). The infant is now 10 days old and the most recent blood phenylalanine concentration is 600 μmol/L (10 mg/dL) and phenylalanine needs to be re-introduced into the diet. Based on the blood phenylalanine concentration at newborn screening (1,800 μmol/L), the recommended amount of phenylalanine to introduce into the diet is 45 mg/kg.

Patient information	Nutrient intake goals (per day)
Ten (10) day old infant male weighing 4.0 kg who was diagnosed with PKU based on elevated blood phenylalanine concentrations. Patient is doing well and currently drinking 22 oz of PKU Periflex® Early Years formula	**Phenylalanine:** 45 mg/kg (range 25–70 mg/kg or 130–430 mg/d) **Protein:** 3.0 g/kg (range 3.0–3.5 g/kg) **Tyrosine:** 300–350 mg/kg **Energy:** 100–120 kcal/kg **Fluid:** 150 mL/kg Recommended caloric density of formula: 20–25 kcal/oz

Select nutrient composition of formulas for PKU diet calculation example (using standard infant formula as the source of whole protein)

Medical food	Amount	PHE (mg)	TYR (mg)	PROTEIN (g)	ENERGY (kcal)
PKU Periflex® Early Years[a]	100 g	0	1440	13.5	473
Enfamil Premium ® powder[b]	100 g	430	500	10.8	510

[a]Nutricia North America (Rockville, MD; nutricia-na.com)
[b]Mead Johnson Nutrition (Evansville, IN; meadjohnson.com)

Step-by-Step Calculation

Step 1: Calculate the amount of Phe required each day.

Phe Goal × Infant Weight = mg Phe per day

45 mg Phe × 4 kg = 180 mg/day Phe

Step 2: Calculate amount of standard infant formula needed to meet daily Phe requirement.

Amount of Phe required per day ÷ Amount of Phe in 100 g of standard infant formula

180 mg Phe ÷ 430 mg Phe = 0.42

0.42 × 100 = 42 g standard infant formula needed to meet daily Phe requirement.

Step 3: Calculate protein and energy provided from standard infant formula.

Amount of standard formula × protein provided in 100 g of standard formula

0.42 × 10.8 g protein = 4.5 g protein in standard infant formula

Step 4: Calculate amount of protein to fill the diet prescription.

Protein goal x Infant weight = daily protein requirement

3.0 g protein × 4 kg = 12 g daily protein requirement

Daily protein requirement − protein provided by standard infant formula

12 g − 4.5 g = 7.5 g protein needed from medical food to fill in the diet prescription

Step 5: Calculate amount of protein required from Phe-free medical food.

Protein needed to fill prescription ÷ protein provided in 100 g of medical food

7.5 g ÷ 13.5 g = 0.56

0.56 × 100 = 56 g Phe-free medical food required to fill the diet prescription

Step 6: Calculate amount of tyrosine provided from standard infant formula and Phe-free medical food.

Amount of standard formula × Tyr in 100 g of standard formula

0.42 × 500 mg Tyr = 210 mg Tyr

Amount of Phe-free medical food × Tyr in 100 g of Phe-free medical food

0.56 × 1,440 mg Tyr = 806 mg Tyr

Add standard formula + Phe-free medical food for total Tyr provided in diet prescription.

210 mg + 806 mg = 1,016 mg

1,016 mg/4 kg = 254 mg Tyr/kg

Step 7: Calculate total energy provided from standard infant formula and Phe-free medical food.

Amount of standard infant formula × kcal in 100 g of standard formula.

0.42 × 510 kcal = 214 kcal

Amount of phe-free medical food × kcal of 100 g of phe-free medical food.

0.56 × 473 kcal = 265 kcal

Add standard formula + Phe-free medical food for total kcal provided in diet prescription.

214 kcal + 265 kcal = 479 kcal

479 kcal/4 kg = 120 kcal/kg

Step 8: Calculate the final volume of formula to make a concentration of 20 kcal per ounce.

Amount of total calories provided by diet prescription ÷ 20 fluid ounces = number of ounces of formula needed to provide caloric concentration of 20 kcal/oz

479 kcal ÷ 20 kcal/oz = 23.95 oz of formula

(Note: If final volume prescribed is 24 oz, caloric concentration will be 20 kcal/oz; if final volume prescribed is 23 oz caloric concentration will be 20.8 kcal/oz- either is acceptable)

Diet prescription summary for sample calculation of PKU diet (using standard infant formula as the source of whole protein)[a]

Medical food	Amount	PHE (mg)	TYR (mg)	PROTEIN (g)	ENERGY (kcal)
PKU Periflex® Early Years[b]	56 g		806	7.6	265
Enfamil® Premium powder[c]	42 g	181	210	4.5	214
Total per day		181	1016	12.1	479
Total per kg		45 mg/kg	254 mg/kg	3.0 g/kg	120 kcal/kg

[a]Rounded to nearest whole number for amount of formula powders, phenylalanine, tyrosine and energy and rounded to the nearest 0.1 g for protein
[b]Nutricia North America (Rockville, MD; nutricia-na.com)
[c]Mead Johnson Nutrition (Evansville, IN; meadjohnson.com)

Example 3:

Infant with PKU using breast milk as the source of whole protein.

Patient History

A newborn infant tested positive for PKU upon the newborn screen. The initial blood phenylalanine concentration was 1,800 μmol/L (30 mg/dL). Based on this result, all phenylalanine was removed from the diet for 72 h ("wash-out" period). The infant is now 10 days old and the most recent blood phenylalanine concentration is 600 μmol/L (10 mg/dL) and phenylalanine needs to be introduced back into the diet. Based on the blood phenylalanine concentration at newborn screening (1,800 μmol/L), the recommended amount of phenylalanine to introduce into the diet is 45 mg/kg.

Patient information	Nutrient intake goals (per day)
Ten (10) day old infant male weighing 4.0 kg who was diagnosed with PKU based on elevated blood phenylalanine concentrations. Patient is doing well and currently drinking 22 oz of PKU Periflex® Early Years formula	**Phenylalanine:** 45 mg/kg (range 25–70 mg/kg or 130–430 mg/d) **Protein:** 3.0 g/kg (range 3.0–3.5 g/kg) **Tyrosine:** 300–350 mg/kg **Energy:** 100–120 kcal/kg **Fluid:** 150 mL/kg Recommended caloric density of formula: 20–25 kcal/oz

Select nutrient composition of formulas for sample PKU diet calculation (using breast milk as the source of whole protein)

Medical food	Amount	PHE (mg)	TYR (mg)	PROTEIN (g)	ENERGY (kcal)
PKU Periflex® Early Years[a]	100 g	0	1440	13.5	473
Breast milk	100 mL	40	50	1.05	72

[a]Nutricia North America (Rockville, MD; nutricia-na.com)

Step-by-Step Calculation:

Step 1: Calculate the amount of Phe required each day.

Phe Goal × Infant Weight = mg Phe per day

45 mg Phe × 4 kg = 180 mg/day Phe

Step 2: Calculate amount of breast milk needed to meet daily Phe requirement.

Amount of Phe required per day ÷ Amount of Phe in 100 mL of breast milk

180 mg Phe ÷ 40 mg Phe/100 mL = 4.5 mL

4.5 mL × 100 = 450 mL breast milk needed to meet daily Phe requirement

450 mL is 15 oz. Since we know the infant is taking 22 oz, 15 oz will be supplied by breast-milk and we determine the remaining 7 oz should come Phe-free medical food (We will use 8 oz in calculations since it is likely that slightly more volume will be needed going forward as the infant grows).

Step 3. Calculate the number of grams of Phe-free medical food needed to make 8 oz of formula between 20 and 25 kcal/oz.

8 oz × 20 kcal/oz = 160 kcal

8 oz × 25 kcal/oz = 200 kcal

160 kcal ÷ 473 kcal/100 g = 34 g Phe-free medical food for a 20 kcal/oz formula

200 kcal ÷ 473 kcal/100 g = 42 g Phe-free medical food for a 25 kcal/oz formula

We will use 40 g in this example:

Step 4: Calculate amount of protein provided by 40 g Phe-free medical food®

40 g × 13.5 g protein/100 g powder = 5.4 g protein from Phe-free medical food

Step 3: Calculate protein provided from breast milk.

Amount of breast milk x protein provided in 100 mL of breast milk

4.5 mL × 1.05 g protein = 4.7 g protein in 450 mL breast milk

Step 6: Calculate amount of tyrosine provided from breast milk and Phe-free medical food.

Amount of breast milk × Tyr in 100 mL of breast milk

4.5 mL × 50 mg Tyr = 225 mg Tyr in breast milk

Amount of Phe-free medical food × Tyr in 100 g of Phe-free medical food

0.40 × 1,440 mg Tyr = 576 mg Tyr in Phe-free medical food

Add breast milk + Phe-free medical food for total Tyr provided in diet prescription.

225 mg + 576 mg = 801 mg in total diet prescription

801 mg/4 kg = 200 mg Tyr/kg

Step 7: Calculate total energy provided from breast milk and Phe-free medical food.

Amount of breast milk × kcal in 100 mL of breast milk.

4.5 mL × 72 kcal/mL = 324 kcal

Amount of Phe-free medical food x kcal of 100 g of Phe-free medical food.

0.40 × 473 kcal = 189 kcal

Breast milk + Phe-free medical food

324 kcal + 189 kcal = 513 kcal total in diet

Diet Prescription: 450 ml (15 oz) of breast milk, and 40 g PKU Periflex® Early Years powder mixed with water to make 8 oz.

Diet prescription summary for sample calculation of PKU diet (using breast milk as the source of whole protein)[a]

Medical food	Amount	PHE (mg)	TYR (mg)	PROTEIN (g)	ENERGY (kcal)
Breast milk	450 mL	180	225	4.7	324
PKU Periflex® Early Years [a, b]	40 g	0	576	5.4	189
Total per day		180	801	10.1	513
Total per kg		45 mg/kg	200 mg/kg[c]	2.5 g/kg[d]	128 kcal/kg

[a]Values rounded to nearest whole number for amount of formula powders, phenylalanine, tyrosine and energy and rounded to the nearest 0.1 g for protein
[b]Nutricia North America (Rockville, MD; www.nutricia-na.com)
[c]Tyrosine/kg is slightly lower than recommended. Monitor blood tyrosine results, but it is unnecessary to add supplemental L-tyrosine to this diet prescription
[d]Protein/kg is lower than protein goal but greater than DRI and since the majority of the protein is being provided by breast milk (protein of high biologic value) rather than free amino acids, the usual increase in recommended protein intake for patients on metabolic medical food does not apply

The diet could be provided in three different ways:

Option 1. Mother can express 15 oz of breast milk and add it to 8 oz of PKU Periflex® Early Years (using recipe above) to make one 23-oz mixture of formula/breast milk and feed the infant from a bottle (only use this method if mother chooses NOT to feed the infant from the breast but prefers to pump and feed from bottle).

Option 2. Determine feeding schedule that will provide 8 oz of PKU Periflex® Early Years and 15 oz of breast-milk. Since 8 oz (out of 23 total ounces in diet) is about 1/3 of total volume and breast milk is about 2/3 of total volume, allow infant to alternate 2 breast feedings with 1 PKU Periflex® Early Years feeding (Breast feed, breast feed, PKU Periflex® Early Years, feed around the clock). PKU Periflex® Early Years feedings should be 2–3 oz per feeding at this age.

Option 3. Limit the PKU Periflex® Early Years feedings to 8 oz per day and ask mother to adlib breastfeed at other times. This is similar to choice #2 except rather than a specific feeding regimen, the mother has a goal (and a limit) on the amount of PKU Periflex® Early Years to provide in 1 day and can determine when during the day or night the infant will have bottle feedings. Bottle feedings should be 2–3 oz per day at this age.

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11 Understanding Large Neutral Amino Acids and the Blood-Brain Barrier

Steven Yannicelli

Contents

Core Messages

- Imbalances of amino acid concentrations in the brain may alter brain protein synthesis, disturb neurotransmitter synthesis, and be responsible for many of the signs and symptoms of aminoacidopathies.
- Using knowledge of competitive inhibition at the blood-brain barrier provides insights into new approaches of nutrition management in inborn errors of metabolism.
- Nutrition management using large neutral amino acid (LNAA) may be an option for some patients with phenylketonuria (PKU).

11.1 Background

The onset of newborn screening, early diagnosis, and nutrition management has been successful in preventing lifelong intellectual disability and has led to an improved quality of life and normal cognition for many individuals with phenylketonuria (PKU). Despite these significant clinical outcomes, individuals with PKU may still present with some cognitive deficits if not properly treated into adulthood [1–4].

"Diet for life" is now a recognized goal of nutrition management for all patients with PKU

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[5]. However, diet acceptability and adherence to a lifelong strict regimen is often unattainable [6]. In support of "diet for life," alternative nutrition approaches should be considered in patients with PKU who cannot maintain adherence to the diet [5, 7].

Many patients with PKU relax dietary control with age [6, 8] and discontinue attending a metabolic clinic [9]. Many adults with PKU are either "off diet" or on a "relaxed diet"; neither is recommended for optimal clinical outcomes. Getting patients back on a "classic" strict phenylalanine-restricted diet is difficult. Challenges to long-term nutrition management include nonadherence, financial costs for medical foods, lifestyle, quality of life, and psychosocial issues. New approaches to nutrition management, including glycomacropeptide protein medical foods and large neutral amino acids, offer alternatives to standard approaches [7, 10].

11.2 Review of LNAA and the Blood-Brain Barrier

Large neutral amino acids (LNAA) are comprised of aromatic amino acids (phenylalanine, tyrosine, tryptophan) and branched-chained amino acids (leucine, isoleucine, valine, as well as methionine, histidine, and threonine (Box 11.1). All LNAA are essential amino acids, except tyrosine, which is conditionally essential in phenylketonuria.

Box 11.1: Large Neutral Amino Acids

- Histidine (HIS)
- Isoleucine (ILE)
- Leucine (LEU)
- Methionine (MET)
- Phenylalanine (PHE)
- Threonine (THR)
- Tryptophan (TRP)
- Tyrosine (TYR)
- Valine (VAL)

Each of these amino acids shares the same transporters across the blood-brain barrier and gut mucosal cells. At the blood-brain barrier, the LAT-1 transporter is responsible for transport of LNAA from the blood into the brain [11, 12]. The blood-brain barrier also actively regulates amino acid content of the brain. Selective affinity for phenylalanine over other LNAA through the LAT-1 transporter means that phenylalanine is efficiently transported across the blood-brain barrier [12, 13]. The result is increased brain phenylalanine concentrations at the expense of other LNAA (Fig. 11.1). Pardridge et al. reported that even modest increases in blood phenylalanine (i.e., 200–500 μmol/L) can reduce non-phenylalanine LNAA uptake [12]. Individual genetic variations in LAT-1 transporter may influence clinical outcomes in individuals with PKU [15, 16].

Brain LNAA transport in individuals with PKU is severely affected whenever high blood phenylalanine concentrations are present. The neurotoxicity associated with PKU is considered a combination of direct toxicity of high brain phenylalanine concentrations along with deficits in serotonin and dopamine (Chap. 9). Consistently elevated phenylalanine concentrations in the brain, along with low concentrations of tyrosine, tryptophan, and brain chemicals resulting in decreased protein synthesis, may negatively affect executive function and behavior in individuals with PKU [3, 17–19]. Based on this knowledge, the approach to management using the understanding of amino acid transporters is the basis for LNAA intervention.

The basic principle of LNAA supplementation is to competitively inhibit the uptake of phenylalanine at the blood-brain barrier. When successful, LNAA supplementation can reduce the ratio of Phe:LNAA brain concentrations and improve cerebral neurochemistry and brain protein synthesis [17, 20, 21] (Fig. 11.2). Hoeskma and colleagues reported a significantly negative correlation with blood phenylalanine concentrations greater than 600–800 μmol/L and decreased cerebral protein synthesis [17].

The overall goal of LNAA management is to supplement sufficient LNAA to correct brain neurochemical aberrations that may be harmful. The potential benefit in using LNAA is to improve behavioral and neuropsychological function by

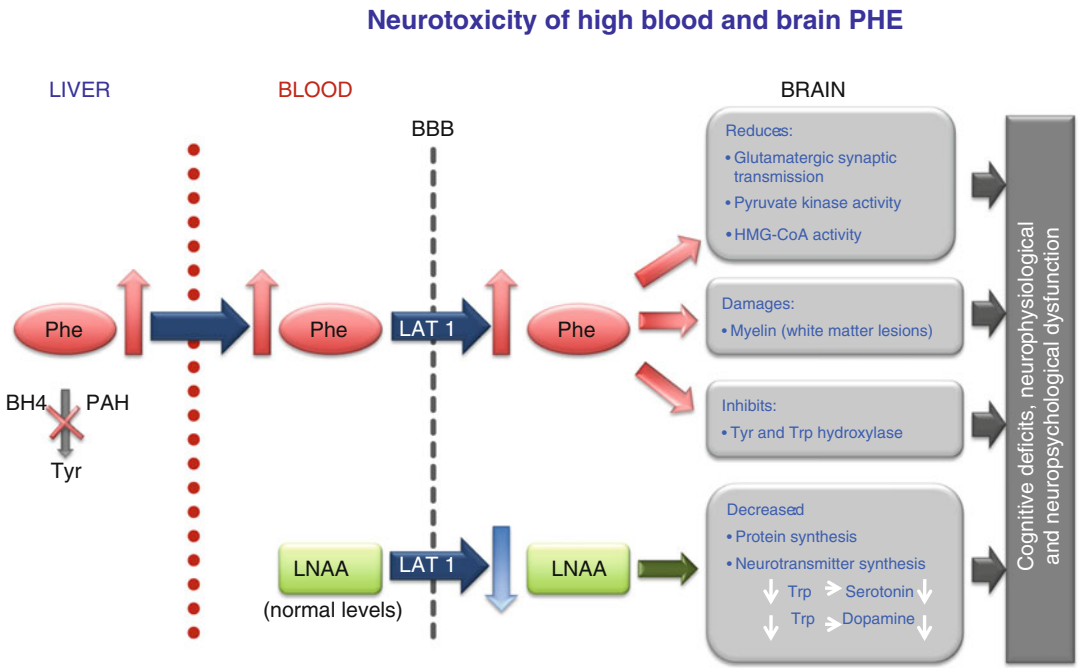

Fig. 11.1 Consequences of high blood and brain phenylalanine concentrations (Adapted from [14])

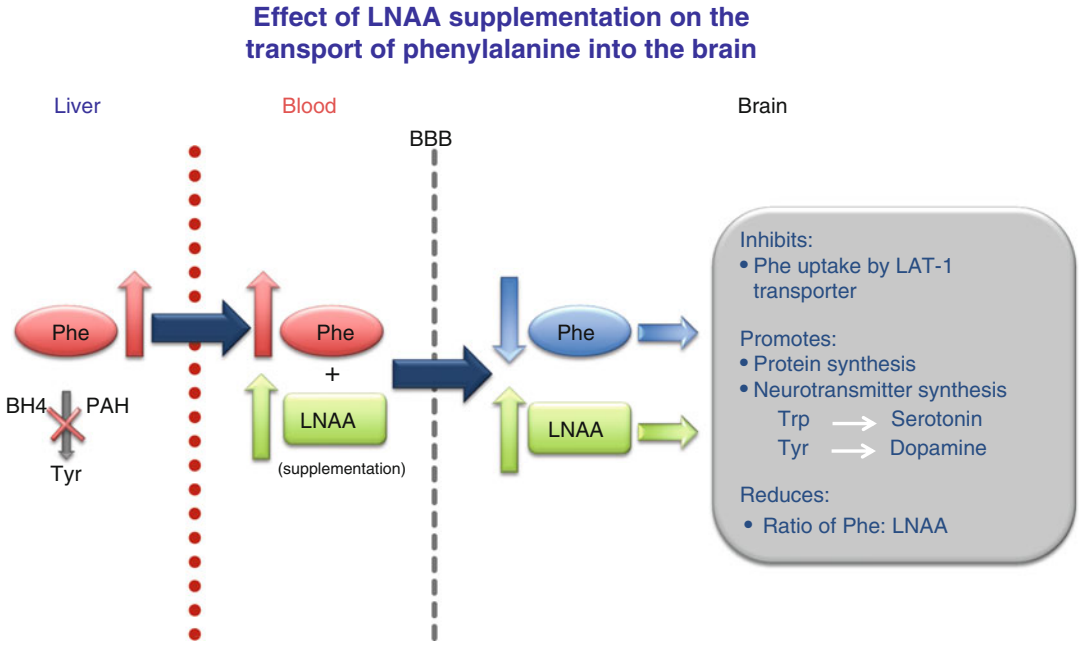

Fig. 11.2 Effects of LNAA therapy on the transport of phenylalanine into the brain

reducing brain phenylalanine concentrations and increasing brain LNAA concentrations, especially the neurotransmitter precursors tyrosine and tryptophan [22–26].

LNAA supplementation targeting the blood-brain barrier was first suggested as an alternative therapy over 40 years ago [27]. In artificially induced hyperphenylalaninemic rats, LNAA supplementation resulted in lower brain phenylalanine concentrations but no change in serum phenylalanine concentrations. Conclusions from this study were the basis for considering the use of increasing LNAA to compete with phenylalanine as an alternative nutrition management tool in individuals with PKU.

Pietz et al. (1999) reported in a pivotal study the positive effects of use of LNAA to block phenylalanine transport into the brain [28]. Six male adults with moderate and severe PKU were given a dose of 100 mg L-PHE/kg and assessed with and without LNAAs (150 mg LNAA/kg). Diet was not changed. Mean pre-load blood phenylalanine was 1,000 μmol/L and brain phenylalanine was 250 μmol/L. In response to an oral phenylalanine challenge, without LNAA, brain concentrations of phenylalanine increased to 400 μmol/L. Concurrent LNAA supplementation completely blocked phenylalanine uptake with no noted change in brain activity as measured in electroencephalogram (EEG) spectral analysis. This work set the groundwork for future studies. Other selected clinical trials are noted in Fig. 11.3.

While the main function of LNAA is blocking phenylalanine uptake at the brain, a secondary effect may be the reduction of dietary phenylalanine uptake through intestinal mucosa [29, 30]. Sanjurjo and others [30] reported that oral L-threonine supplementation had a blood phenylalanine-reducing effect [30]. Despite the effect of threonine on blood phenylalanine, a mechanism of action could not be confirmed.

In 2007, Matalon and associates reported that LNAA supplementation with high leucine content reduced blood phenylalanine by 39 % [29]. Subjects were given 0.5 g LNAA/kg/day divided into three doses taken with meals. Diet was not altered during the 2-week trial. Baseline blood phenylalanine concentrations were variable with some subjects in treatment range while others >1,200 μmol/L. Neuropsychologic tests were not measured. Authors concluded that for any effect on blood phenylalanine reduction, high amounts of LNAA must be provided. Other factors besides LNAA supplementation alone may account for decreased blood phenylalanine concentrations [10].

Author	Subject group	Intervention	Findings
* Crowley et al. (1990)	10 Classical PKU	Open-label study 1 year	Behavioral improvements. Suggested decrease plasma Phe when subjects were compliant.
* Cleary et al. (2002)	5 Classical PKU	Cross-over study, placebo-controlled. Unrestricted diet. MRS	Suggested reduction in brain Phe when taking LNAA supplement
Koch et al. (2003)	6 Classical PKU	Open-label study 6 months	No change in plasma Phe concentrations Behavioral improvements
Sanjurjo et al. (2003)	8 Classical PKU; 4 mild HPA patients	Placebo-controlled cross over study. 8 weeks. 50 mg Thr/kg/d	Significant decrease of plasma PHE levels by 26 %
Kalkanoglu et al. (2005)	19 Untreated PKU	Placebo-controlled cross over study for 6 months	Improvements in behavior and quality of life.
Matalon et al. (2007)	20 PKU patients	Double-blind, placebo controlled, cross over study. 1 week	Decreases plasma Phe by 39 %
* Schindeler et al. (2007)	16 Classical PKU	Double-blind, placebo-controlled, cross over study. 2 week	Increased LNAA intake showed decreased plasma phe Cognitive improvements

Fig. 11.3 Selected clinical trials of LNAA

In a classic double-blinded crossover clinical trial, Schindeler and colleagues reported a positive effect on executive function in 16 subjects with treated PKU [26]. Either placebo or LNAA supplementation (250 mg/kg/day) was given over four 2-week phases, with a 4-week washout period. Results showed positive impact of LNAA supplementation on several specific executive functions, especially on measures of attention. Higher plasma phenylalanine concentrations were reported in phases where LNAA intakes were lowest. A secondary effect was noted in that subjects given the most LNAA, by supplement and medical food, had the lowest blood phenylalanine concentrations (24.9 % reduction).

A recent meta-analysis of the effectiveness of LNAA compared to standard PKU diet therapy [31] concluded that LNAA lacked a clinical impact; however, the review only included studies with measures of cognition as the outcome. More well-designed clinical trials assessing the effectiveness and nutrition status of LNAA therapy are needed.

11.3 The LNAA Diet: Applications for Use

The majority of clinicians agree that LNAA is not recommended for young children nor pregnant women but should be considered for adults with PKU who are not in good metabolic control and do not adhere to other treatment options [7]. This includes individuals with PKU who are considered "off diet" – not drinking medical food and eating high-phenylalanine foods. Other individuals who may be struggling with the diet (i.e., consuming some or all medical food but eating higher-phenylalanine foods) and who cannot maintain recommended blood phenylalanine concentrations may also be considered for the LNAA diet. The LNAA diet is considered a reasonable alternative for adult patients off diet or never treated [25]. The LNAA diet is NOT intended to replace the standard phenylalanine-restricted diet for all patients and is NOT intended for individuals who are considered in "good" metabolic control [5].

Box 11.2: Contraindications for the Use of LNAA in PKU Diet Management

- Individuals who are considered in "good" metabolic control
- Pregnant women or women planning pregnancy
- Infants and young children

Contraindications for use of LNAA diet management include women with PKU either pregnant or planning pregnancy because the LNAA diet is not specifically intended to decrease blood phenylalanine concentrations, which is paramount for optimal fetal outcome in pregnant women with PKU [29]. In these women, a strict PKU diet regimen must be implemented and carefully monitored. The LNAA diet is also not indicated for young children (Box 11.2).

Individuals on psychotropic medications (such as selective serotonin reuptake inhibitors or SSRIs, monoamine oxidase inhibitors, and methylating agents) being managed for depression and anxiety should be carefully counseled before starting the LNAA diet. LNAA products contain significant amounts of tyrosine and tryptophan, precursors to dopamine and serotonin, respectively. Taking too much LNAA may exacerbate the brain serotonin systems.

11.4 The LNAA Diet Plan

The LNAA diet differs from the standard low-phenylalanine diet. Whereas the low-phenylalanine diet depends on approximately 80 % of daily protein requirements being supplied from medical foods, the LNAA diet consists of approximately 70–80 % protein from whole protein foods with 20–30 % of protein requirements from LNAA. Calculations should be based on a daily protein intake of 0.8 g protein/kg/day. When prescribing the diet, first, calculate the total daily protein requirement, and then subtract the 70–80 % of total protein from food sources. The remaining 20–30 % of protein equivalents should be supplied as LNAA (Box 11.3).

Box 11.3: Initiating LNAA Dietary Treatment for an Individual with PKU: Example

Calculation based on providing 0.25 g LNAA/kg body weight

1. Obtain actual body weight of patient (e.g., 60 kg).
2. Calculate grams of LNAA required each day.

60 kg × 0.25 g = 15 g LNAA per day*

Calculation based on using a ratio of 70–80 % whole protein to 20–30 % from LNAA

1. 60 kg × 0.8 g protein/kg = 48 g protein per day
2. 80 % of protein from whole protein = 38 g
3. 20 % of protein equivalents from LNAA = 10 g LNAA*

*Divide the total grams of LNAA by the number of meals.

Variance in amount of prescribed LNAA using different methods during initiation phase is common. Modify prescription as needed to meet goals.

The dose of LNAA in the diet depends on current blood phenylalanine concentrations, age, and tolerance to whole protein foods. Reported doses range from 0.25 g LNAA to 0.5 g LNAA/kg body weight/day [25, 26, 29]. Van Spronsen and colleagues [32] showed the LNAA content of the diet in standard low-phenylalanine diet, LNAA supplementation alone, and the combination of both LNAA and low-phenylalanine diet. LNAA intake consumption with LNAA supplementation is higher than on a conventional low-phenylalanine diet [32]. The most beneficial effect on clinical outcomes occurs when individuals with PKU consume increased dietary LNAA [26]. Although the LNAA diet was intended to be used in place of standard low-phenylalanine diet, LNAA may also be added to a standard low-phenylalanine diet using other medical foods [32].

LNAA products come in either powdered form or capsules/tablets. Powdered LNAA products are reconstituted and consumed like standard medical foods. An advantage of powdered forms is that they can contain a full range of vitamins and minerals that may be missing in the diet. For individuals familiar with the taste of standard medical foods, LNAA products will be complimentary. For individuals who prefer capsules and tablets, LNAA in these forms are more acceptable. For individuals consuming LNAA capsules/tablets, it is vital that a daily vitamin and mineral supplement be prescribed. Additional calcium with vitamin D is a major consideration because the LNAA diet may still lack foods containing these important nutrients. Calcium in standard vitamin-mineral supplements only typically provides 25 % of the recommended amounts. Vitamin D supplementation alone may be prescribed for some individuals.

LNAA products differ in their amino acid content based on which transporter is targeted. Some products contain high leucine with a goal of inhibiting phenylalanine at the gastrointestinal brush border. Other LNAA products contain high tyrosine and tryptophan to have most effect at the blood-brain barrier. For LNAA amino acid profiles targeting the blood-brain barrier, blood phenylalanine concentrations may not significantly change.

A typical LNAA diet will include grains, standard breads, and unlimited fruits and vegetables along with some high biological value proteins. Unlimited amounts of potatoes and other starches are also included. Adding whole protein foods, especially protein dense foods, into the LNAA diet should be titrated slowly. Some individuals with PKU may interpret the LNAA diet as *carte blanche* to consume all food types and amounts. Monitoring dietary food intake is important as in the standard low-phenylalanine diet to avoid overconsumption of high-protein foods and to assure that nutrient-rich foods, such as vegetables and fruits, are adequately eaten. Consuming LNAA along with protein-containing foods is important to have the best effect. Currently, there are no reported data on LNAA dosage per grams of food protein. A rule of thumb to consider would be to offer one dose of LNAA at each

protein-containing meal throughout the day. Modifications to this approach should be tailored to each individual with PKU. Consuming large doses of LNAA on an empty stomach may result in gastric distress. However, amounts of 900 mg LNAA/kg/day have not resulted in any side effects [26].

11.5 Assessing the Response to LNAA Supplementation

Accurately measuring brain phenylalanine concentrations remains elusive to clinicians. Brain magnetic resonance spectroscopy to quantify brain phenylalanine concentrations has been reported [33, 34]. Moats and colleagues [33] reported a linear relationship between brain and blood phenylalanine concentrations using magnetic resonance imaging [33]. However, this correlation was accurate only when blood phenylalanine concentrations exceeded 1,200 μmol/L. Blood phenylalanine concentrations below 1,200 μmol/L are not exact [26, 35, 36]. Attempts to replicate these findings have not been successful. Consequently, using magnetic resonance spectroscopy as a tool to measure changes in brain phenylalanine requires further research.

Blood phenylalanine concentrations do not accurately reflect *brain* phenylalanine concentrations; therefore, monitoring blood phenylalanine in patients on LNAA therapy is not useful. However, despite little or no change in blood phenylalanine concentrations, individuals with PKU on LNAA therapy have shown improved behavior and cognitive benefits [25, 26]. Qualitative changes in behavior and cognitive health can be assessed using behavioral assessment tools [37]. Standard questionnaires such as the Beck Depression Inventory and Beck Anxiety Inventory [38] are ideally administered before a new therapy is initiated and periodically thereafter.

Measurement of melatonin synthesis has been reported as a potential surrogate marker to quantify the effect of LNAAs on the brain [39]. Melatonin is only synthesized in the brain from serotonin (e.g., urinary sulfatoxymelatonin) [40] and may reflect effects of diet intervention. In a double-blinded crossover study in individuals with PKU, those individuals given a LNAA supplement compared to placebo showed statistically higher tyrosine:LNAA and tryptophan:LNAA ratios, and elevated serum melatonin, urine melatonin and urine dopamine. Blood phenylalanine concentrations were not significantly different between placebo and LNAA supplementation. These results indicate the possibility of using melatonin biomarkers to reflect brain chemistry changes from LNAA supplementation [39].

11.6 Monitoring Individuals on a LNAA Diet

Suggested parameters for monitoring individuals with PKU on taking LNAA are the same as for individuals on a standard PKU diet and/or tetrahydrobiopterin supplementation. Frequency of monitoring and specific parameters to be monitored should be individualized and depend on factors such as whether LNAA supplementation is used alone or in combination with medical food and whether the LNAA contains vitamins and minerals, to name a few.

Monitoring should include blood concentrations of phenylalanine and tyrosine as well as other amino acids. Assessing dietary phenylalanine intake and overall nutrient content of diet is essential because although the LNAA diet is more liberal than a standard PKU diet, the amount of high biological protein allowed is generally quite limited based on the protein guidelines and the nutrients associated with intakes of animal protein (B_{12}, zinc, iron, vitamin D) should not be assumed to be adequate. When instituting the LNAA diet plan, it is important to closely monitor the individual, especially in the first several weeks. Weekly blood phenylalanine and tyrosine concentrations and changes in behavior/mood and cognition should be considered. Any purported side effects should be noted. Reports of increased anxiety have been reported [26]. Using behavior assessment tools for monitoring LNAA therapy is important considering that blood phenylalanine concentrations alone do not correlate with clinical improvements.

11.7 New Frontiers with LNAA

Manipulation of amino acids in the diet to impact uptake across the blood-brain barrier has been used in patients with maple syrup urine disease [41, 42] and glutaric acidemia [42]. A review of LNAA supplementation [43] points out that advances in diets for inherited metabolic diseases will include targeting the concentrations of amino acids in the brain.

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Tetrahydrobiopterin Therapy for Phenylketonuria

12

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Contents

Core Messages

- Sapropterin dihydrochloride is a pharmaceutical preparation of BH_4 available in 100 mg tablets or powder, which can be administered at doses of 5–20 mg/kg/day, for those individuals with BH_4-responsive PKU.
- In clinical trials, 20–61 % of individuals with phenylketonuria (PKU) have been found to respond to tetrahydrobiopterin (BH_4) as demonstrated by a decrease in blood phenylalanine concentration.
- Children with PKU ≤4 years of age that were determined to be responsive to BH_4 have demonstrated a favorable safety profile to this medication.
- Clinical studies on BH_4 therapy have shown responders to BH_4 therapy to have improved phenylalanine to tyrosine ratios and less variability in blood phenylalanine concentration.

12.1 Background

Phenylketonuria (PKU) is an inborn error of metabolism caused by a genetic mutation resulting in dysfunction in the hepatic enzyme phenylalanine hydroxylase (PAH) responsible for metabolizing the essential amino acid

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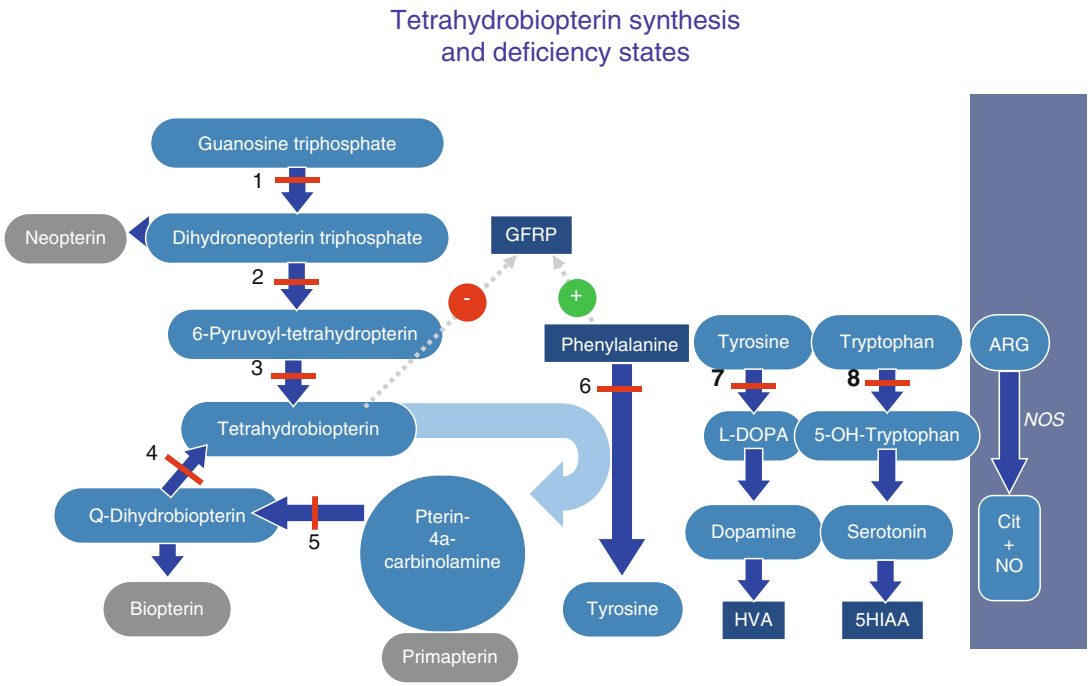


Fig. 12.1 Tetrahydrobiopterin synthesis and recirculation (*left* side) and enzyme reactions requiring BH_4 as a cofactor (*right* side). Each enzyme that has the potential to create a deficiency is noted with a corresponding number: (*1*) GTP cyclohydrolase 1 (GTPCH); (*2*) 6-pyruvoyltetrahydropterin synthase (PTPS); (*3*) sepiapterin reductase (SR); (*4*) dihydropteridine reductase (DHPR); (*5*) pterin-4a-carbinolamine dehydratase (PCD); (*6*) phenylalanine hydroxylase (PAH); (*7*) tyrosine hydroxylase (TH); (*8*) tryptophan hydroxylase (TPH)

phenylalanine to tyrosine. Patients with this condition have elevated blood phenylalanine concentrations, which can cause devastating neurological damage, if not identified and treated at birth with a phenylalanine-restricted diet. PAH requires the presence of a cofactor, 6R-tetrahydrobiopterin (BH_4), which also acts as a cofactor for the enzymes mediating the rate-limiting steps in the synthesis of dopamine, serotonin, and nitric oxide synthases.

BH_4 has been used with patients found to have deficiencies in synthesizing this cofactor due to a defect in any one of the six enzymes required to make it [1] (Fig. 12.1).

Historically, in some countries, newborns identified with elevated blood phenylalanine concentrations would receive a BH_4 loading test to differentiate whether they had a primary BH_4 deficiency or PKU. In 1999, Dr. Kure first reported the benefit of administering BH_4 to individuals with PKU when he found that three PKU infants responded to BH_4, after receiving an oral dose of 10 mg/kg body weight, by demonstrating a decrease in blood phenylalanine concentrations [2] (Fig. 12.2).

Sapropterin dihydrochloride (sapropterin) is a pharmaceutical formulation of BH_4, approved for treating individuals with PKU by the Food and Drug Administration (FDA) in 2007 and by the European Medicines Agency in 2008. In some PKU patients, sapropterin can activate residual PAH activity to lower the blood phenylalanine concentration [3].

12.2 Clinical Studies Leading to Approval of Sapropterin Treatment in PKU

Two phase II studies and four phase III studies have evaluated sapropterin in patients with elevated blood phenylalanine (Fig. 12.3).

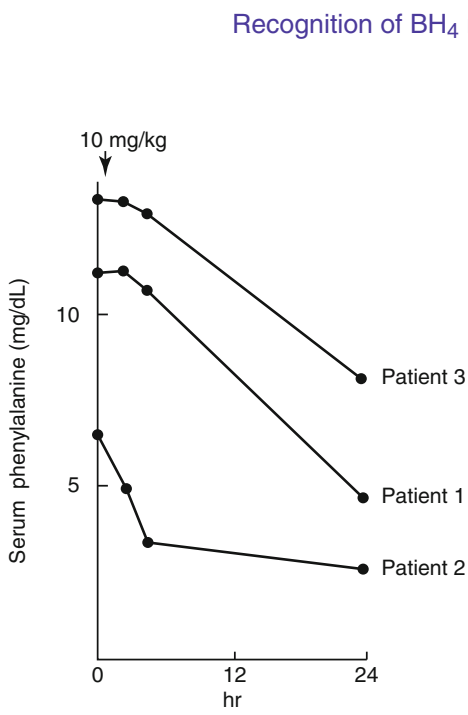


Fig. 12.2 Recognition of BH_4 responsiveness in three infants with PKU (Kure et al. [2])

The PKU001 study was an 8-day phase II study designed to evaluate the short-term safety and efficacy of sapropterin, at a dose of 10 mg/kg body weight, in 490 PKU patients ≥8 years of age to identify those who demonstrated a ≥30 % decrease in blood phenylalanine concentration. Of the 98 (20 %) subjects who demonstrated a 30 % reduction in blood phenylalanine, 89 participated in PKU003, a phase III randomized, double-blind, multicenter, placebo-controlled evaluation of 10 mg/kg/day sapropterin over 6 weeks. The mean blood phenylalanine concentration decreased by 236 (±257) μmol/L on sapropterin versus an increase of 3 (±240) μmol/L on placebo ($p<0.0001$) (Fig. 12.4); 44 % on sapropterin versus 9 % on placebo demonstrated a reduction in blood phenylalanine of ≥30 % from baseline [4]. During a 22-week extension trial, PKU004, 80 of the 89 patients completing PKU003 participated in a forced titration study of sapropterin at 5, 20, and 10 mg/kg/day for 2 weeks each, followed by 12 weeks of treatment with a constant dose. After 2 weeks of treatment, the highest dose of sapropterin at 20 mg/kg/day showed the greatest decrease in blood phenylalanine by a mean of 263 μmol/L, with dose-dependent decreases demonstrated at the other doses [5] (Fig. 12.5).

A second phase III study, PKU006, was conducted in children with PKU, ages 4–12 years, with blood phenylalanine concentrations ≤480 μmol/L at screening. In the first part of the study, 90 children were given a dose of 20 mg/kg/day of sapropterin; after 8 days, 56 % demonstrated a decrease in blood phenylalanine of ≥30 % from baseline. In the second part of the study, 46 of the subjects who demonstrated this decrease in blood phenylalanine were randomized to evaluate 10 weeks of sapropterin treatment added to a phenylalanine-restricted diet, with additional phenylalanine supplementation provided in accordance to blood phenylalanine concentrations. Daily dietary phenylalanine tolerance increased from 0 to 21 (±15) mg/kg/day on sapropterin versus 0 to 3 (±4) mg/kg/day on placebo. This was a significant mean treatment difference of 17.7 (±4.5) mg/kg/day ($p<0.001$) [6] (Fig. 12.6).

An open, multicenter, 3-year extension study, PKU008, enrolled 111 patients who completed

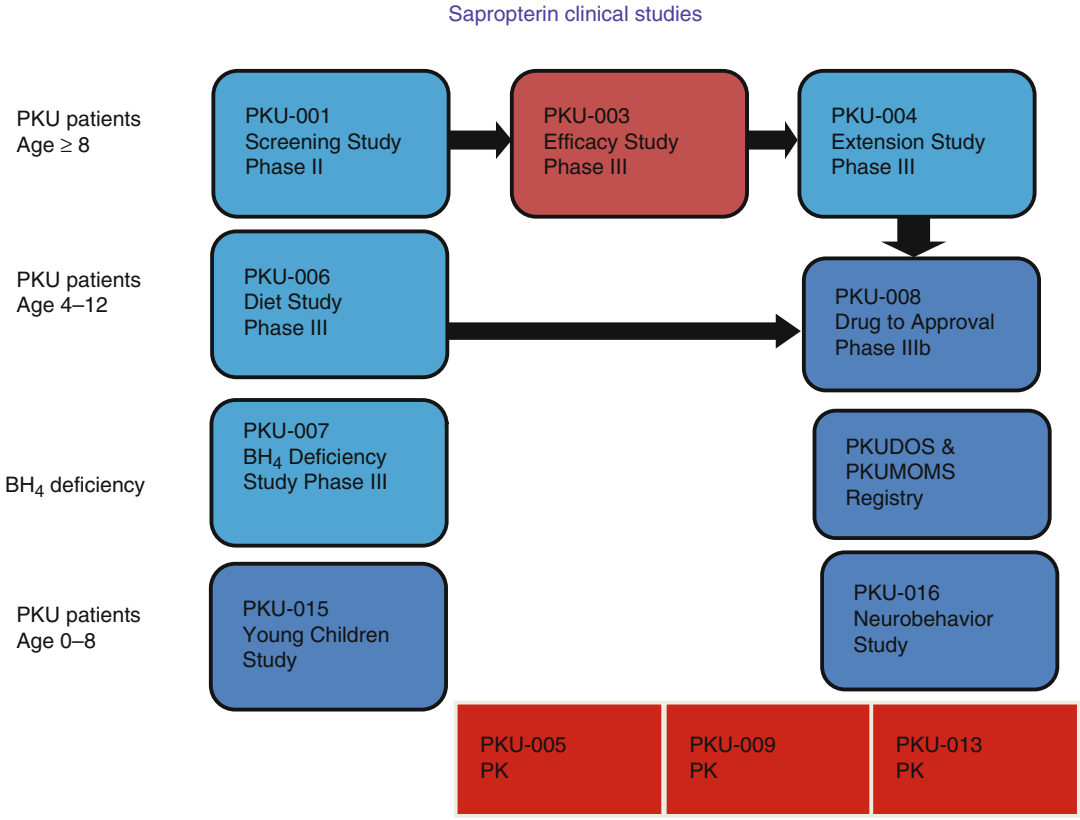


Fig. 12.3 Clinical studies of sapropterin use in patients with PKU or BH_4 deficiency. This figure illustrates the clinical studies that support the development of Kuvan and how these studies are interrelated. (*PKUDOS* PKU Demographic Outcomes and Safety Registry, *PKU MOMS* The Maternal PKU Observational Program sub-registry, *PK* pharmacokinetic study)

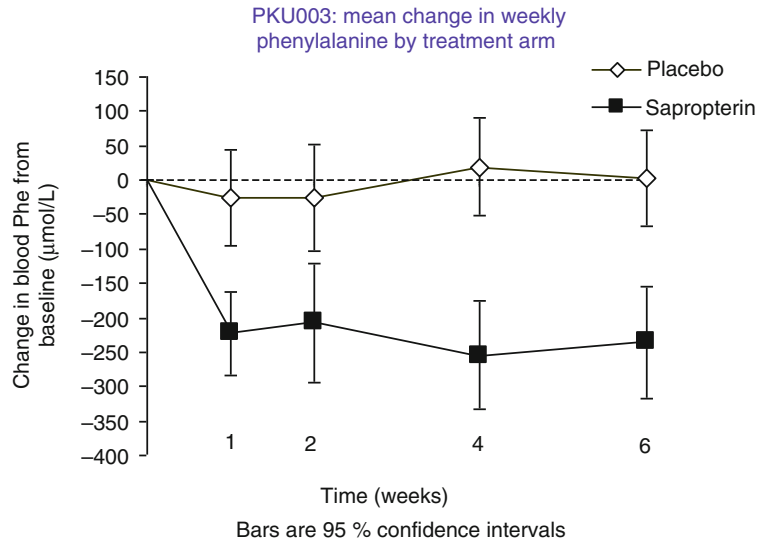
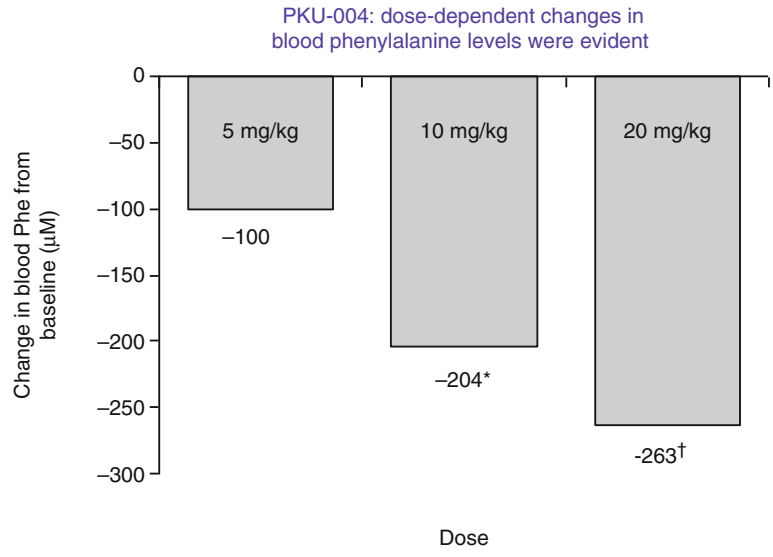
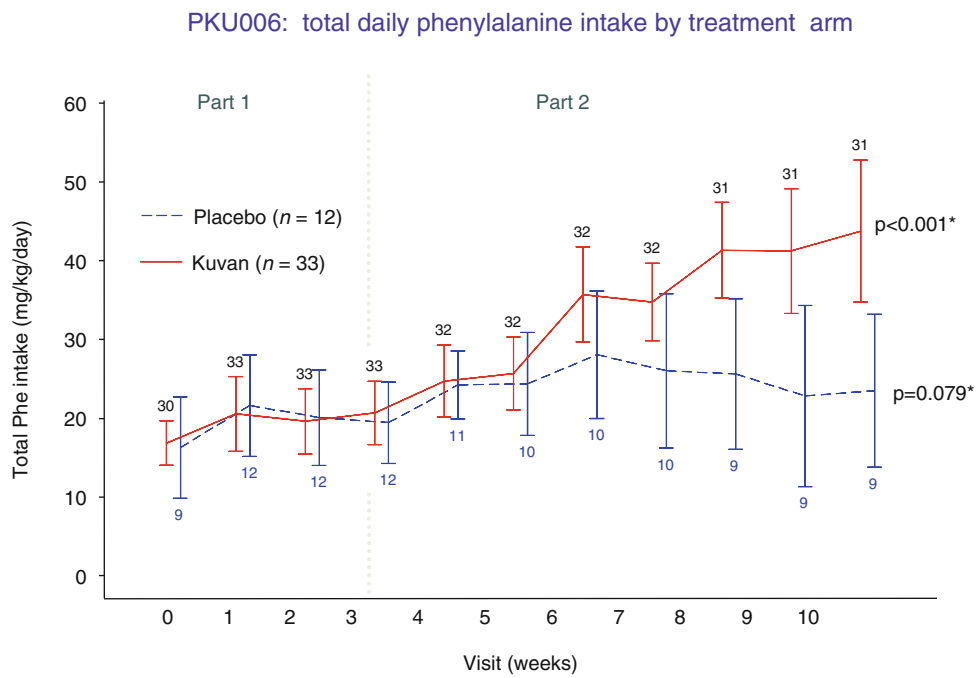


Fig. 12.4 Blood phenylalanine concentrations in patients given sapropterin or placebo. *Horizontal axis* is time in weeks, and *vertical axis* is the change in blood phenylalanine concentration from baseline as reported in µmol/L. *Open diamonds* are the placebo arm and *black squares* are the sapropterin arm (Levy et al. [4])



*P< 0.0001 compared to 5 mg/kg
†P< 0.01 compared to 10 mg/kg
N = 80 for all doses

Fig. 12.5 Dose-dependent changes in blood phenylalanine concentrations in patients with PKU on sapropterin therapy (Lee et al. [5])



Total daily Phe = supplemental Phe + dietary Phe
*Mean change in total Phe intake from week 0 to week 10
Bars indicate 95 % confidence intervals need to indicate that the numbers indicate number of subjects at that visit week

Fig. 12.6 Total daily phenylalanine intake in patients with PKU given sapropterin or placebo (Trefz et al. [6])

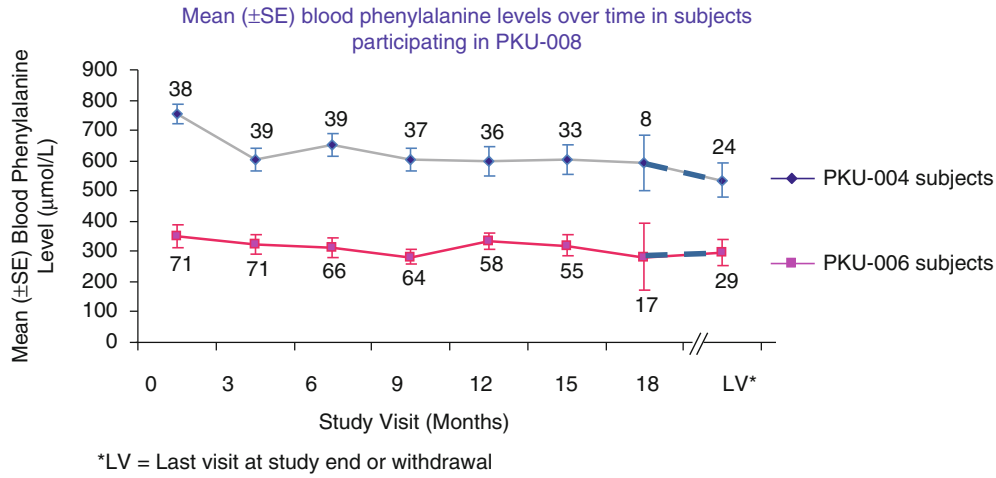


Fig. 12.7 Mean blood phenylalanine concentrations over time in patients with PKU receiving sapropterin therapy (Burton et al. [7])

either the PKU004 or PKU006 studies, to further evaluate the safety of sapropterin. The median exposure to sapropterin was 595 days on an average sapropterin dose of 16 mg/kg/day. The overall incidence of adverse events (AEs) in subjects receiving sapropterin was similar to that reported in subjects receiving placebo. For those AEs that were considered study drug related, the majority were of mild to moderate severity; three subjects discontinued for treatment-related AEs and 1 of 7 serious AEs was considered treatment-related due to gastroesophageal reflux. Blood phenylalanine was well maintained for most patients [7] (Fig. 12.7).

In a separate open-label, 10-week, phase II study, 12 subjects with primary BH_4 deficiency (9 with BH_4 synthesis defects and 3 with recycling defects) were evaluated to determine the impact of sapropterin on blood phenylalanine at doses up to 20 mg/kg/day. Many of the subjects were on other forms of BH_4 treatment prior to the study. Mean blood phenylalanine concentrations changed from 72.2 (±13.4) µmol/L to 75.0 (±11.5) µmol/L in those with primary BH_4 synthesis defects and from 315 (±180.9) µmol/L to 347.7 (±187.7) µmol/L in those subjects with BH_4 recycling defects, suggesting no statistical difference in blood phenylalanine control from baseline as compared to after 10 weeks of sapropterin treatment [1].

The PKU005, PKU009, and PKU013 studies were designed to assess the pharmacokinetics of sapropterin in healthy volunteers. Absorption was rapid, within 0.6–2.9 (±1.5) hours, with a half-life of 6.7 (range 3.9–16.6) hours, and determined sufficient to support once daily dosing without evidence of accumulation. Body weight was the only clinical factor found to influence the pharmacokinetics of sapropterin. Administration of food increased the absorption of sapropterin by 40–85 % and was greater following ingestion of intact tablets. Blood phenylalanine concentrations decreased within 24 h after administration of sapropterin, although maximal effect on phenylalanine concentrations may take up to a month, depending on the individual. A single daily dose is adequate to maintain stable blood phenylalanine concentrations over a 24-h period [8].

12.3 Administration of Sapropterin

Sapropterin is currently available in 100 mg tablets or in 100 mg packets of powder (both containing 76.8 mg of the active ingredient, sapropterin). The recommended starting dose of sapropterin is 10 mg/kg/day in children 1 month to 6 years of age and 10–20 mg/kg/day

in ≥7 years, to be taken once daily. Sapropterin tablets can be taken whole or dissolved in 4–8 oz water or apple juice and consumed within 15 min of dissolving. Sapropterin powder for oral solution should be dissolved in 4–8 oz of water or apple juice and consumed within 30 min of preparation. This medication can be mixed in small amounts of soft foods, such as applesauce or pudding, and should be taken with a meal. For infants weighing ≤10 kg, sapropterin can be dissolved in as little as 5 mL of water or apple juice, and a portion of this solution, corresponding to the correct dose, may be administered orally via an oral dosing syringe [3].

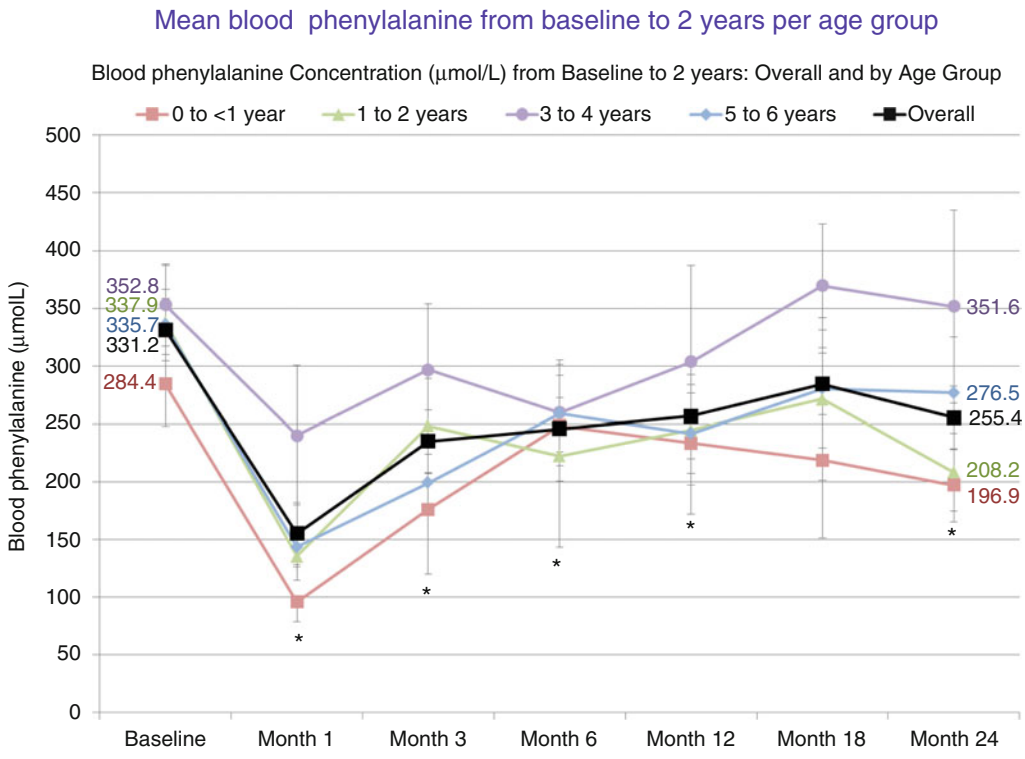
In the clinical trials leading to the approval of sapropterin, approximately 20–56 % of the subjects with PKU were determined to respond to sapropterin, at doses of 10–20 mg/kg/day, based on a ≤30 % decrease in blood phenylalanine concentrations. The recently published PKU guidelines by the American College of Medical Genetics recommend that all individuals with PKU receive a trial of sapropterin to determine if they will benefit from this therapy [9]. While assessing whether individuals benefit from sapropterin, close monitoring is required. Frequent assessments of diet, blood phenylalanine concentration, and other factors that can impact blood phenylalanine, such as activity level, stress, growth, and illness, should be obtained prior to starting the medication and throughout the trial period [10]. The sapropterin dose can be adjusted from 5 to 20 mg/kg/day to achieve optimal blood phenylalanine control. Individuals with PKU who are taking sapropterin should continue consuming a phenylalanine-restricted diet, though dietary phenylalanine intake may need to be adjusted to maintain blood phenylalanine within the recommended range of 120–360 μmol/L and to avoid hypophenylalaninemia. The prescribed amount of medical food protein should also be adjusted to ensure an adequate total protein intake. These individuals should continue to be followed regularly by a PKU clinic. Detailed dietary management guidelines for PKU subjects on sapropterin have been published [11].

12.4 Clinical Experience of BH_4 Use in Patients Under 4 Years of Age

The clinical trials conducted prior to receiving approval from the FDA included PKU individuals from 4 to 49 years of age. After approval, an open-label, single-arm multicenter trial was conducted on 93 PKU patients aged 1 month to 6 years. The children who entered this study had blood phenylalanine concentrations of ≥360 μmol/L at screening and were given sapropterin at 20 mg/kg/day. After 4 weeks of treatment, 57 (61 %) were identified as responders based on a ≥30 % decrease in blood phenylalanine. These children continued to receive sapropterin and maintained phenylalanine-restricted diets while participating in this study that evaluated the safety of sapropterin and its effect on neurocognitive function, blood phenylalanine concentration, and growth. In an interim analysis, 55 children who completed at least 2 years of the study were evaluated. Sapropterin lowered blood phenylalanine concentrations to ≤360 μmol/L in 84 % of subjects and to ≤240 μmol/L in 64 % of subjects (Fig. 12.8), with increased prescribed dietary phenylalanine intakes. The 25 children who had both baseline and 2-year full-scale IQ assessments demonstrated no significant change in intelligence over time, with a mean full-scale IQ of 103 ± 12 at baseline and 104 ± 10 at 2-year follow-up.

For the children <30 months of age, development remained within the average range, as determined by the Bayley Scales of Infant and Toddler Development. Mean z-scores for height, weight, and head circumference, as determined by the Center for Disease Control reference values, were maintained with no statistically significant change from baseline. Sapropterin had a favorable safety profile and was well tolerated, based on dose adherence and absence of serious adverse events. The reported AEs and drug-related AEs were consistent with adverse reactions reported in the earlier studies of sapropterin in PKU subjects 4–8 years of age [12].

There is increasing clinical experience with use of sapropterin in PKU children ≤4 years



* $P<0.05$ for change from baseline in overall population (black line)

Values are mean ± SE at each time point with the overall group including all patients.

Fig. 12.8 Mean blood phenylalanine concentration over time in children with PKU age 1–6 years at enrollment in study of BH_4 supplementation (Longo et al. [12])

of age. In a retrospective study, long-term BH_4 therapy was reported in 15 patients who were initiated on the medication before 18 months of age after exhibiting an 80 ± 12 % decrease from baseline in blood phenylalanine. BH_4 treatment was reported to significantly decrease mean blood phenylalanine concentrations (352 ± 85 vs. 254 ± 64, µmol/L, $p<0.05$) and raise the percentage of blood phenylalanine concentrations within the therapeutic target of 120–360 µmol/L (35 ± 25 vs. 64 ± 16 %, $p<0.05$). These children had increased dietary phenylalanine tolerance and reduced variability in blood phenylalanine concentrations [13]. Eleven of 16 (69 %) PKU patients ≤4 years of age, on BH_4 treatment for an average of 5 years, showed mean blood phenylalanine concentrations ≤360 µmol/L, and all 16 demonstrated increased dietary phenylalanine tolerance. The investigators did not assess any side effects with this treatment [14]. Six case reports of children, aged 7 months to 4 years, reported sapropterin to be generally well tolerated with reductions in blood phenylalanine concentration and increases in dietary phenylalanine tolerance reported [15]. The cases illustrated some of the difficulties, such as administering the medication, determining responsiveness with low blood phenylalanine concentrations at baseline, and identifying if a change in behavior could be due to an intolerance to the medication, encountered when using sapropterin in infants and children with recommendations on how to overcome these issues and allow continuation of treatment in this age group.

12.5 Impact of BH_4 on Neurocognition

The impact of BH_4 on neurocognition has been the topic of recent research studies. Gassio et al. assessed cognitive function after long-term treatment of BH_4 in 9 PKU subjects, 6–18 years of age on doses of 5–9 mg/kg/day administered for >5 years and compared to 28 PKU subjects treated by a phenylalanine-restricted diet [16]. No significant differences were found between the BH_4-treated and diet-treated groups in measurements assessing full-scale intelligent quotient, attention deficit hyperactivity disorder, or executive functioning. Mean (SD) blood phenylalanine concentrations were 314 μmol/L (±53) in the BH_4-treated group as compared to 478 μmol/L (±185) in the diet-treated group. The authors suggested caution in the interpretation of these results given the small sample size of BH_4-treated subjects [16].

White et al. evaluated the impact of sapropterin at 20 mg/kg/day on cognition and neuroimages in 12 subjects with PKU, ages 6–35 years, who demonstrated a ≥20 % decrease in blood phenylalanine from baseline. This group was compared to 9 age-matched unaffected individuals that served as the control group. Baseline diffusion tension imaging (DTI) showed widespread reduction in microstructural white matter integrity in the PKU group as compared to the control group, which appeared to be related to higher blood phenylalanine concentrations. As compared to the control group, the PKU group also demonstrated poorer executive abilities as measured by tasks to assess working memory and strategic processing. Significant improvements in microstructural white matter integrity were seen after 6 months of sapropterin treatment in the PKU subjects. Despite improvements in neuroimages, they did not find any improvement in executive abilities at this time point. The researchers concluded that 6 months of sapropterin treatment may be too brief of a period to detect changes in cognition and that longer-term follow-up on a larger number of PKU subjects was needed [17]. In another study, the impact of BH_4 on neural activity patterns as measured by functional magnetic resonance imaging (fMRI) was observed in 12 PKU subjects while performing working memory tasks. These findings were compared to 12 demographically matched, unaffected control subjects. The baseline evaluation revealed impaired working memory and atypical brain activity as measured by fMRI in the subjects with PKU as compared to controls. BH_4 treatment with sapropterin at 20 mg/kg/day was associated with improvement in brain activation based on neural changes after 4 weeks of treatment, with the greatest improvement seen in the group exhibiting a ≥20 % decrease in blood phenylalanine from baseline. Improvement in working memory was demonstrated after 6 months of treatment, though this was measured in only five subjects who completed the study. The investigators concluded that future research with a larger sample size was needed to help understand if the improvements seen were due to improvement in phenylalanine metabolism as demonstrated by lower blood concentrations versus a potential secondary therapeutic pathway of BH_4 [18].

A placebo-controlled study evaluated the effects of sapropterin therapy on PKU-associated symptoms of attention deficit-hyperactivity disorder (ADHD) and executive and global functioning in the 118 of 206 individuals who had a therapeutic blood phenylalanine response to sapropterin therapy. In the 38 individuals with sapropterin-responsive PKU and ADHD symptoms at baseline, sapropterin therapy resulted in a significant improvement in ADHD inattentive symptoms after 4 weeks of treatment, which were maintained over the 26 weeks duration of the study. Aspects of executive functioning (including initiation, working memory, planning/organizing, organizing materials, and monitoring) also improved in the subjects demonstrating a blood phenylalanine decrease to sapropterin therapy. The improvements in ADHD inattentive symptoms and aspects of executive functioning in response to sapropterin therapy noted in a large cohort of individuals with PKU indicate that these symptoms are potentially reversible when blood Phe levels are reduced [19].

12.6 Long-Term Experience with BH_4 Therapy

The effect of BH_4 on the phenylalanine to tyrosine ratio and variation in blood phenylalanine concentrations were assessed in blood tests collected over an 8-year period in 9 patients with PKU and compared to 25 non-BH_4-treated patients with PKU [20]. The BH_4-treated patients had smaller variations in blood phenylalanine concentrations, with a mean (95 % CI) of 358 (350–366) μmol/L versus 370 (364–376) μmol/L in non-BH_4 treated, and tighter phenylalanine to tyrosine ratios, with a mean (95 % CI) of 5.44 (5.3–5.6) versus 6.12 (5.9–6.3) in non-BH4 treated. The authors recommended long-term neuropsychological testing to ascertain the clinical benefit of these biochemical findings. In another study, 14 of 16 patients with PKU initially determined to be responsive to BH_4 were able to achieve long-term blood phenylalanine control with a mean blood phenylalanine concentration of 321 ± 236 μmol/L, for a mean duration of 56 months (range 24–110 months) [6]. The mean decrease in blood phenylalanine from baseline in these 14 patients was 54.6 %. The other two subjects were considered "pseudo-responders" and did not continue BH_4 treatment. Factors reported to cause fluctuation in phenylalanine concentrations in these patients included genotype, age, phenylalanine intake, treatment adherence, illnesses or infections, and periods of catabolism or anabolism.

The impact of BH_4 treatment on phenylalanine tolerance, medical food consumption, and nutrition status in six PKU children, 5–12 years of age, was assessed over a 2-year period. Mean dietary phenylalanine tolerance increased from 421 ± 128 to 1470 ± 455 mg/day resulting in greater intakes of intact protein and less medical food protein. Height z-scores significantly improved from 0.25 ± 0.99 at baseline to 0.53 ± 1.16 at 2 years (p for trend < 0.001) [21]. In another retrospective longitudinal study, anthropometric characteristics were reported on a cohort of 38 PKU patients on BH_4 and/or phenylalanine-restricted diets as compared to 76 subjects only on phenylalanine-restricted diets. Despite the higher natural protein intake in the BH_4-treated subjects, no differences were found between the groups in the growth measures collected for 2 or 5 years. In the BH_4-treated cohort, z-scores were consistent with pretreatment values [22].

A retrospective analysis on 147 European PKU patients receiving BH_4 treatment for up to 12 years was reported [23]. A questionnaire was developed to assess outcomes including blood phenylalanine concentration, quality of life (QoL), and treatment adherence. Median blood phenylalanine was well controlled as demonstrated by an average value within the recommended range of 120–360 μmol/L. About half of the respondents reported improved QoL (50 %) and improved adherence to diet (47 %) and treatment (63 %). Five subjects discontinued sapropterin due to pregnancy in one and poor adherence in the others. Based on these survey results, the investigators concluded that sapropterin was safe and effective over the long term for individuals with PKU who respond to this treatment. In ten patients with PKU who completed validated generic and chronic health-condition QoL questionnaires, improvements in these measures were not demonstrated after 1 year of treatment with BH_4 [24]. In another study, a PKU-specific self-report QoL questionnaire was used to assess QoL outcomes at baseline and after 1 year in PKU individuals aged 10–49 years, comparing sapropterin responders ($n = 17$) to nonresponders ($n = 19$). Improvements in QoL outcomes were reported to be most pronounced in those patients able to increase dietary phenylalanine tolerance [25]. In summary, treatment with sapropterin has resulted in clinically significant and sustained reductions in blood phenylalanine concentrations in individuals with PKU found to be responsive to this treatment. These benefits have been seen in individuals as young as 1 month of age. Additional long-term benefits have been reported, including decreased variability in blood phenylalanine, increased dietary phenylalanine tolerance, improved blood phenylalanine to tyrosine ratio, as well as improved

growth and QoL, although further studies on a larger number of PKU subjects are needed to confirm these findings.

12.7 Summary

There are three long-term observational registries following sapropterin-treated patients. The PKU Demographic Outcomes and Safety Registry (PKUDOS, clinicalTrials.gov NCT00778206) is designed to include data on up to 3,500 individuals with hyperphenylalaninemia due to PKU who are taking sapropterin, have previously taken sapropterin, or plan to take sapropterin in the next 90 days, with final data collection in 2023. The Maternal PKU Observational Program subregistry (PKU MOMs) follows pregnant women who have taken sapropterin prior to or during their pregnancy. There is also a registry in Europe following sapropterin-treated patients with hyperphenylalaninemia due to PKU or BH_4 deficiency in 625 patients for up to 15 years (KAMPER, clinicalTrials.gov NCT01016392). Results from these long-term studies will help to further the understanding of sapropterin treatment in PKU.

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Maternal Phenylketonuria

13

Fran Rohr

Contents

Core Messages

- Children born to mothers with phenylketonuria (PKU) who are not on a phenylalanine-restricted diet during pregnancy are at high risk of intellectual disability, microcephaly, congenital heart defects, low birth weight, and facial dysmorphism.
- Women with PKU should maintain blood phenylalanine between 120 and 360 μmol/L (2–6 mg/dL) before and during pregnancy for the best pregnancy outcomes.
- The maternal PKU diet includes a PKU medical food as a source of protein, limited amount of phenylalanine from whole protein, and sufficient energy, fat, tyrosine, vitamins, and minerals to support fetal growth.
- Maternal diets low in protein, vitamin B_{12}, and folate increase the risk of congenital heart disease in the infant.

13.1 Background

Maternal PKU (MPKU) refers to pregnancy and childbearing in a woman with phenylketonuria (PKU). Phenylalanine is teratogenic to the developing fetus, and therefore, in MPKU, the infant is at risk because of the mother's metabolic disorder.

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Table 13.1 Outcomes in maternal PKU in relationship to timing of maternal blood phenylalanine control[a]

Problem	Prior to conception [7]	By 10 weeks gestation [7]	Untreated [1]
Congenital heart defects	0	2	12
Microcephaly	3.6	5	73
IQ < 85 at 7 years	5	24	90

[a]In this study, good control was defined as maternal blood phenylalanine concentration of <600 μmol/L [7]

This is unlike the effect in maple syrup urine disease (MSUD), organic acidemias (PROP or MMA), or urea cycle disorders (UCD) where the mother is at risk (Chap. 21).

Children born to mothers with PKU who are not consuming a strict, phenylalanine-controlled diet during pregnancy may be born with intellectual disability, microcephaly, congenital heart defects (CHD), low birth weight, and facial dysmorphism [1, 2]. The frequency of adverse outcomes in MPKU is related to maternal blood phenylalanine concentration. The recommended maternal blood phenylalanine concentration range throughout pregnancy is 120–360 μmol/L (2–6 mg/dL) [4–6]. This recommendation is based on the MPKU Collaborative Study, a 12-year study of 413 pregnancies which showed lower intelligence in offspring of mothers whose average blood phenylalanine concentration exceeded 360 μmol/L (6 mg/dL) [7]. Table 13.1 shows the incidence of congenital heart defects, and microcephaly is the highest in children born to mothers who were untreated or do not achieve blood phenylalanine concentrations at or below 600 umol/L until later in pregnancy.

The British Registry of 228 live births found a negative correlation between intellectual outcomes and blood phenylalanine concentrations exceeding 300 μmol/L; therefore, in the United Kingdom, it is recommended that blood phenylalanine be maintained between 100 and 250 μmol/L for optimal outcomes [8]. The Australians, Ng et al. [9], recommend lower blood phenylalanine concentrations during pregnancy [9]. Some centers in the United States also counsel women to maintain blood phenylalanine under 240 μmol/L [6]. However, there is evidence that very low (<120 μmol/L; 2 mg/dL) blood phenylalanine concentrations may be associated with poor fetal growth [10] suggesting that hypophenylalaninemia should be avoided.

Stability of blood phenylalanine throughout pregnancy was associated with better development in the offspring of MPKU in one study [11], which showed that the variability in blood phenylalanine concentration had impact on intellectual outcome at 1, 8, and 14 years, even in women who had good metabolic control. Variability of blood phenylalanine may be a marker for the severity of PKU; women who have classic PKU are less able to tolerate day-to-day changes in dietary phenylalanine intake and therefore have greater variation in blood phenylalanine concentrations. In the MPKU study, women were given a severity score based on genotype, untreated blood phenylalanine concentration, and dietary phenylalanine tolerance. The score was the strongest predictor of both maternal blood phenylalanine during pregnancy and of variability in maternal blood phenylalanine concentrations [12].

In addition to phenylalanine, other nutrients are of importance in MPKU outcomes, including protein, fat, energy, and vitamin B_{12}. Maternal protein, fat, and energy intake is negatively correlated with blood phenylalanine concentration [13]. Inadequate energy intake was associated with poor maternal weight gain and lower birth measurements. A higher incidence of congenital heart defects is seen in children born to women with lower-protein intakes, especially when both low vitamin B_{12} and folate intake were also observed [14].

13.2 Nutrition Management of MPKU

The principles of nutrition management in MPKU are to control blood phenylalanine concentrations within 120–360 μmol/L, support normal weight gain for pregnancy (Table 13.2), and provide adequate nutrients for pregnancy. Other than phenylalanine, protein, and tyrosine,

Table 13.2 Recommendations for total and rate of weight gain during pregnancy by prepregnancy BMI [19]

Prepregnancy BMI	BMI[a] (kg/m^2)	Total weight gain (pounds)	Rates of weight gain 2nd and 3rd trimester[b] (pounds/week)
Underweight	<18.5	28–40	1 (1–1.3)
Normal weight	18.5–24.9	25–35	1 (0.8–1)
Overweight	25.0–29.9	15–25	0.6 (0.5–0.7)
Obese (includes all classes)	>30.0	11–20	0.5 (0.4–0.6)

[a]To calculate BMI, go to www.nhlbisupport.com/bmi/
[b]Calculations assume a 0.5–2 kg (1.1–4.4 lbs) weight gain in the first trimester (Based on Siega-Riz et al. 2004; Abrams et al. 1995; Carmichael et al. 1997)

Table 13.3 Recommended daily intake of phenylalanine, tyrosine, and protein in pregnancy and lactation for women with MPKU [26]

	Phenylalanine (mg)	Tyrosine (mg)	Protein (g)
Trimester 1	265–770	6,000–7,600	≥70
Trimester 2	400–1,650	6,000–7,600	≥70
Trimester 3	700–2,275	6,000–7,600	≥70
Lactation	700–2,275	6,000–7,600	≥70

Box 13.1: Points to Consider if Blood Phenylalanine Is Too High

- Is medical food intake sufficient?
- Is phenylalanine intake excessive?
- Is energy intake sufficient?
- Has there been adequate weight gain?
- Has there been an illness?

the nutrient needs of a pregnant woman with PKU do not differ from the Dietary Reference Intakes (Table 13.3) [3]; however, obtaining adequate nutrition for pregnancy while on a phenylalanine-restricted diet can be a challenge.

13.2.1 Phenylalanine and Tyrosine

Phenylalanine should be provided in the amount needed to meet the target range of 120–360 μmol/L in blood. For a woman with PKU who comes to attention after pregnancy, it is important to reduce phenylalanine intake as soon as possible, and some centers suggest a "washout" period where only medical food, fruits, low phenylalanine veggie, and low protein foods are included in the diet until the blood phenylalanine concentration decreases to within the desired range. In severe PKU, the average phenylalanine intake is 250–300 mg/day; if a patient's phenylalanine tolerance is not known, this is a reasonable goal to begin with. Phenylalanine intake in the first trimester ranges from 265 to 770 mg/day [6].

With frequent monitoring of blood phenylalanine and food intake records, dietary phenylalanine can be adjusted until the target range is reached. If blood phenylalanine concentrations are not in good control within a few days, consider whether the woman is getting enough protein (medical food) and/or energy (Box 13.1). Morning sickness or hyperemesis gravidarum can also be a cause of high blood phenylalanine. Prolonged morning sickness can be treated with antiemetics. In cases where metabolic control is compromised due to hyperemesis gravidarum, hospitalization may be necessary in order to reverse catabolism and reduce blood phenylalanine concentrations. Hospitalization may also be necessary for intensive diet education.

If blood phenylalanine concentration becomes too low, additional phenylalanine is added to the diet. If blood phenylalanine is between 60 and 120 μmol/L, increase phenylalanine by 10 %; if less than 60 μmol/L, increase phenylalanine by 25 %; and if undetectable, increase phenylalanine by 50 % for 1 day followed by a 25 % increase and recheck blood phenylalanine concentration in 3 days. As pregnancy progresses and the woman gains weight, phenylalanine tolerance will increase. This is especially true in the second and third trimesters when the fetus is growing rapidly and phenylalanine intake doubles or triples over prepregnancy intake [6].

Table 13.4 Comparison of select nutrients in medical foods for MPKU (when providing 75 g protein)

Medical food name	Quantity	Energy (kcal)	Iron (mg)	Zinc (mg)	Vitamin A (IU)	Vitamin D (IU)	Phenylalanine (mg)	Tyrosine (mg)
PKU Lophlex LQ® [a]	3.75 pouches	435	19	14	3,559	540	0	7,126
Phenylade® 60 Drink Mix[a]	7.5 pouches	368	23	15	2,920	651	0	8,076
Periflex® LQ[a]	5 drinks	800	27	18	4,165	650	0	8,652
PKU Cooler™ 10/15/20[b]	3.75 coolers	464	27	27	3,480	660	0	8,925
Glytactin RTD™ 15[c]	5 drinks	1,000	23	16	4,500	1,250	135	5,750
Camino Pro BetterMilk™ [c]	5 packets	693	22	15	3,356	650	115	6,713
Phenex-2™ [d]	250 g	1,025	32	32	5,500	750	0	7,500
Phenyl-Free® 2[e]	340 g	1,394	44	41	4,862	986	0	7,800
Phenyl-Free® 2 HP[e]	188 g	733	30	30	3,760	733	0	7,520

[a]Nutricia North America (Rockville, MD; nutricia-na.com)
[b]Vitaflo USA (Alexandria, VA; vitaflousa.com)
[c]Cambrooke Therapeutics (Ayer, MA; cambrookefoods.com)
[d]Abbott Nutrition (Columbus, OH; abbottnutrition.com)
[e]Mead Johnson Nutrition (Evansville, IN; meadjohnson.com)

Tyrosine is a conditionally essential amino acid in the MPKU diet. Medical food is the major source of dietary tyrosine; therefore if a woman has low blood tyrosine, check to make sure she is taking all of her medical food. Blood tyrosine fluctuates diurnally and is lowest after an overnight fast. Before adding a tyrosine supplement, monitor non-fasting blood tyrosine concentrations to assess whether supplementation is necessary [15].

13.2.2 Protein

The Dietary Recommended Intake (DRI) for protein in pregnancy is 71 g/day [3]. This recommendation provides an additional 21 g over nonpregnancy protein recommendations in order to support the growth of the placenta and fetal tissue. Medical food is the major source of protein for individuals with PKU. When protein is supplied as medical food containing L-amino acids, it is oxidized more rapidly than whole protein, and therefore the amount of protein needed is greater than normal (1.2 times the DRI or 85 g/day). In severe PKU, medical food provides about 80 % of the protein or about 68 g protein per day. A simple way to assure that adequate protein is being provided is to meet the DRI for protein from amino acid-based medical food alone.

The nutrient content of medical foods varies widely (Table 13.4). If high-protein, lower-calorie medical foods are used, the volume of medical food required is lower, but fat and energy content is also lower and sufficient energy must be supplied elsewhere in the diet. Conversely, when lower-protein, higher-fat medical foods are used, a higher volume of medical food is necessary to meet protein requirements. The choice of medical food is made on an individual basis depending on the needs and preferences of the pregnant woman, and sometimes combination of medical foods is best. Additional protein is often needed as the pregnancy progresses and should be added if plasma prealbumin or plasma amino acids are low for pregnancy (Table 13.5).

Large neutral amino acids (LNAA) are contraindicated as a sole source of protein in women with maternal PKU because LNAA do not sufficiently lower blood phenylalanine to within the desired treatment range of 120–360 μmol/L [16]. The proposed mechanism of action of LNAA is to block uptake of phenylalanine into the brain by supplementing other amino acids that share the LAT-1 transport system across the blood-brain barrier (Chap. 11). Some reduction in blood phenylalanine has been seen with LNAA use but not to the degree necessary to protect the fetus [17].

Table 13.5 Plasma amino acid concentrations during pregnancy in unaffected women (μmol/L) [7]

	<20 weeks	20–30 weeks	>30 weeks
Isoleucine	53 ± 23	53 ± 15	46 ± 15
Leucine	114 ± 38	107 ± 30	91 ± 23
Methionine	34 ± 54	20 ± 7	27 ± 7
Phenylalanine	67 ± 30	60 ± 18	54 ± 12
Threonine	118 ± 34	168 ± 42	193 ± 50
Tyrosine	55 ± 22	50 ± 11	50 ± 17
Valine	196 ± 60	179 ± 43	162 ± 43

13.2.3 Energy

Energy requirements in pregnancy are the same for the women with PKU as other individuals [18]. Sufficient energy is especially important in maternal PKU to prevent protein from being used as an energy source, thereby increasing blood phenylalanine concentrations. Energy intake is sufficient if the woman with PKU is gaining weight appropriately (Table 13.2) [19].

13.2.4 Fat and Essential Fatty Acids

Fat is needed in pregnancy to supply sufficient energy as well as precursors for essential fatty acids needed for fetal brain development. In pregnancy, about 30–35 % of calories should come from fat. For a 2,400-cal diet, this translates to 93 g of fat, the equivalent to approximately six tablespoons of fat. For expectant mothers on a fat-free or low-fat medical food, special attention must be paid to providing other sources of dietary fat.

The type of fat is also important to consider to ensure the requirement for the essential fatty acids, linoleic, and α-linolenic acid is met (Box 13.2). Essential fatty acids compete for the same desaturase enzymes, and omega-6 and omega-3 fatty acids must be provided in the proper ratio of approximately 5:1, or docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) synthesized from the omega-3 fatty acids may be low. In order to ensure that sufficient DHA is provided, 650 mg of omega-3 fatty acids, of which 300 mg is DHA, is recommended [20].

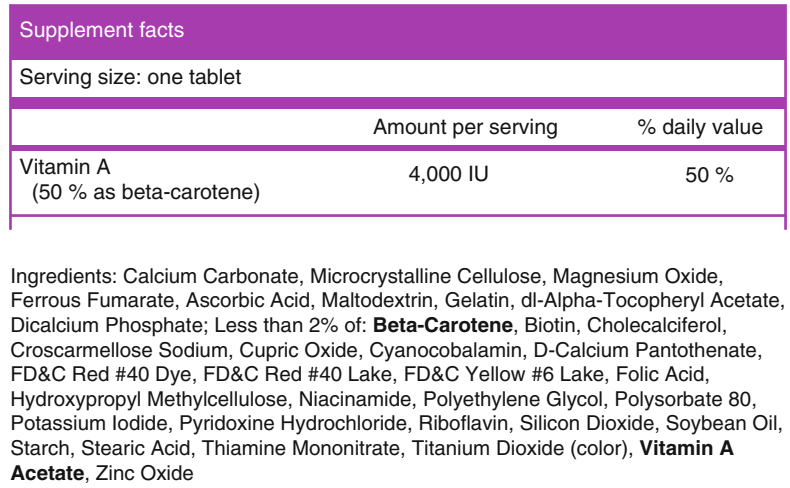
Box 13.2: Facts About Fat in the MPKU Diet

- Provide 30–35 % of energy as fat.
- DRI for essential fatty acids in pregnancy [3]:
  - Linoleic acid (omega-6) – 13 g/day
  - α-Linolenic acid (omega-3) – 1.4 g/day
- Soybean and canola oils are good, readily available essential fatty acid sources.
- DHA intake of 300 mg/day is recommended.

13.2.5 Vitamins and Minerals

The DRI for pregnancy should be met for all vitamins and minerals. Medical food is the source of many vitamins and minerals in the maternal PKU diet; however, if not taken as prescribed or if the medical food does not contain a full complement of vitamins and minerals, intakes may be low. Vitamins and minerals that are of particular concern in maternal PKU are vitamin B_{12} and folate [14]. Low intakes have been correlated with increased risk of CHD. Deficiencies in zinc, iron, and vitamin B_{12} may be seen in MPKU as these nutrients are most often found in high-protein foods that individuals with PKU do not usually consume. Prenatal supplements or specific vitamins and mineral supplementation may be necessary if monitoring of intake and/or if nutritional biomarkers indicate a problem.

Excessive intakes of vitamin A intake leads to hypervitaminosis A that has been associated with birth defects, including malformations of the eye, skull, lungs, and heart [21]. High intakes are possible in the diet for MPKU if a medical food containing vitamin A is taken along with a prenatal supplement or fish oil. The upper safe limit for vitamin A intake during pregnancy is 2,800–3,000 μg/day, or approximately equal to 10,000 IU (1 μg retinol



Supplement facts

Serving size: one tablet

	Amount per serving	% daily value
Vitamin A (50 % as beta-carotene)	4,000 IU	50 %

Ingredients: Calcium Carbonate, Microcrystalline Cellulose, Magnesium Oxide, Ferrous Fumarate, Ascorbic Acid, Maltodextrin, Gelatin, dl-Alpha-Tocopheryl Acetate, Dicalcium Phosphate; Less than 2% of: **Beta-Carotene**, Biotin, Cholecalciferol, Croscarmellose Sodium, Cupric Oxide, Cyanocobalamin, D-Calcium Pantothenate, FD&C Red #40 Dye, FD&C Red #40 Lake, FD&C Yellow #6 Lake, Folic Acid, Hydroxypropyl Methylcellulose, Niacinamide, Polyethylene Glycol, Polysorbate 80, Potassium Iodide, Pyridoxine Hydrochloride, Riboflavin, Silicon Dioxide, Soybean Oil, Starch, Stearic Acid, Thiamine Mononitrate, Titanium Dioxide (color), **Vitamin A Acetate**, Zinc Oxide

Fig. 13.1 Prenatal vitamin supplement label indicates that 50 % of the 4,000 IU of vitamin A supplied by is beta-carotene, and the remainder is from vitamin A acetate; thus, 50 % of the total vitamin A supplied or 2,000 IU would need to be considered toward the total of 10,000 IU considered to be toxic to the developing fetus

activity equivalent is equal to 3.3 IU) [22]. Vitamin A from animal sources (fish oil, or vitamin A palmitate, retinol and acetate) is of concern, but vitamin A supplied as carotenoids does not cause hypervitaminosis A because the conversion of beta-carotene to the active form of vitamin A is highly regulated by the body. Supplements often specify the source of vitamin A (Fig. 13.1).

13.3 Sapropterin Dihydrochloride Use in Pregnancy

Sapropterin dihydrochloride is a Category C drug meaning there are no adequate and well-controlled studies of its use in pregnant women. Preliminary evidence from a registry of women who have been on sapropterin during pregnancy shows that they tolerated the drug well and have maintained blood phenylalanine in good control during pregnancy [23]. In general, the sapropterin dose was 20 mg/kg at the start of pregnancy and not adjusted for weight gain during pregnancy. Because of the well-known effects of high blood phenylalanine on the developing fetus, sapropterin should be considered for women who are not able to keep blood phenylalanine within treatment range [5].

13.4 Nutrition Management in Lactation and the Postpartum Period

Women with PKU are counseled to be on diet for life including in the postpartum period. It is possible for the woman with PKU to breast-feed her infant. If the woman chooses not to be on diet after pregnancy yet is breast-feeding, there will be a slightly higher phenylalanine content in her breast milk, but this has no effect on the infant's blood phenylalanine concentration, as long as the infant does not have PKU. Even then, limited amounts of breast milk would be allowed in combination with a phenylalanine-free infant formula. While staying on the phenylalanine-restricted diet is not necessary for breast-feeding, it is encouraged in order for the mother to maintain optimal neuropsychological functioning, which is important for coping with the demands of caring for an infant [24].

The nutrient requirements for breast-feeding are the same as in the third trimester of pregnancy due to the high-protein, phenylalanine, and energy demands of producing breast milk. There is no evidence regarding the safety of sapropterin dihydrochloride use in lactation [4]. Monitoring of blood phenylalanine and continued support of the woman with PKU are needed, but these are often difficult to accomplish once the mother's attention turns from her diet and pregnancy to caring for an infant.

13.5 Monitoring

Careful metabolic and nutritional monitoring of a pregnant woman with PKU is important to ensure that the fetus is not exposed to high blood phenylalanine and sufficient nutrition is provided for proper fetal development. Frequent (once or twice weekly) monitoring of blood phenylalanine is especially important, as is routine monitoring of protein status including plasma amino acids and prealbumin levels (Box 13.3). The reference ranges for many laboratory tests, including amino acids, differ for pregnancy. The laboratory monitoring can be done by the metabolic clinic or by the obstetrician if the woman lives far from the clinic or if traveling becomes difficult later in pregnancy, as long as communication between providers occurs. Weight gain should be monitored regularly and ultrasounds performed twice during pregnancy, once early in pregnancy to establish that the fetus is viable and once at 18 weeks gestation to rule out cardiac and other anomalies [25].

Box 13.3: Nutrition Monitoring of a Patient with Maternal PKU[a]

- Routine assessments including anthropometrics, dietary intake, and physical findings (Appendix F)
- Laboratory Monitoring
  - Diagnosis Specific
    - Plasma amino acids
      - Phenylalanine
      - Tyrosine
  - Nutrition laboratory monitoring of patients on phenylalanine-restricted diets may include markers of:
    - Protein sufficiency[b] (plasma amino acids, prealbumin, albumin)
    - Nutritional anemia (hemoglobin, hematocrit, MCV, serum vitamin B_{12} and/or methylmalonic acid, total homocysteine, ferritin, iron, folate, total iron binding capacity)
    - Vitamin and mineral status: 25-hydroxy vitamin D, zinc, trace minerals, and folic acid
    - Essential fatty acid sufficiency: plasma or erythrocyte fatty acids
    - Others as clinically indicated

[a]For suggested frequency of monitoring see GMDI/SERC PKU Nutrition Guideline (www.southeastgenetics.org/ngp) or ACMG guideline (Appendix P).
[b]Further described in Chap. 7.

13.6 Summary

Women with PKU must maintain blood phenylalanine between 120 and 360 μmol/L before and during pregnancy to prevent the maternal PKU syndrome, a constellation of effects of high blood phenylalanine on the developing fetus that includes microcephaly, low birth weight, and congenital heart disease. The phenylalanine-restricted diet must provide sufficient protein, fat, and vitamins and minerals to support a developing fetus. Medical food for PKU provides protein without phenylalanine, and, depending on the nutrient profile of the medical food, supplemental energy, fat, essential fatty acids, and vitamins and minerals may be needed. Insufficient protein and vitamin B_{12} in the diets of women with PKU are associated with congenital heart defects. Close monitoring of blood phenylalanine and other laboratory values, as well as assessment of weight gain and nutrient intake is recommended. Women with PKU are encouraged to breast-feed their infants and to stay on the diet in the postpartum period.

13.7 Example of a MPKU Diet

Patient information	Nutrient intake goals (per day)
The patient is a 29 year-old woman with moderate PKU who has been on an unrestricted diet and comes to attention at 4 weeks gestation. She weighs 80 kg and has a BMI of 29 kg/m^2. Her blood phenylalanine at this visit is 960 μmol/L (16 mg/dL)	**Protein:** 75 g **Phenylalanine:** 250 mg **Energy:** ≥2,100 kcal **Fat:** 70 (30 % of energy) **DHA:** 200–300 mg **Vitamins and Minerals:** DRI for pregnancy

Table 13.6 shows the nutrient analysis of a diet for MPKU that meets the goals defined in the case example. In some centers, patients are encouraged to count milligrams of phenylalanine. Another approach that would meet these goals is to instruct the patient to consume the medical food and "free" foods (low-protein foods, fruits, non-starchy vegetables, fats, and sugars).

Table 13.6 Example of a diet for MPKU[a]

Medical food	Units	Quantity	Energy (kcal)	Protein (g)	Fat (g)	Phe (mg)
PKU Lophlex LQ, liquid, tropical[b]	1 Pouch (125 mL)	4	464	80.0	4	0
Cinnamon Raisin Swirl Bread – low protein[c]	1 slice	2	280	0.5	3	28
Butter, with salt	1 pat	4	143	0.2	16	8
Strawberries, raw	1 cup, halves	0.5	24	0.5	0	14
Cranberry juice cocktail	1 fl oz	8	144	0.3	0	3
Lettuce, red leaf, raw	1 cup shredded	2	9	0.7	0	38
Tomatoes, red, ripe, raw, year and average	1 wedge	2	11	0.6	0	17
Carrots, raw	1 cup grated	0.25	11	0.3	0	17
Salad dressing, Italian dressing, comm, reg	1 tbsp	3	106	0.2	9	5
Cheese pizza – low protein[c]	1 pizza	0.5	150	0.5	4	22
Applesauce, cnd, unswtnd, w/vit C	1 cup	1	102	0.4	0	12
Loprofin rice – low protein[b]	0.75 cup (Dry)	1	205	0.2	1	8
Vegetable oil	1 Tbsp	3	371	0.0	42	0
Green sweet peppers, raw	1 cup, sliced	0.5	9	0.4	0	42
Onions, sweet, raw	1 onion	0.25	26	0.7	0	20
Celery, raw	1 stalk, large	1	10	0.4	0	13
Popsicle twin	1 piece	1	60	0.0	0	0
Total			2,128	85.7	81	247

[a]Diet analysis of a diet for MPKU containing approximately 250 mg of phenylalanine
[b]Nutricia North America (Rockville, MD; nutricia-na.com)
[c]Cambrooke Therapeutics (Ayer, MA; cambrookefoods.com)

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Homocystinuria: Diagnosis and Management

14

Janet A. Thomas

Contents

Core Messages

- Homocystinuria is caused by a deficiency in the enzyme, cystathionine-β-synthase (CBS), and results in the accumulation of homocysteine and methionine.
- Homocystinuria is a multisystem disorder with significant morbidity and mortality if untreated.
- The goal of therapy is the reduction of total homocysteine levels.
- Treatment is multifaceted with dietary restriction of methionine and supplementation with betaine, B_6, B_{12}, and folate.
- Outcome is improved with early diagnosis via newborn screening and treatment.

14.1 Background

Homocystinuria (OMIM# 236200) was first reported in 1962 by Carson, Neill, and colleagues [2]. Two years later, the enzymatic defect was identified [5]. The incidence of homocystinuria is reported as quite variable anywhere from 1 in 50,000 to 1 in a million with an overall incidence estimated to be approximately 1 in 200,000 to 300,000 [6–8].

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Homocystinuria is an autosomal recessive condition caused by a deficiency of the enzyme, cystathionine-β-synthase (CBS), which results in the accumulation of homocysteine and methionine and a deficiency of cystathionine and cysteine. There are other disorders to consider when an elevated homocysteine concentration is identified. These disorders include vitamin B_{12} uptake or activation defects, which may or may not have associated elevated methylmalonic acid, severe 5,10-methylenetetrahydrofolate reductase deficiency, and 5-methyl-THF-homocysteine methyltransferase deficiency. The latter two are typically associated with an elevated homocysteine, but low methionine concentrations, so it is relatively easy to discriminate these conditions from homocystinuria. It is also important to consider that nongenetic causes of hyperhomocysteinemia exist, such as dietary deficiencies, end-stage renal disease, and administration of several drugs [6].

Pyridoxine (vitamin B_6) is a cofactor for the enzyme, cystathionine-β-synthase. Hence, two forms of homocystinuria are characteristically described: one form in which individuals are responsive to treatment with vitamin B_6 (B_6-responsive homocystinuria) and another form in which individuals are not (B_6-nonresponsive homocystinuria). Pyridoxine-responsive patients always have some residual enzyme activity [7]. Homocystinuria is diagnosed via newborn screening. Although tandem mass spectrometry (MS/MS) is more sensitive for identifying elevated methionine concentrations than past methods, B_6-responsive patients may still be missed on newborn screening [7].

14.2 Biochemistry

Homocysteine is an intermediate metabolite generated during the metabolism of methionine, an essential sulfur-containing amino acid. The biochemical pathways involved in homocystinuria perform two important processes: transsulfuration and remethylation (Fig. 14.1).

Transsulfuration is facilitated by the action of two vitamin B_6-dependent enzymes, cystathionine-β-synthase (CBS), the enzyme deficient in homocystinuria, and cystathionine-γ-lyase (CTH). CBS catalyzes the condensation of homocysteine and serine to cystathionine, and CTH subsequently catalyzes the hydrolysis of cystathionine to cysteine and α-ketobutyrate. Cysteine is important in protein synthesis and taurine synthesis and is a precursor to glutathione, a strong antioxidant and essential compound in detoxification of many xenobiotics [8, 10, 11].

The remethylation cycle allows the conversion of homocysteine back to methionine by two pathways. The first and major pathway is catalyzed by the enzyme, methionine synthase, and links the folate cycle with homocysteine metabolism. Methionine synthase requires the cofactor, methylcobalamin. The second pathway utilizes the enzyme, betaine-homocysteine methyltransferase [8]. This pathway remethylates homocysteine using a methyl group derived from betaine, formed via oxidation of choline, and is presumably responsible for up to 50 % of homocysteine remethylation [10]. Both methionine and homocysteine play important roles in protein synthesis, folding, and function.

14.3 Clinical Presentation

Homocystinuria involves four major organ or body systems (Box 14.2).

14.3.1 Eyes

Ectopia lentis is often the first sign recognized in an undiagnosed patient and is usually present between 5 and 10 years of age [6, 7]. Classically, the lens dislocates downwards, in contrast to Marfan syndrome, a condition often considered in the differential diagnosis of homocystinuria, where the lens classically dislocates upwards. Exceptions occur. Other eye findings may include retinal detachment, optic atrophy, and cataracts.

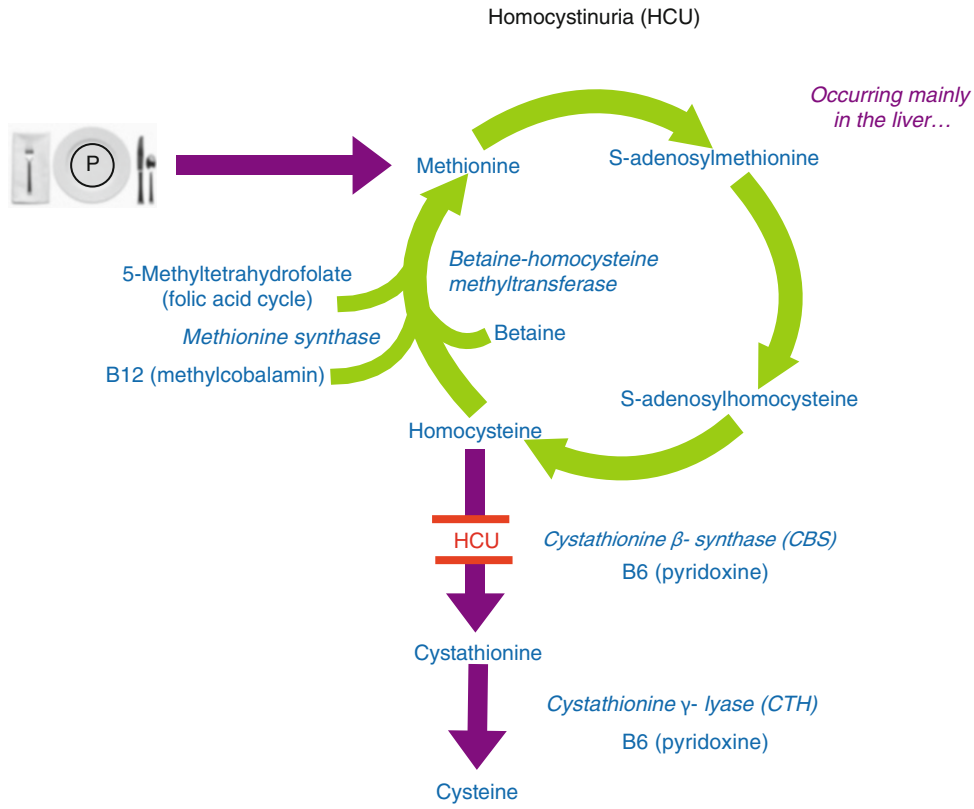

Fig. 14.1 Transsulfuration and remethylation in the biochemical pathway of homocystinuria

Box 14.1: Principles of Nutrition Management of Homocystinuria
Restrict: Methionine
Supplement: Vitamins B_6[1] and B_{12}, folate, L-cystine, and betaine
Toxic metabolite: Homocysteine

14.3.2 Skeletal

The skeletal system is also characteristically involved and the features quite prominent. Individuals with homocystinuria are frequently, but not always, of tall stature with long extremities

[1] Some individuals are pyridoxine responsive and require no other treatment.

Box 14.2: Organ Systems Involved in Homocystinuria
- Eye
  - Ectopia lentis (dislocated lens), myopia, glaucoma, retinal detachment, optic atrophy, and cataracts
- Skeleton
  - Osteoporosis, scoliosis, fractures, tall stature and long extremities, genu valgum, pes cavus, pectus, and restricted joint mobility
- CNS
  - Intellectual disability, seizures, and psychiatric disease
- Vascular
  - Thromboembolic disease, thrombophlebitis, pulmonary embolism, and ischemic heart disease

and long appearing fingers and toes. They are frequently described as having a marfanoid habitus, and hence, homocystinuria should be considered in any individual being evaluated for tall stature and/or Marfan syndrome. Osteoporosis is almost invariably detected after childhood with a tendency to fracture. Other skeletal features include scoliosis, genu valgum (knocked kneed), pes cavus (high instep), pectus carinatum or excavatum, and restricted joint mobility [7]. Notably, there is a significant connective tissue component in the clinical features of individuals with homocystinuria.

14.3.3 Central Nervous System

Developmental delay and mental retardation affect about 60 % of patients to a variable degree [6]. Seizures, EEG abnormalities, and psychiatric disease are also reported. Psychiatric symptoms, such as schizophrenia, depression, and personality disorder, were observed in more than half in one series of 63 patients [16]. Focal neurologic signs may be seen as a consequence of a thromboembolic event [6].

14.3.4 Vascular System

The largest cause of morbidity and mortality comes from involvement of the vascular system, particularly from thromboembolic events which can occur in both arteries and veins and in all sizes of vessels [6]. Thrombophlebitis and pulmonary embolism are the most frequent vascular accidents, whereas thromboses of large and medium arteries, especially carotid and renal arteries, are frequent causes of death [7]. Ischemic heart disease is less common. Neuroimaging may demonstrate evidence of infarction or thrombosis. Association with other genotypes linked to increased risk of vascular disease, such as factor V Leiden and thermolabile methylenetetrahydrofolate reductase, may increase the risk of thrombosis in individuals with homocystinuria [17, 18].

14.3.5 Other

Spontaneous pneumothorax, pancreatitis, lower gastrointestinal bleed, and spontaneous perforation of the small bowel are rare findings reported in homocystinuria [19–21]. In addition, acute liver failure with neurologic involvement has also been reported [22, 23].

14.4 Natural History

At birth, individuals with homocystinuria appear normal, typically without symptoms in the newborn period or early childhood. This feature makes homocystinuria an excellent candidate condition for newborn screening. Undiagnosed, the condition is progressive with involvement of the eyes, skeleton, central nervous system, and vascular system over time. The spectrum of clinical abnormalities is broad. Treated, however, risks of the complications can be reduced significantly, likely directly related to the reduction in total homocysteine.

Time to event curves, based on detailed information on 629 patients, were calculated by Mudd et al. for the main clinical manifestations of homocystinuria [24]. The data demonstrated that the risk for a vascular event was 25 % by age 16 years and 50 % by age 30 years for both B_6-responsive and B_6-unresponsive forms of homocystinuria (Fig. 14.2). Of the patients in whom events occurred, 51 % had peripheral vein thrombosis (with 25 % having pulmonary embolism), 32 % had cerebral vascular accidents, 11 % had peripheral arterial occlusion, 4 % had myocardial infarction, and 2 % had other ischemic events [24].

The data by Mudd et al. also demonstrated that ectopia lentis occurred by age 6 years in 50 % of patients with B_6-unresponsive homocystinuria and by age 10 years in B_6-responsive disease [24]. Eighty-six percent of patients with homocystinuria were ascertained on the basis of ectopia lentis. Finally, the time to event curves demonstrated a 50 % occurrence of radiographic spinal osteoporosis by approximately age 16 years.

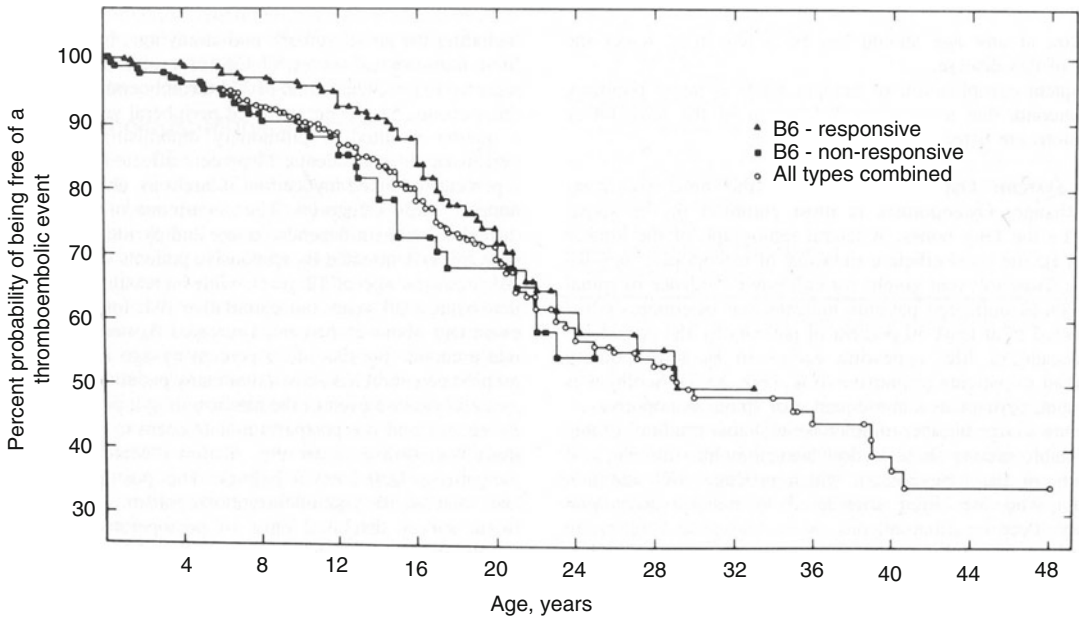

Fig. 14.2 Time to event for initial thromboembolic event in untreated patients

It is notable that the aforementioned natural history study that resulted in the data from which the time to event graphs were calculated was published in 1985, and advances in therapy as well as newborn screening have subsequently occurred. Hence, it is probable that the natural history of homocystinuria has changed. A comprehensive natural history study of homocystinuria has not been repeated. New reports, however, suggest that many individuals with homocystinuria may be asymptomatic or may present only with vascular disease later in life [25–27]. Population studies using known common mutations increase the estimate of disease frequency. There are, however, fewer known patients with homocystinuria than would be suggested by known gene mutation rates [25, 26]. This suggests that many patients may be asymptomatic. This also suggests that perhaps the older data represents an ascertainment bias for the natural history of homocystinuria.

14.5 Diagnosis

The diagnosis of homocystinuria is based on the recognition of the clinical phenotype in conjunction with the identification of an elevated total plasma homocysteine and elevated plasma methionine concentrations (via quantitative plasma amino acid analysis). Low cystine and low cystathionine are also seen (Box 14.3). In addition, increased urinary excretion of homocysteine as well as cysteine-homocysteine disulfide can be identified on urine amino acid analysis. Confirmation of the diagnosis can be done via enzyme assay, typically performed on cultured skin fibroblasts, lymphocytes, or liver tissue, or via molecular studies.

14.6 Pathophysiology

The pathophysiology of homocystinuria appears to be highly complex and is incompletely understood. Much of the pathophysiology is likely due

Box 14.3: Biochemical Features of Untreated Homocystinuria

Disorder	Methionine	L-cystine	Total homocysteine	Cystathionine
Homocystinuria (CBS deficiency)	↑	↓	↑↑↑	↓

to accumulating homocysteine. Homocysteine, itself, is a known risk factor for early atherosclerosis [28, 29]. It is known that homocysteine-induced abnormalities of platelets, endothelial cells, and coagulation factors contribute to the hypercoagulable state and/or altered stability of the arterial walls seen in this condition [6, 29]. In addition, there is also evidence that homocysteine enhances smooth muscle proliferation [30] and may enhance oxidative damage with decreased oxidative antioxidant defenses resulting in DNA damage [31–33]. Hence, there is good evidence to suggest that elevated homocysteine contributes to the thromboembolic events that complicate homocystinuria. Further, it is known that lowering the homocysteine concentration reduces the risk of such events from occurring. Homocysteine is also known to disrupt collagen cross-linking which may contribute to the skeletal and connective tissue features of this condition [6].

Cysteine is also an important feature of collagen, and hence, a reduction in available cysteine results in weakened collagen. Weak collagen then contributes to the clinical features of lens subluxation, osteoporosis, and skeletal features such as pectus excavatum and marfanoid appearance [34]. In addition, disruption of disulfide bonds by the formation of homocysteine-cystine mixed disulfides in fibrillin, a protein important in the lens of the eye, may contribute to the feature of ectopic lentis [35]. Other features may also play a role in the pathophysiology of homocystinuria. For example, Keating et al. recently demonstrated evidence of chronic inflammation suggesting that aberrant cytokine expression may be contributing to the pathogenesis of the disease [36], and there is continued controversy as to the role played by altered lipid metabolism [37].

Finally, several hypotheses have been proposed to explain the neurological manifestations seen in individuals with homocystinuria. Orendác et al. proposed a decrease in serine concentration, secondary to an increased remethylation rate, as the cause due to serine's role in the synthesis of myelin [23, 38]. Mudd et al. suggested that the altered S-adenosylmethionine to S-adenosylhomocysteine ratio inhibits transmethylation reactions, including myelin synthesis, contributing to the neurologic manifestations [23, 35].

14.7 Management

The goal of the management of homocystinuria is to reduce or normalize plasma homocysteine concentrations. Management is multifaceted and necessarily individualized and is understandable when one considers the biochemical pathway. Following diagnosis, all patients with homocystinuria require a trial of B_6. It is estimated that ~50 % of patients with homocystinuria are responsive or partially responsive to B_6 [6]. Responsiveness is chiefly determined by the individual's underlying mutations. Doses of B_6 vary greatly, typically beginning at 100 mg/day and progressively increasing to 500–1,000 mg/day pending response. Doses higher than 1,000 mg/day should be avoided due to an association with sensory neuropathy [39]. In responsive patients, the dose of B_6 should be kept at the lowest dose able to achieve adequate metabolic control [10]. Total homocysteine concentrations and plasma methionine concentrations can be used to monitor response. Response to B_6 is also influenced by folate depletion; thus, folic acid (5–10 mg/day) or folinic acid (1–5 mg/day) should be given [6, 10]. Low doses of B_6 (50–200 mg/day) are often continued even in those patients determined not to be B_6 responsive due to its role as a cofactor for cystathionine-β-synthase [10, 40].

Box 14.4: Initiating Nutrition Management in a Patient with B_6-Nonresponsive Homocystinuria

Goal: Reduce or normalize plasma homocysteine.

Step-by-Step:

1. Restrict methionine using a methionine-restricted medical food [1].
2. Supply adequate cystine (from medical food and/or supplements) [1].

Age	Methionine (mg/kg/d)	Cystine (mg/kg/d)
0–6 mo	15–60	85–150
6 mo-1 year	12–43	85–150
1–4 yr	9–28	60–100
>4 yr- adults	5–22	20–80

3. Provide sufficient protein: 120–140 % DRI for protein is often used when medical food containing L-amino acids as protein source is used [3, 4]
   (ex. infant DRI for protein: 2.2 g/kg/d × 120–140 % = 2.6 – 3.1 g/kg/d).
4. Provide adequate energy to meet the DRI and fluid to meet normal requirements [9].
5. Ensure adequate intake of vitamins and minerals to meet DRI, except where noted below:[a]
   Folic acid (5–10 mg/d) or Folinic acid (1–5 mg/d) [5, 8]
   Vitamin B_6 (50–100 mg/d) [10]
   Vitamin B_{12} (varies)
   Vitamin C (1 g/d) [5, 11]
6. Other supplements:
   [b]Betaine (150–250 mg/kg/d divided into 3 doses); 6–9 g/d in adults [12–14].

[a] Vitamin supplementation amounts included here have been reported; variation in practice exists [15]. Some centers supplement routinely and others only if blood concentrations are low.

[b] Age of patient and clinical condition are considered by medical team to determine when and how much betaine to supplement.

For individuals who do not respond to B_6, a methionine-restricted diet is necessary (Box 14.4). Synthetic methionine-free amino acid medical foods are commercially available. The natural requirement for methionine is met by dietary intake of regular foods or, in infancy, standard infant formulas. The diet is restrictive and requires the use of low-protein products to be truly successful. Cystine is prescribed as necessary to obtain normal cysteine concentrations. Small amounts of vitamin B_{12} may aid in the remethylation of homocysteine to methionine due to its use as a cofactor by methionine synthase [10, 40].

The other mainstay of therapy is the use of betaine (N,N,N-trimethylglycine) [14]. It is often used in conjunction with a methionine-restricted diet and can improve metabolic control even in individuals with optimal diet control [12, 13]. Betaine is a substrate for the enzyme, betaine-homocysteine methyltransferase, and works to remethylate homocysteine to methionine which consequently lowers homocysteine concentrations but raises methionine concentrations. Moderately elevated methionine concentrations do not appear to have physiological consequences; however, concentrations >1,000 nmol/ml have been associated with cerebral edema [41, 42]. Hence, high concentrations of methionine >1,000 nmol/ml should be avoided. Betaine is given orally, typically at doses of 150–250 mg/kg/day divided three times daily (6–9 g/day for adults; up to 20 g/day) [6, 10].

The decision of what modality to begin first, diet versus betaine, is often at the discretion of

the treating physician. Unfortunately, achievement of normal total homocysteine concentrations, even with a combination of therapies, is very difficult in most patients. Prevention of long-term consequence of homocystinuria requires lifelong therapy.

Additional management recommendations vary and remain to be proven. Considerations include a daily aspirin, other antiplatelet aggregation medications (dipyridamole), or anticoagulation therapy, all used to reduce hypercoagulability and thromboembolic risks, and vitamin C supplementation (1 g/day) to ameliorate endothelial dysfunction [6, 11]. Liver transplantation has been reported as treatment for homocystinuria in two individuals [22, 43]. To further reduce thromboembolic risk, it is important to ensure adequate hydration during times of illness or surgery and to avoid long periods of sitting or inactivity. These considerations are likely most important in an individual with elevated homocysteine concentrations. Management should also include a frequent discussion of the signs and symptoms of potential complications, such as stroke, deep vein thrombosis, and pulmonary embolism, with the patient, family, or care providers. Ongoing ophthalmalogic care is important.

Further, if surgery is required for an individual with homocystinuria, it is recommended that dextrose-containing intravenous fluids be started preoperatively and continued throughout the procedure to maintain circulating fluid volume and avoid hypoglycemia. Nitrous oxide should be avoided as postoperative cardiac ischemic episodes have been reported after its administration, and use may increase the risk of vascular thrombosis and raise homocysteine concentrations [34, 44–46]. Regional anesthetic techniques may be contraindicated: nerve blocks may be complicated by damage to adjacent blood vessels with the potential for vascular thrombosis, and spinal or epidural analgesia may lead to vascular stasis [34]. Surgical management may also include elastic stockings and intermittent foot compression with a pneumatic system to aid in the prevention of thromboembolism [46, 47].

Box 14.5: Nutrition Monitoring of a Patient with Homocystinuria

- Routine assessments including anthropometrics, dietary intake, and physical findings (Appendix F).
- Laboratory monitoring:
  - Diagnosis specific
    - Plasma amino acids:
      - Methionine (goal <1,000 μmol/L), others within normal limits
      - Total homocysteine (goal <50 μmol/L)
      - Cystine (goal normal levels)
  - Nutrition-related laboratory monitoring of patients on a methionine-restricted diet may include markers of:
    - Protein sufficiency[a] (plasma amino acids, prealbumin)
    - Nutritional anemia (hemoglobin, hematocrit, MCV, serum vitamin B_{12} and/or methylmalonic acid, ferritin, iron, folate, total iron-binding capacity)
    - Vitamin and mineral status (25-hydroxy vitamin D, zinc, trace minerals)

[a] Further described in Chap. 7.

14.8 Monitoring and Outcome

Monitoring of an individual with homocystinuria includes the responsiveness to therapeutic interventions as well as monitoring for potential complications. In addition, a patient on a methionine-restricted diet should have consistent monitoring of laboratory values (Box 14.5).

The outcome of homocystinuria has improved with current therapeutic regimes and with early diagnosis via newborn screening [48]. The prognosis is directly associated with the occurrence of vascular ischemia since, as noted, the majority of morbidity and mortality are associated with thromboembolic events. Outcome is also

determined by B_6 responsiveness with B_6-responsive patients having an improved prognosis [7, 24]. Historically, almost 25 % of individuals with homocystinuria died before the age of 30 years most commonly from thromboembolism. Lowering homocysteine concentrations significantly reduces the risk of vascular events [12, 49]. Therapy with betaine has contributed to the ability to lower homocysteine concentrations adequately and improve prognosis. Initiation of therapy in the newborn period appears to reduce the incidence of mental retardation, delay the start and progression of lens dislocation, and reduce the incidence of seizures and thromboembolic events [24, 48, 49]. Family and social support is imperative for successful management and optimal outcome.

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Nutrition Management of Urea Cycle Disorders

15

Fran Rohr

Contents

Core Messages

- Urea cycle disorders (UCD) differ widely in their presentation and severity.
- Correcting hyperammonemia is the priority in treating UCD.
- Dietary protein is restricted in UCD. The amount of protein provided as whole protein versus medical food protein (essential amino acids) varies.
- Preventing catabolism by providing sufficient energy is a critical part of nutrition management.
- Medications that remove nitrogen by alternative pathways help to prevent hyperammonemia and increase protein tolerance.
- Outcomes are guarded and depend on severity of the disease.
- Liver transplantation is recommended for infants with severe forms of the disorder.

15.1 Background

A urea cycle disorder is caused by a deficiency in any one of six enzymes in the urea cycle [1] (Fig. 15.1). Collectively, urea cycle disorders (UCD) are relatively common, with an incidence of 1:35,000 births [2]. The primary function of the urea cycle is to remove nitrogen produced

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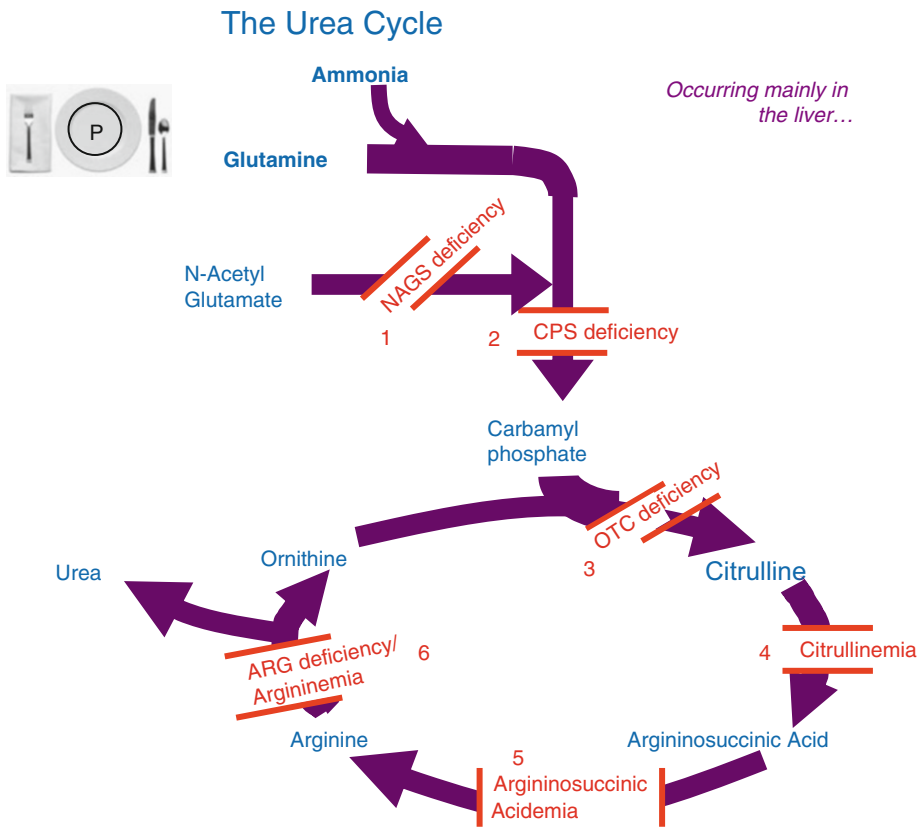

Fig. 15.1 Metabolic pathway of urea cycle disorders
The urea cycle contains six enzymes:
1. *NAGS* N-acetylglutamate synthase – activates CPS
2. *CPS* Carbamoyl phoshate synthetase – adds bicarbonate to ammonia along with a phosphate group to form carbamoyl phosphate, starts the urea cycle
3. *OTC* Ornithine transcarbamylase – combines carbamoyl phosphate with ornithine to produce citrulline
4. *ASS* Arginiosuccinate synthetase – combines citrulline and aspartate to form arginosuccinic acid
5. *ASL* Argininosuccinate lyase – breaks down argininosuccinic acid into arginine and fumarate
6. *Arginase* Cleaves arginine to form urea and ornithine which then feeds back into urea cycle

from amino acid metabolism so that it does not accumulate as ammonia. Waste nitrogen is produced when protein intake exceeds the amount needed for protein synthesis or when endogenous protein stores are broken down to produce energy (catabolism). Nitrogen is cleaved from an amino acid, and the remaining molecule is used as a source of energy (if needed) or stored as fat (if not needed). Excess nitrogen is normally converted to ammonia which enters the urea cycle and, through a series of enzymatic reactions, is converted to urea and excreted.

Ammonia is neurotoxic [3, 4]. The pathophysiology of UCD and the cause of neurotoxicity are complex. It involves not only ammonia but also glutamine and other pathways involving neurotransmitters, nitrogen oxide, and ion channels [5, 6]. The acute effects of hyperammonemia include poor feeding, vomiting, seizures, and lethargy that can rapidly progress to coma and death. Chronic effects of milder elevations of ammonia are less well understood, but may be a cause of impaired neurocognition seen in children with UCD [7]. The potential consequences of increased ammonia concentrations are presented in Table 15.1.

Table 15.2 presents the six enzymes of the urea cycle and the disorder associated with a

deficiency of each. UCDs that are at the beginning of the urea cycle (NAGS, CPS, OTC) tend to be associated with marked elevations in ammonia, where UCDs that are more distal (ASS, ASL, ARG) are less likely to have severe hyperammonemic episodes associated with them; however, all individuals with UCDs are at risk of developing hyperammonemia, especially if stressed by infection and/or poor energy intake leading to catabolism of endogenous protein [9]. UCD can be differentiated on the basis of whether citrulline is low (OTC and CPS), elevated (ASS and ASL), or normal (ARG).

Table 15.1 Ammonia concentrations and potential consequences

Ammonia concentration		
μmol/L	mcg/dL	Interpretation and Symptoms
<35	<60	Normal concentration[a]
36–60	60–100	Mild elevation; not always associated with symptoms
61–200	150–350	Elevation: poor feeding, vomiting, irritability, lethargy, confusion
>250	>350	Hyperammonemic crisis; potentially leading to coma

Caveat: Norms for ammonia concentration and units used (mcg/dL or μmol/L) may vary according to laboratory used. Individual symptoms may vary – some patients are more sensitive to elevations in ammonia than others. Therefore, treatment should not be based on ammonia concentration alone, but will include patient history, clinical, and laboratory assessments

[a]In newborns normal ammonia concentrations are 64–107 μmol/L (90–150 mcg/dL) and in infants age 0–2 weeks 56–92 μmol/L (79–129 mcg/dL) [8]

UCD can present at any age [1, 10]. Typically, a neonate with a severe form of UCD will present with rapidly progressive symptoms of hyperammonemia. In infants and children, presenting symptoms may include failure to thrive, cyclic vomiting, liver dysfunction, seizures, and developmental delay [1, 11]. Children and adults may present after the newborn period and have a more mild course [10]. In some cases, adults are diagnosed with UCD after an encephalopathic crises following catabolic stress, including infection, surgery, and the postpartum period [12]. In addition, approximately 15 % of females who are carriers for OTC deficiency are symptomatic and require treatment [1]. Adolescents and adults who are diagnosed with a UCD often have had history of chronic neurological and/or psychiatric symptoms [1] as well as a diet history indicating avoidance of high-protein foods.

Not all individuals with UCD come to attention clinically. Some may be identified through newborn screening (NBS) [2]. NBS detects high concentrations of metabolites in the blood; therefore, ASS and ASL can be identified because these disorders result in increased concentrations of citrulline and arginase deficiency because of high concentrations of arginine. However, OTC, the most common UCD, is not identified through NBS because in OTC the metabolite citrulline is lower than normal. For individuals who come to attention through NBS, the challenge is to deter-

Table 15.2 Urea cycle disorders, associated enzymes, and altered laboratory values

Disorder	Enzyme	Abbreviation	Ammonia concentration	Citrulline concentration
NAGS deficiency	N-acetylglutamate synthetase	NAGS	Markedly elevated	Absent to low
CPS deficiency	Carbamoyl phosphate synthetase	CPS	Markedly elevated	Absent to low
OTC deficiency	Ornithine transcarbamylase[a]	OTC	Markedly elevated	Absent to low
ASS deficiency; citrulliinemia I	Argininosuccinic acid synthetase	ASS	Elevated	Elevated
ASL deficiency; argininosuccinic aciduria (ASA)	Argininosuccinic acid lyase[b]	ASL	Elevated	Elevated
Arginase deficiency; argininemia	Arginase	ARG	Rarely elevated	Normal

[a]Urine orotic acid is also present and is pathognomonic for OTC deficiency

[b]Urine and plasma argininosuccinic acid are elevated in ASL deficiency

mine how aggressively to treat infants who are asymptomatic. Algorithms for guidance in diagnosing specific UCD are available [1].

Overall, outcomes in UCD are improving due to newborn screening for several disorders, advances in medications and the diet, and liver transplantation [13, 14]. Traditionally, however, outcomes have been suboptimal and characterized by early mortality, growth failure, chronic liver disease, and poor development [15]. Outcomes are better for those with late onset (11 % mortality) compared to neonatal onset (24 % mortality) [14]. Because of shortcomings of traditional therapies, liver transplantation is becoming a more viable and attractive option for many patients with UCD [16].

15.2 Nutrition Management

15.2.1 Chronic Nutrition Management

Treatment of UCD includes limiting dietary protein, providing sufficient energy to prevent catabolism, supplementing with specific amino acids, and using nitrogen-scavenging drugs [12, 17–20] (Box 15.1). These strategies are typically used in combination depending on the severity of the disease. In an infant who presents with a severe form of UCD, for example, a male with OTC deficiency, emergency management is indicated [21, 22] (Sect. 15.2.2).

The goals of nutrition management are to prevent the accumulation of ammonia, normalize plasma amino acids, and promote normal growth and development (Box 15.2). The treatment of UCD differs from other metabolic disorders with respect to protein intake. In UCD total protein is limited, unlike in other many metabolic disorders where total protein is not limited, but is provided as medical food without offending amino acid(s). The steps to initiating a diet in a newborn are presented (Box 15.3). Sources of protein in the diet for UCD include whole protein (breast milk or standard infant formula in infancy, baby food, table foods) and medical foods containing essential amino acids.

Box 15.1: Components of Management of UCD

- Limit protein
- Prevent catabolism
- Use nitrogen-scavenging drugs
- Supplement amino acids

Box 15.2: Principles of Nutrition Management in UCD

Restrict: Protein

Supplement: Essential amino acids, arginine in ASS and ASL deficiency, and citrulline in OTC and CPS deficiency

Toxic: Ammonia in all UCD
Argininosuccinic acid in ASL deficiency
Arginine in arginase deficiency

Limit protein. Practice varies widely with respect to the balance between whole protein and medical food protein. Many recommend that approximately half of the total protein allowance be given as essential amino acids and half as whole protein [25]. Others suggest that protein be limited to the WHO recommendation [26] and 20–30 % of protein requirement be given as essential amino acids [12]. In some centers, protein restriction alone (without the use of medical foods containing essential amino acids) is used [19, 27]. However, protein restriction without essential amino acid supplementation may lead to chronic protein insufficiency [28]. Once the diet goals are established, the amount of medical food and the amount of standard infant formula (or breast milk) necessary to meet these goals are calculated. Medical foods for the treatment of UCD are listed in Table 15.4. They provide essential amino acids as the protein source but differ in energy, vitamin, and mineral profiles.

Providing sufficient calories often necessitates the use of special protein-free medical foods, such as Pro-Phree® (Abbott Nutrition, Columbus, OH), PFD® (Mead Johnson, Glenview, IL), Polycal® (Nutricia North America, Gaithersburg, MD),

Box 15.3: Initiating Nutrition Management in an Asymptomatic Infant with UCD

Goals:

- Prevent hyperammonemia.
- Normalize plasma amino acids.
- Promote normal growth and development.

Step-by-Step:

1. Establish goals for total protein intake and the percent of protein to be supplied as medical food (essential amino acids) (Table 15.3).
2. Determine the amount of medical food needed to meet goal in step 1 and which medical food to use.
3. Determine the amount of whole protein needed to meet the protein goal in step 1 and whether the source will be breast milk or standard infant formula.
4. Determine if DRI for energy is met by the combination of medical food and standard infant formula/breast milk. If not, add a protein-free energy source to meet needs.
5. Determine how much water to add to make a volume of formula that will meet the infant's fluid needs and have a caloric density of 20–25 kcal/oz.
6. In consultation with metabolic team, determine the amount of supplemental citrulline or arginine needed.

See Sect. 15.6 for diet calculation examples

Table 15.3 Recommended nutrient intakes for patients with UCD. Intakes for energy, vitamins, and minerals should meet the DRI [23] and fluid maintenance requirements [24]

Age (year)	Total protein (g/kg/day)	Protein from medical food (essential amino acids) (g/kg/day)	Whole protein (g/kg/day)
0–1	1.2–2.2	0.6–1.1	0.6–1.1
1–7	1.0–1.2	0.6–0.7	0.4–0.5
7–19	0.7–1.4	0.4–0.7	0.3–0.7
>19	0.5–1.0	0.3–0.5	0.2–0.5

Adapted from Singh [25]

Table 15.4 Energy and protein content of medical foods (essential amino acids) for the treatment of UCD (per 100 g powder)

Medical food	Energy (kcal)	Protein (g)
Cyclinex®-1 [a]	510	7.5
Cyclinex®-2 [a]	440	15
EAA Supplement™ [b]	288	40
Essential Amino Acid Mix[c]	316	79
Milupa® UCD 2[c]	290	67
UCD Anamix® Junior[c]	385	12
UCD Trio™ [b]	393	15
WND® 1[d]	500	6.5
WND® 2[d]	410	8.2

[a]Abbott Nutrition (Columbus, OH; abbottnutrition.com)
[b]Vitaflo USA (Alexandria, VA; vitaflousa.com)
[c]Nutricia North America (Rockville, MD; nutricia-na.com)
[d]Mead Johnson Nutrition (Evansville, IN; meadjohnson.com)

Duocal® (Nutricia North America, Gaithersburg, MD), or SolCarb® (Solace Nutrition, Pawcatuck, CT). For toddlers and older patients with UCD, low-protein foods are an important energy source.

Prevent Catabolism. Catabolic stress is a major source of waste nitrogen. Episodes of hyperammonemia are often precipitated by an acute infectious illness coupled with poor feeding. It can be challenging to provide sufficient calories to patients with UCD because they often have very poor appetites. The reason for anorexia in UCD may be twofold. One theory is that elevated blood glutamine concentrations often seen in sick patients with UCD cause high brain glutamine concentrations. In the brain, glutamine is a carrier for tryptophan, which is a precursor for serotonin, the neurotransmitter that is associated with a feeling of satiety [29]. It is also likely that patients with UCD have food aversions because they have been conditioned to associate intake of high-protein foods with episodes of vomiting, headaches, and/or lethargy. Also, patients with UCD have been shown to have higher than normal concentrations of the hormone peptide tyrosine tyrosine (also referred to as peptide YY or PYY) that is associated with feeling of satiety [30]. Half of the patients with UCD in one study had feeding

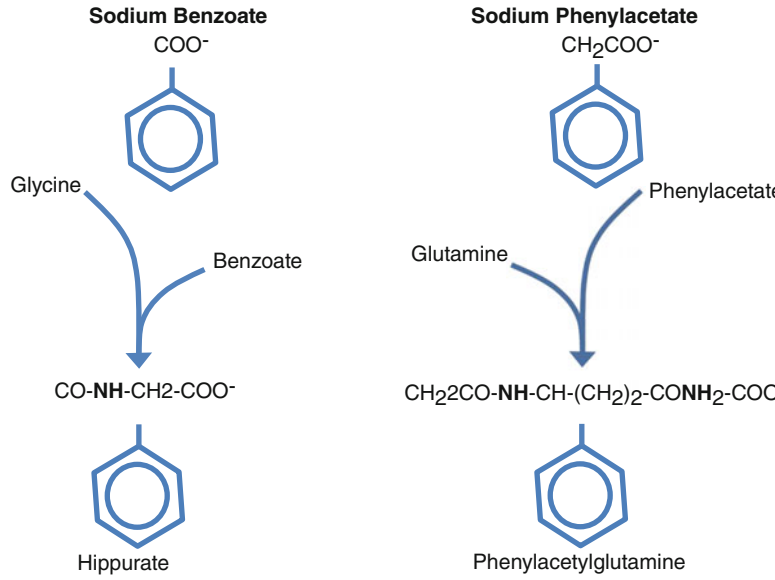

Fig. 15.2 Alternate pathways for the removal of nitrogen using sodium benzoate or sodium phenylacetate. Sodium phenylbutyrate (Buphenyl®), and glycerol phenylbutyrate (Ravicti®) are both converted to sodium phenylacetate in the liver

problems including poor appetite, food refusal, protein aversion, or vomiting [31]. It is common for children with UCD, especially those with severe form, to require nasogastric or gastrostomy tubes (G-tubes) in order to provide sufficient calories. G-tubes are especially helpful for providing medications and extra calories, especially during illness when appetites may be further diminished.

Use of Nitrogen-Scavenging Drugs. Although there are several scavenging drugs available, they remove nitrogen by one of two pathways (Fig. 15.2). Sodium benzoate binds with glycine and forms hippurate and is excreted. This reaction removes one nitrogen atom. Similarly, in the second reaction, sodium phenylacetate binds with glutamine and forms phenylacetylglutamine that is excreted. In this reaction, two nitrogen atoms are bound and excreted. Sodium phenylacetate is available as Buphenyl® (Hyperion Therapeutics Inc., Brisbane, CA). Glycerol phenylacetate (Ravicti®, Hyperion Therapeutics Inc.) works by the same mechanisms as sodium phenylacetate, and some find it easier to administer because the dose is lower and the taste is better than sodium phenylacetate [32]. Ammonul® (Ucyclyd Pharma, Inc., Scottsdale, AZ) is an IV form of nitrogen-scavenging medication that contains a combination of sodium phenylacetate and sodium benzoate.

Carbaglu®(Orphan Europe SARL, Paris, France) is a medication for treating deficiency of the first enzyme of the urea cycle, NAGS deficiency, but it is not a nitrogen-scavenging drug. Carbaglu® is chemically similar to N-acetylglutamine, which activates CPS. In a study of 20 patients receiving Carbaglu®, 12 had NAGS deficiency and their hyperammonemia resolved [20]. Carbaglu® is under investigation for treatment of secondary hyperammonemia in organic acidemias.

Supplement Amino Acids. For all UCD except arginase deficiency, arginine becomes an essential amino acid. Arginine or citrulline supplements are given to replace the arginine that is normally produced by the urea cycle. Often, L-arginine is used in ASS and ASL deficiency, whereas L-citrulline is used in CPS and OTC deficiency because it has the advantage of incorporating aspartate into the pathway and removing one additional nitrogen molecule [33]. The goal in supplementing amino acids is to keep plasma concentrations normal, and the doses vary as higher amounts are used in acute illness [21]. Traditionally for ASS and ASL deficiency, doses have ranged from 0.4 to 0.7 g/kg/day; in ASL there is evidence that lower-dose arginine supplementation (0.1 g/kg/day) results in less accumulation of ASA and perhaps improved outcome since ASA may contribute to the liver and neurological disease [34] that are often complications of ASA. The typical maintenance dose of citrulline in OTC and CPS is 170 mg/kg/day.

15.2.2 Acute Nutrition Management

15.2.2.1 Nutrition Management During Hospitalization

A hyperammonemic crisis is treated as a medical emergency whether it is in a sick neonate or an older child with an acute illness. All patients should have an emergency department protocol (http://newenglandconsortium.org/for-professionals/acute-illness-protocols/urea-cycle-disorders/) to ensure they receive prompt and proper treatment. If hospitalized, protein feeds should be discontinued and IV access obtained for administration of nutrition support and medications including the nitrogen-scavenging drug, Ammonul®(Ucyclyd Pharma, Inc., Scottsdale, AZ) and arginine. In severe cases of hyperammonemia, dialysis is usually required to normalize ammonia concentrations. Often patients are not able to tolerate enteral feedings, and parenteral nutrition (PN) is required. The PN solution focuses on providing as much energy as possible by using 20 % dextrose solution and 2–3 g/kg Intralipid® and eliminating or severely restricting protein intake (Box 15.4). If the patient is to be dialyzed, a small amount of an amino acid solution may also be given, since dialysis will remove amino acids as well as ammonia, and the infant runs the risk of becoming catabolic. PN solutions that contain essential amino acids, such NephrAmine® (B. Braun Medical, Inc., Bethlehem, PA), may be used alone or in combination with standard PN solutions to provide a higher percentage of essential amino acids.

In the patient who is not dialyzed, protein should be limited for no more than 24–48 h to prevent further catabolism [21]. Some advocate providing essential amino acids even during an acute illness in order to prevent branched-chain amino acid deficiency that can occur with hyperammonemia and be further exacerbated by nitrogen-scavenging medications [28]. There is no consensus about whether protein should be reintroduced in the diet in 24 or 48 hours in acutely ill patients with UCD, and the decision is made by the medical team depending on the patient's ammonia, plasma amino acids, nutritional intake, and neurological status.

Once a neonate who presented with hyperammonemia is stabilized, he or she will usually experience a period of metabolic stability or "honeymoon period" [14]. During this time, the infant is growing rapidly and has a relatively higher protein tolerance. The diet is less complicated in the first few months since solid foods have not been introduced and protein does not yet have to be counted. In addition, the infant has innate immunity and limited exposure to infections.

Box 15.4: Example of Parenteral Nutrition for a Patient with UCD in Hyperammonemic Crisis[a]

Glucose infusion rate: 10–12 mg/kg/min
Intralipid: 2–3 g/kg/day (up to 3–4 g/kg/day in neonate)
Amino acids: (after 24 h or if dialyzed): 0.25–0.5 g/kg/day and advanced as tolerated
Arginine HCl: 210 mg/kg/day

[a]Management in conjunction with metabolic physician

15.2.2.2 Nutrition Management During Illness at Home

Intercurrent illness is the most common cause of hyperammonemia outside of the newborn period, and prompt treatment can prevent catabolism and be lifesaving [14]. Patients should have a MedicAlert® bracelet (MedicAlert, Turlock, CA). Patients with mild illnesses may be managed at home if the metabolic physician assesses that it is safe to do so. In such cases, whole protein intake is reduced or eliminated, depending on how sick the child is and other factors (how well child has managed at home in the past, distance to clinic, etc.). Many patients have sick-day diet prescriptions containing no whole protein or half of whole protein. However, medical food containing essential amino acids is still provided in sick-day formulas, as well as additional energy from fat or carbohydrate. Sick-day diets should be used for no more than 24–48 hours [12, 17]. Protein can be reintroduced slowly, starting with half of whole protein intake for 1 or 2 days and advancing. Because fluid and calorie needs are higher than normal when sick, patients need to eat more when they often feel like eating less. For patients who do not have G-tubes, getting sufficient energy and

fluids by mouth may not be possible, and admission for IV fluids and calories is often needed.

15.3 Monitoring

Nutrition monitoring includes assessment of anthropometrics, dietary intake, and laboratory parameters (Box 15.5). These include measurement of ammonia and plasma amino acids, particularly glutamine, branched-chain amino acids, alanine, and glycine. Glutamine is often a harbinger of high ammonia because it is a reservoir for ammonia (glutamine is synthesized from glutamate and ammonia). Both ammonia and glutamine are markers for neurocognitive outcomes [14]. Branched-chain amino acids, if low (either as a consequence of dietary restriction or Buphenyl® therapy) [35], indicate the need for more protein. This can be given as whole protein (if tolerated), essential amino acids, or specific branched-chain amino acid supplements [35]. Alanine may be elevated if energy intake is low. High glycine concentration is often seen if catabolism occurs [1]. Periodic laboratory monitoring to ensure that the patient is receiving adequate protein, vitamins, and minerals is recommended.

Box 15.5: Nutrition Monitoring of Patients with UCD

- Routine assessments including anthropometrics, dietary intake, and physical findings (Appendix F)
- Laboratory monitoring:
  - Diagnosis specific
    - Ammonia
    - Plasma amino acids, including:
      - Glutamine
      - Arginine (in arginase deficiency)
      - Citrulline (in ASA synthetase deficiency)
      - Argininosuccinic acid (in ASA lyase deficiency)
      - BCAA (often low if on Buphenyl therapy)
  - Nutrition laboratory monitoring of patients on protein-restricted diets may include markers of:
    - Protein sufficiency[a] (plasma amino acids, prealbumin)
    - Nutritional anemia (hemoglobin, hematocrit, MCV, serum vitamin B_{12} and/or MMA, total homocysteine, ferritin, iron, folate, total iron-binding capacity)
    - Vitamin and mineral status (25-hydroxy vitamin D, zinc, trace minerals)
    - Others as clinically indicated

[a]Further described in Chap. 7

15.4 Transplantation

In patients with severe UCD, outcomes are often poor despite treatment, and liver transplantation is often the treatment of choice [27]. The Urea Cycle Consortium recommends that patients with UCD, other than those with NAGS or arginase deficiency, and who have absent or very low enzyme activity be stabilized, aggressively managed, and placed on the liver transplant list as early as is practical [14]. Even in arginase deficiency, liver transplantation has been shown to halt the progression of neurological damage [36]. European guidelines are similar, especially for patients who have severe OTC or CPS1 deficiency [22].

United Network for Organ Sharing data shows there was higher mortality when patients with UCD were transplanted before age 2 years [16]. This may be explained by the fact that historically only the most severely affected patients were referred for transplantation, but current recommendations are for the transplant to occur at the earliest possible time [14, 22]. Transplantation outcomes in patients with UCD have been good. In a study of

23 patients (none of whom had arginase deficiency), there was 100 % survival and 96 % graft survival [37]. Developmental outcomes were stable or improved.

Prior to transplantation, the goal is to keep the patient in good metabolic control and nutritional status. Better surgical outcomes are correlated to pre-transplant weight and protein status both of which can be difficult to attain on a protein-restricted diet. A minimum weight of 5 kg is usually recommended before transplantation can be performed [22]. Maintaining metabolic control is of paramount importance in order to preserve neurocognition because transplantation does not reverse neurocognitive damage [14, 38].

Pre- and postoperative nutrition protocols vary. At one center [37], patients with UCD continue to receive their usual protein-restricted diet up to approximately 6 h prior to surgery. During surgery, they are typically given 10 % dextrose with electrolytes and Intralipid® (2 g/kg/day). After surgery, intravenous amino acids (1.5–2 g/kg/day) are added to the parenteral nutrition. After transplantation, patients with UCD no longer need a protein-restricted diet, medical food, or nitrogen-scavenging drugs [37]. While a normal diet can be followed, it may be difficult for patients who have not had high-protein foods in the past to readily accept unfamiliar foods.

Some patients still require arginine or citrulline supplementation, depending on plasma amino acid profile. Follow-up of liver transplant patients by the metabolic dietitian in collaboration with the transplant dietitian is best. The transplant dietitian can best address issues common to all transplant patients, including possible nutrition-related side effects of antirejection drugs, food safety concerns, and prevention of obesity, which is common in pediatric patients who have undergone liver transplantation [39].

15.5 Summary

The primary function of the urea cycle is to rid the body of waste nitrogen. Deficiency in the activity of any of the six enzymes in the urea cycle may result in the accumulation of ammonia, often to toxic concentrations. Treatment involves restricting protein, preventing catabolism, supplementing amino acids that are normally produced by the urea cycle, and promoting the excretion of nitrogen via alternative pathways. Outcomes are guarded and appear to be better for patients identified by NBS compared to patients identified clinically. Liver transplantation is a treatment option, especially for patients with a severe form of the disorder.

15.6 Diet Calculation Examples

Example 1:
Infant with UCD transitioning from parenteral nutrition (PN) to enteral feeding

Patient information	Nutrient intake goals (per day)
This patient is a 3-week old male with OTC deficiency who presented acutely with hyperammonemia. He has been treated with dialysis, Ammonul and parenteral nutrition. He is currently tolerating 1 g protein PN and is ready to transition to enteral feeding. His current weight is 2.9 kg	**Energy:** 120 kcal/kg **Protein:** 1.5 g/kg (half as whole protein and half as essential amino acids) **Fluid:** 100 mL/kg Recommended caloric density of formula: 20–25 kcal/oz

Steps in diet calculation: This child will be placed on Cyclinex-1 powdered formula. An interactive step-by-step guide of this diet calculation is available at www.imd-nutrition-management.com

Diet prescription summary for sample calculation of UCD diet

Diet	Amount	Protein, total (g)	Protein, whole (g)	Protein, medical food (g)	Energy[a] (kcal)	Fluid[b] (mL)
Goals		4.4	2.2	2.2	348	290
Cyclinex®-1 powder[c]	29 g	2.2	0	2.2	148	
Similac® powder[c]	20 g	2.2	2.2		104	
Pro-Phree® powder[c]	18		0	0	92	
Totals		4.4	2.2	2.2	344	
Add water to make	14–17 oz[d]					420–510

[a]Dietary Reference Intake [37]
[b]Fluid requirement [38]
[c]Abbott Nutrition (Columbus, OH; abbottnutrition.com)
[d]Formula concentration of 20-25 kcal/oz

Example 2:
Child with ASA requiring 2 different types of "sick-day" diets

Patient information	Nutrient intake goals		
This patient is a 6 year-old girl with ASA deficiency who is provided with 2 "sick day" diet prescriptions to be used for illnesses when deemed appropriate by the metabolic medical team. She weighs 24 kg and is fed orally and by gastrostomy tube	**When well:**	**During mild illness:**	**During moderate illness:**
	Energy: DRI **Protein:** 1.0 g/kg 0.5 g/kg as whole protein 0.5 g/kg as essential amino acid medical food	**Energy:** 20 % above DRI **Protein:** Half of usual whole protein; and all of usual essential amino acid medical food	**Energy:** 20 % above DRI **Protein:** None of usual whole protein and all of usual essential amino acid medical food

Selected nutrient composition of formulas®

Medical food	Amount (g)	Protein, total (g)	Protein, whole (g)	Protein, medical food (g)	Energy (kcal)
UCD Anamix® Junior[a]	100	7.5	0	7.5	510
Pro-Phree® powder[b]	100	trace	trace	0	510

[a]Nutricia North America (Rockville, MD; nutricia-na.com)
[b]Abbott Nutrition (Columbus, OH; abbottnutrition.com)

Diet prescription	Amount	Protein, whole[a] (g)	Protein, medical food[a] (g)	Energy (kcal)
Diet prescription: Usual full protein		0.5 g/kg (12 g)	0.5 g/kg (12 g)	1,600[b]
UCD Anamix® Junior[c]	100 g	0	12	385
Pro-phree®[d]	80 g	0	0	408
Add water to make	28 oz			
Food/beverages[e]		12	0	807
Diet prescription: Half whole protein, 20 % more energy		0.25 g/kg (6 g)	0.5 g/kg (12 g)	1,900
UCD Anamix® Junior[c]	100 g	0	12	385
Pro-phree®[d]	150 g	0	0	765
Add water to make	40 oz[e]			
Food/beverages		6	0	750[f]
Diet Prescription: No whole protein, 20 % more energy		0	0.5 g/kg (12 g)	1,900
UCD Anamix® Junior[c]	100 g	0	12	385
Prophree®[d]	200 g	0	0	1,020
Add water to make	48 oz[e]			
Food/beverages				495[f]

[a]Singh [17, 25]
[b]Dietary Reference Intake [23]
[c]Nutricia North America (Rockville, MD; nutricia-na.com)
[d]Abbott Nutrition (Columbus, OH; abbottnutrition.com)
[e]Total amount of fluid provided in the formula is greater than usual diet in order to keep caloric density similar to usual diet and because additional fluid is required during illness
[f]If patient is not able to consume sufficient amounts of food or fluids to reach energy goal, additional Pro-Phree® and water may be added to the formula instead

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Nutrition Management of Maple Syrup Urine Disease

16

Sandy van Calcar

Contents

Core Messages

- Maple syrup urine disease (MSUD) is caused by a deficiency in the branched-chain keto acid dehydrogenase enzyme complex that metabolizes the keto acids of leucine, isoleucine, and valine.
- Infants with classical MSUD can present with intoxication syndrome and require aggressive nutrition support to prevent or reverse catabolism.
- Nutrition management includes use of medical foods devoid of branched-chain amino acids, dietary leucine restriction, supplemental valine and isoleucine, and provision of adequate energy, protein, vitamins, and minerals.
- The goal of therapy is to maintain plasma leucine concentrations of 100–200 μmol/L for infants and children <5 years and 100–300 μmol/L for those over 5 years of age.

16.1 Background

Maple syrup urine disease (MSUD) is an inborn error of the branched-chain keto acid dehydrogenase (BCKDH) enzyme complex required for the catabolism of the branched-chain amino acids (BCAA) leucine, valine, and isoleucine (Fig. 16.1).

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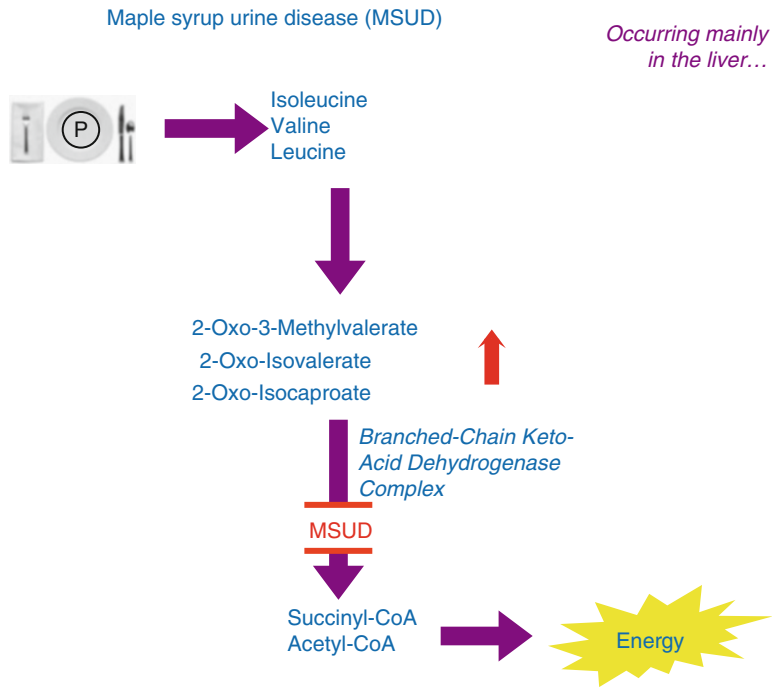

Fig. 16.1 Metabolic pathway in the metabolism of the branched-chain amino acids isoleucine, leucine, and valine

Box 16.1: Principles of Nutrition Management for MSUD

Restrict: Branched-chain amino acids, especially leucine

Supplement: Valine and isoleucine (if plasma concentrations are below target range), thiamine[a]

Toxic metabolite: Leucine and its keto acid, 2-oxo-isocaproic acid

[a]Trial of 100–1,000 mg thiamine is completed by some metabolic centers for patients with variant forms of MSUD [1, 2].

Of these, leucine and its corresponding keto acid, 2-oxo-isocaproic acid, are the primary toxic compounds in this disorder (Box 16.1). Maple syrup urine disease is so named because patients with this disorder have a characteristic sweet odor detectable in the urine or cerumen.

Infants with severe MSUD come to attention shortly after birth with symptoms such as poor feeding, weak suck, weight loss, and a high-pitched cry progressing to a metabolic intoxication crisis (Chap. 5) characterized by lethargy, irritability, vomiting, and fluctuating muscle tone (e.g., floppy, then rigid). If the infant is not treated, seizures, metabolic acidosis, coma, and death may result. There are several classifications of disease severity, including classical, thiamine responsive or intermittent, and mild or variant forms [3]. BCKDH is a thiamine-dependent enzyme, and individuals with residual enzyme activity may benefit from thiamine supplementation, but those with classic MSUD do not. Nevertheless, a trial of thiamine is often performed (doses 100–1,000 mg for 1–4 weeks) [2]. Patients with variant forms of MSUD may present later in infancy or childhood with poor growth or developmental delay [4]. Newborn screening identifies infants with high blood leucine, although infants with the classical form can be symptomatic before newborn screening results are available. The diagnosis is made based on clinical symptoms, biochemical findings (elevated blood concentrations of leucine, valine, and isoleucine and presence of alloisoleucine), and genetic testing. MSUD is rare in the general population with an incidence of 1 in 185,000 live births, but in the Old Order Mennonite population, the incidence is much higher at 1 in 380 live births and is caused by the common c. 1312T>A

mutation in the BCKDHA gene [3]. Nutrition management for MSUD has improved greatly and with vigilant care can result in good developmental outcomes, especially if illnesses or other catabolic events are aggressively managed. Liver transplantation is an option for treatment of this disorder [5].

16.2 Nutrition Management

16.2.1 Chronic Nutrition Management

Patients who present with symptoms in the newborn period and have been medically stabilized, as well as those who are asymptomatic at diagnosis, require a leucine-restricted diet. Table 16.1 provides suggested goals for nutrient intakes for infants with MSUD.

The steps for initiating a diet in an infant are outlined below (Box 16.2).

Once the diet has been established, adjustments in leucine, valine, and isoleucine prescriptions should be based on blood BCAA concentrations rather than maintaining a specific mg/kg intake goal (Box 16.3). The amount of the BCAA required per kilogram of body weight decreases as the infant matures.

Solid foods can be introduced to infants with MSUD at the typical age recommended for all infants, unless motor delays are present. To allow for solid food introduction, the volume of regular infant formula or breast milk is decreased and leucine from these sources is replaced with leucine from solid foods. High-protein foods contain too much leucine to be incorporated into the diet in all but the mildest forms of MSUD. Foods with a moderate protein content such as starchy vegetables and regular grain products will

Box 16.2: Initiating Nutrition Management of an Infant with MSUD

Goal: Reduce or normalize plasma leucine.

Step-by-step:

1. Establish intake goals based on the infant's diagnostic blood leucine, clinical status, and laboratory values.
2. Determine amount of standard infant formula or breast milk required to meet the infant's leucine needs. Determine the amount of protein and energy that will be provided by this amount of formula.
3. Subtract the protein provided by the standard infant formula or breast milk from the infant's total protein needs.
4. Calculate amount of BCAA-free medical food required to meet the remaining protein needs.
5. Calculate valine and isoleucine intake provided by the infant formula/breast milk. Determine the amount of supplemental valine and isoleucine to add to meet recommended intakes (Table 16.1). The recommended concentration of valine and isoleucine supplements is 10 mg/mL (1 g amino acid in 100 mL water).
6. Determine the number of calories provided by both the infant formula/breast milk and BCAA-free medical food. If more energy is required, provide the remaining calories from a BCAA-free medical food (as long as the additional medical food does not cause the total protein intake to be excessive).
7. Determine the amount of fluid required to provide a caloric density of 20–25 kcal/oz.
8. Divide total volume into appropriate number of feedings over a 24-h period.

Table 16.1 Suggested daily nutrient intakes for acutely ill or asymptomatic infants with classic MSUD [1, 6]

	Acute [6]	Asymptomatic [1, 6]
Energy (kcal/kg)	120–140	100–120
Lipid (% of energy)	40–50 %	DRI
Protein (g/kg)	3–4	2–3.5
Leucine (mg/kg)	0	40–100 mg/kg
Isoleucine (mg/kg)	80–120	30–95
Valine (mg/kg)	80–120	30–95

Box 16.3: Recommendations for Adjusting the MSUD Diet Prescription

1. Determine the estimated increase or decrease in leucine, isoleucine, and valine intake that will be needed to improve the blood concentrations. Changes in 10 % increments are typical but can be higher or lower based on plasma amino acid concentrations [7].
2. Adjust the amount of infant formula or breast milk to increase or decrease leucine in the diet.
3. Recalculate the valine and isoleucine content provided by the infant formula or breast milk.
4. Recalculate the amount of supplemental isoleucine and valine needed to meet your intake goals.
5. Recalculate the amount of MSUD medical food required to meet the energy goal.
6. Recheck plasma amino acid concentrations.

provide the majority of leucine in the diet prescription. Modified low-protein foods made from wheat or other starch can be introduced to allow for a greater volume of food with a very low leucine content.

In MSUD, only the leucine content of foods and beverages need to be counted (Box 16.4). There is no need for caregivers to calculate the valine and isoleucine content of foods. Unless there is a concern about low energy intake, caregivers do not need to count calories from foods or beverages; the medical food provides the majority of energy for infants.

Low-protein recipes from cookbooks designed for PKU can be useful for MSUD as well. The leucine content can be estimated from the protein content of foods (60 mg leucine per gram of protein). For older individuals with MSUD, counting protein rather than leucine may be appropriate and easier for the patient, if metabolic control can be maintained with this less accurate method.

Box 16.4: Counting Leucine Intake in the MSUD Diet

Only dietary leucine must be counted

- The valine and isoleucine content of food is about half that of the leucine content.
- Patients will not get too much valine and isoleucine if they consume the prescribed amount of leucine.
- Each gram of protein contains approximately 60 mg leucine.
- A food list of leucine content is available [8].

A medical food designed for MSUD (Table 16.2) is required for life. In patients with classical MSUD, medical foods provide up to 80–90 % of protein needs and a majority of energy needs in infancy and beyond.

Infants with MSUD will consume a complete medical food containing all amino acids except BCAA with a fat, carbohydrate, and micronutrient content similar to standard infant formulas. Toddlers and young children are transitioned to complete medical foods designed for those over age 2 years containing all amino acids except BCAA with age-appropriate fat, carbohydrate, and micronutrient profiles. There are several other medical foods on the market, including those more concentrated in amino acids with little or no fat and some with both reduced fat and carbohydrate sources. These can be used to decrease the amount of medical food required to meet an individual's protein needs and thus can be helpful if excess energy intake is a concern. However, decreasing energy from medical food may lead to excessive intake of leucine-containing foods or may decrease energy intake to a point that the individual is losing weight. Both of these situations can cause elevations in blood leucine and poor metabolic control. The vitamin and mineral content of medical foods varies. Intake needs to be assessed and supplemental vitamins and minerals provided, if necessary.

Table 16.2 Selected medical foods for the treatment of MSUD

Infant/toddler (complete[a])	Older child/adult (complete[a])	Older child/adult (incomplete[b])	
Complex Junior MSD[c] Ketonex-1[d] Ketonex-2[d] BCAD-1[e] BCAD-2[e] MSUD Early Years[c]	Complex Essential MSD[c] Ketonex-2[d] MSUD Lophlex LQ[c] BCAD-2[e]	Complex MSD Amino Acid Blend[c] Camino Pro MSUD Drink[f] MSUD Maxamaid[c] MSUD Maxamum[c] Milupa MSUD 2[c]	Complex MSD Amino Acid Bar[c] MSUD Gel[g] MSUD Cooler 15[g] MSUD Express[g]

[a]Contains L-amino acids (without BCAA), as well as fat, carbohydrate, vitamins, and minerals
[b]Contains L-amino acids (without BCAA) low in or devoid of fat, carbohydrate, vitamins, or minerals. See company websites for specific nutrient composition
[c]Nutricia North America (Rockville, MD; nutricia-na.com)
[d]Abbott Nutrition (Columbus, OH; abbottnutrition.com)
[e]Mead Johnson Nutrition (Evansville, IN; meadjohnson.com)
[f]Cambrooke Therapeutics (Ayer, MA; cambrookefoods.com)
[g]Vitaflo USA (Alexandria, VA; vitaflousa.com)

16.2.2 Acute Nutrition Management

Symptomatic MSUD in a neonate is a medical emergency and requires the immediate initiation of nutrition support to reverse catabolism. Nonprotein calories are provided by peripheral or central line access. A source of BCAA-free amino acids is needed as soon as treatment begins. Nasogastric feedings of an MSUD medical food can be provided, or if gastrointestinal administration is not tolerated, BCAA-free parenteral solutions are available (Coram Specialty Infusion Services, Denver, Colorado).

Promoting protein anabolism is key to reducing BCAA concentrations and requires energy intake above maintenance requirements, removal or significant reduction in leucine intake, and prevention of valine and isoleucine deficiency through supplementation. It is important to understand that in order to reduce blood leucine concentrations, a sufficient supply of all other amino acids, including valine and isoleucine, must also be present. In MSUD, blood concentrations of isoleucine and valine are typically lower than leucine, and if the patient is consuming a BCAA-free medical food, will normalize before leucine concentrations normalize. Without supplementation of isoleucine and valine to maintain sufficient blood concentrations of these two amino acids, the blood leucine concentration will not decrease. Maintaining isoleucine and valine concentrations above the normal range until leucine concentration sufficiently decreases promotes faster reduction of the leucine concentration [6, 9].

With any intercurrent illness, individuals with MSUD are at risk for metabolic decompensation. During these episodes, development of cerebral edema can be fatal. Families and individuals with MSUD should know the signs of metabolic decompensation and be provided with an emergency protocol that includes both directions for the family and for emergency personnel who may become involved in their care. Information for contacting the on-call metabolic physician must be included in any protocol.

There are many factors to be considered during an illness, and the entire metabolic team must be aware of the potential of an impending emergency. Often, a "sick-day diet" prescription is provided for use at the first sign of an illness. For minor illnesses, use of the sick-day diet may allow for management of the disorder on an outpatient basis, but many factors go into this decision,

Box 16.5: Guidelines for Designing a "Sick-Day" Diet for Patients with MSUD

1. The "sick-day" diet must provide enough energy to meet the individual's estimated requirement.
2. Increase protein by increasing medical food intake to 120 % usual intake. This also supplies more energy from carbohydrate or fat.
3. Decrease leucine prescription by 50% to complete removal from the diet depending on the degree of illness, for 24 h, and reassess with the medical team.
4. Prevent low blood concentrations of isoleucine and valine. Provide the same amount of isoleucine and valine as in the patient's usual diet. Additional isoleucine and valine may be required to prevent low concentrations [6].
5. Provide small, frequent feedings throughout a 24-h period.
6. Monitor plasma concentrations of the BCAA in order to guide appropriate diet adjustments.

Box 16.6: Management During Admission for Illness in MSUD[a]

The initial treatment of intoxication syndrome is a medical emergency and is managed by the metabolic physician[b]. Once the patient's plasma leucine has decreased to an acceptable range, to prevent a "rebound" rise in leucine:

- Provide appropriate nutrients and energy for acute illness (see Table 16.1).
- Maintain blood isoleucine and valine concentrations >200 μmol/L as leucine decreases. When anabolic, leucine decreases very rapidly and isoleucine and valine needs often exceed the patient's usual isoleucine and valine tolerance.
- Do not discharge patient until plasma leucine has decreased sufficiently and patient is tolerating enteral feedings well.
- Reassess plasma amino acids every 12–24 h or as clinically indicated.
- Monitor electrolytes and fluid volume.

[a]In conjunction with medical management by metabolic physician

[b]See Chap. 5

including age of the child, severity of MSUD (i.e., classical vs. variant MSUD), severity and length of illness, and ability of the family to manage a complicated protocol at home. Home monitoring with dinitrophenylhydrazine (DNPH) solution may be used. DNPH specifically reacts with the α-keto acids produced in MSUD. If DNPH is not available, then measuring urine ketones using Ketostix® has been employed by some clinics [1], but it is not as sensitive of a marker. Sick-day diets must be individualized for each patient, and guidelines for designing these diets are outlined below (Box 16.5).

There is a low threshold when deciding if a patient's clinical condition warrants an emergency department visit or admission. Admissions require a medical team specialized in treating inborn errors of metabolism, access to frequent and rapid turnaround time for laboratory results to monitor BCAA concentrations, electrolytes and other critical laboratory values, as well as all components for providing the diet, including medical foods and specialized parenteral solutions, if necessary. General guidelines for nutrition management during hospital admissions are given above (Box 16.6).

Caregivers and individuals with MSUD need to be aware that metabolic decompensation can be precipitated by events other than illness – significant injury and surgery are also catabolic events that need attention from the metabolic team. For surgical procedures, reducing the fasting time, using an energy source (i.e. IV glucose) during and after the procedure until oral intake can be restarted, and developing a plan for monitoring are necessary.

16.3 Monitoring

In addition to frequent monitoring of BCAA concentrations, a full amino acid profile should be periodically evaluated. Monitoring should be completed at a consistent time during the day, either after an overnight fast or approximately 2–3 h after a meal. Table 16.3 provides recommended blood BCAA concentrations.

If leucine concentrations are elevated without signs of illness or other stress, there are several parameters to consider:

Evaluate energy intake. Significant weight loss may increase BCAA concentrations due to catabolism. Increasing energy intake from medical food promotes weight gain (or maintenance in older patients) and also helps to reduce hunger, so the individual may be less tempted to eat a greater volume of food than recommended. If a low fat- or protein-concentrated medical food is prescribed, consider addition of a more complete medical food with greater calorie-to-protein ratio.

Evaluate the distribution of medical food throughout the day. As in PKU, medical food distributed in three or four servings per day and given with some leucine in the diet at the same time will result in better utilization of BCAA and thus lower and more consistent leucine concentrations (Chap. 6).

More protein from medical food may be needed. Given the rapid oxidation of amino acids compared to intact protein sources, protein requirements for patients with metabolic disorders are often higher than recommended for the general population (Chap. 7). If excess energy intake is a concern, adding a medical food concentrated in protein can increase total protein without significantly increasing energy intake from the medical food.

With your metabolic team, consider the possibility of a hidden illness or infection. Urinary tract infections, sinus infections, or dental problems often increase leucine concentrations but may not be obvious to the patient. Because of hormonal effects on protein metabolism, some women with MSUD have higher leucine concentrations just before they menstruate (author's personal clinical experience). Reduction in the leucine prescription and additional calories may be needed during these times.

Evaluate the amount of isoleucine and valine in the diet. Suplementation of these amino acids may need to be adjusted. In the amino acid profile, aim to maintain a 1 to 1 ratio of isoleucine to leucine concentrations and a 2 to 1 ratio of valine to leucine concentrations.

Consider decreasing the patient's leucine prescription. First determine if the patient is taking the prescribed amount of leucine. Typically, decreasing the leucine prescription is the last thing to try since leucine requirements are relatively constant. However, during periods when growth slows, such as late infancy or late adolescence, a decrease in the prescribed amount of leucine may be needed. Adequate calories are crucial when decreasing leucine in the prescription.

Table 16.3 Recommended blood BCAA concentrations in MSUD [1, 6]

	μmol/L	mg/dL	Normal (μmol/L)
Leucine	100–300[a]	1.3–3.9	50–215
Valine	200–400	2.3–4.6	85–200
Isoleucine	100–300	1.3–3.9	25–90

[a]Recommended maximum blood concentration is 200 μmol/L for infants and children <5 years of age and 300 μmol/L for those >5 years of age

Box 16.7: Considerations if Plasma Leucine Concentrations Are Elevated (Without Signs of Illness or Stress)

1. Is the patient consuming enough energy?
2. Is all medical food being consumed and distributed throughout the day?
3. Is protein intake from medical food too low?
4. Are there hidden illnesses or infections?
5. Is the patient taking the prescribed amount of leucine?
6. Are blood concentrations of valine and/or isoleucine too low?
7. Is a decrease in the leucine prescription required?

Box 16.8: Nutrition Monitoring of a Patient with MSUD[a]

- Routine assessments including anthropometrics, dietary intake, and physical findings (Appendix F)
- Laboratory monitoring
  - Diagnosis specific
    - Plasma amino acids
      - Leucine
      - Valine
      - Isoleucine
  - Nutrition-related laboratory monitoring of patients on BCAA-restricted diets may include markers of:
    - Protein sufficiency[b] (plasma amino acids, prealbumin)
    - Nutritional anemia (hemoglobin, hematocrit, MCV, serum vitamin B_{12} and/or methylmalonic acid, total homocysteine, ferritin, iron, folate, total iron binding capacity)
    - Vitamin and mineral status (Total 25-hydroxyvitamin D, zinc, trace minerals)
    - Others as clinically indicated

[a]Suggested frequency of monitoring can be found in the GMDI/SERC MSUD Guidelines [1, 2] (southeastgenetics.org/ngp)

[b]Further described in Chap. 7.

Other laboratory parameters also need to be considered for diet monitoring (Box 16.8).

16.4 Transplantation

Liver transplantation is becoming a common treatment option for patients with MSUD to prevent episodes of cerebral edema associated with high leucine concentrations during illness or other catabolic stress [5, 10]. A donated liver restores 9–13 % of BCKAD activity, which is sufficient to allow for a normal diet [10]. BCKAD activity is not found solely in the liver but is also active in the muscle, heart, kidney, brain, and other tissues. Therefore, a liver that is removed from a patient with MSUD can be donated to another patient (domino liver transplantation) because the recipient of an MSUD liver will use BCKAD from other tissues to metabolize valine, isoleucine, and leucine [11]. For the patient with MSUD, orthotoptic liver transplantation is most common, but partial transplantation of a liver from a living, related donor has been described [12].

Overall, outcomes following liver transplantation in individuals with MSUD have been good. In 54 patients with MSUD who received a liver transplant, the overall survival rate was 98–100 % [10]. After transplantation, patients tolerate normal diets and have stable plasma BCAA concentrations [5]. Patients showed no cognitive improvement over pre-transplant intelligence (mean IQ 78 ± 24) but many had cognitive impairment prior to transplantation. For patients with severe MSUD, receiving a transplant early in life before brain damage is sustained is a viable treatment option [5, 10].

Prior to surgery, the patient with MSUD should have plasma BCAA in the treatment range and should have had no metabolic crises within the previous 3 weeks. They should be fasting for a minimal period prior to surgery and receive IV glucose before and during surgery and in the recovery period. Ideally, BCAA-free parenteral amino acid solutions should be available and amino acid analyses completed frequently to monitor progress. BCAA concentrations should normalize rapidly and a normal diet can be introduced according to usual transplant protocol [13].

16.5 Summary

Early identification, aggressive treatment during catabolic events, and diligent nutrition management with frequent monitoring can result in positive outcomes for patients with MSUD. Treatment includes restricting BCAA intake with the use of medical foods intact protein restriction and isoleucine and valine supplementation as needed to maintain plasma BCAA concentrations in the target ranges. Acute metabolic decompensation in MSUD is a life-threatening illness because of the risk of cerebral edema and should be treated as a medical emergency with rapid, aggressive management to reverse catabolism.

16.6 Diet Calculation Example

Example 1:
Infant diagnosed with MSUD

Patient information	Nutrient intake goals (per day)
Eight (8) day-old infant weighing 4 kg diagnosed with MSUD through newborn screening. This patient presents asymptomatically. Recent labs show plasma leucine concentration is 600 μmol/L, isoleucine is 80 μmol/L and valine is 180 μmol/L. The infant is currently taking 23 oz of Enfamil® Premium powdered formula	**Leucine (LEU):** 90 mg/kg **Isoleucine (ILE):** 50 mg/kg **Valine (VAL):** 50 mg/kg **Protein:** 3.0 g/kg **Energy:** 100–120 kcal/kg **Fluid:** 100 mL/kg Recommended caloric density of formula: 20–25 kcal/oz

Selected nutrient composition of formulas

Medical food	Amount (g)	LEU (mg)	VAL (mg)	ILE (mg)	PROTEIN (g)	ENERGY (kcal)
MSUD Anamix® Early Years[a]	100	0	0	0	13.5	473
Enfamil® Premium powder[b]	100	1,250	640	640	10.8	510

[a]Nutricia North America (Rockville, MD)
[b]Mead Johnson Nutrition (Evansville, IN)

Diet prescription summary for sample calculation of MSUD diet (using standard infant formula as the source of whole protein)[a]

Medical food	Amount	LEU (mg)	ILE (mg)	VAL (mg)	PROTEIN (g)	ENERGY (kcal)
MSUD Anamix® Early Years[b]	66 g	0	0	0	8.9	312
Enfamil® Premium powder[c]	29 g	362	186	186	3.1	148
ILE supplement	1.4 mL[d]		14			
VAL supplement	1.4 mL[d]			14	–	
Total per day		362	200	200	12.0	460
Total per kg		91 mg/kg	50 mg/kg	50 mg/kg	3.0 g/kg	115 kcal/kg

[a]Rounded to nearest whole number for amount of formula powders, leucine, isoleucine, valine, and energy and to the nearest 0.1 g for protein
[b] Nutricia North America (Rockville, MD)
[c] Mead Johnson Nutrition (Evansville IN)
[d] Amino acid solution containing 10 mg/mL

Step-by-Step Calculation

Step 1: Calculate the amount of each nutrient required each day.

Nutrient goal/kg × Infant weight = daily requirement
Leucine: 90 mg/kg × 4 kg = 360 mg/day
Isoleucine: 50 mg/kg × 4 kg = 200 mg
Valine: 50 mg/kg × 4 kg = 200 mg
Protein: 3.0 g protein × 4 kg = 12 g total protein requirement
Energy: 110–120 kcal/kg × 4 kg = 440 – 480 kcal/kg

Step 2: Calculate amount of standard infant formula needed to meet daily LEU requirement.

Amount of LEU required per day ÷ amount of LEU in 100 g of standard infant formula
360 mg ÷ 1,250 mg = 0.29
0.29 × 100 = 29 g standard infant formula needed to meet daily LEU requirement.

Step 3: Calculate protein provided from standard infant formula.

Amount of standard infant formula × protein provided in 100 g of standard infant formula
0.29 × 10.8 g = 3.1 g protein in standard infant formula

Step 4: Calculate the amount of protein required to fill diet prescription

Daily protein requirement – protein provided in standard infant formula
12 g – 3.1 g = 8.9 g protein needed to fill in the diet prescription

Step 5: Calculate amount of BCAA-free medical food required to fill diet prescription

Protein needed to fill diet prescription ÷ protein provided in 100 g of BCAA-free medical food
8.9 g ÷ 13.5 g = 0.66
0.66 × 100 = 66 g BCAA-free medical food required in the diet prescription

Step 6: Calculate amount of isoleucine and valine provided from standard infant formula (note: there is no LEU, ILE, or VAL in BCAA-free medical food)

Amount of standard formula × ILE in 100 g of standard formula
0.29 × 640 mg ILE = 186 mg ILE
Amount of standard formula × VAL in 100 g of standard formula
0.29 × 640 mg VAL = 186 mg VAL

Step 7: Calculate the amount of ILE and VAL that needs to be supplemented by an amino acid solution in order to meet the requirements determined in Step 1.

ILE: 200 mg – 186 mg = 14 mg ILE to be provided by supplement
VAL: 200 mg – 186 mg = 14 mg VAL to be provided by supplement
Amino acid solutions can be made to contain 10 mg/mL by adding 1 g (1,000 mg) of amino acid (ILE or VAL) powder to 100 mL
14 mg ILE divided by 10 mg/mL = 1.4 mL ILE solution (containing 10 mg/mL)
14 mg VAL divided by 10 mg/mL = 1.4 mL VAL solution (containing 10 mg/mL)

Step 9: Calculate total energy provided from standard infant formula and BCAA-free medical food.

Amount of standard infant formula × kcal in 100 g of standard formula.

0.29 × 510 kcal = 148 kcal

Amount of BCAA-free medical food × kcal of 100 g of BCAA-free medical food.

0.66 × 473 kcal = 312 kcal

Add standard formula + BCAA-free medical food for total kcal provided in diet prescription.

148 kcal + 312 kcal = 460 kcal

Step 10: Calculate the final volume of the formula to make a concentration of 20–25 kcal per ounce.

460 kcal ÷ 20 kcal/oz = 23 oz of formula

(Note: if final volume prescribed is 20 oz, caloric concentration will be 23 kcal/oz; if final volume prescribed is 25 oz, caloric concentration will be 18.4 kcal/oz)

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Part III

Organic Acidemias

Organic Acidemias

17

Janet A. Thomas

Contents

Core Messages

- Organic acidemias (OA) are defects in the degradation of leucine, isoleucine, and valine.
- OA can present either as a severe neonatal-onset form (poor feeding, vomiting, lethargy, tachypnea, progressing to acidosis, respiratory distress, coma, death) or late-onset form (usually recurrent ketoacidosis or lethargy with catabolic stress).
- Nutrition treatment involves use of propiogenic amino acid-free medical foods and restriction of natural protein in PROP and MMA and protein restriction with or without leucine-free medical foods in IVA.
- Outcomes in PROP and MMA have been guarded with frequent neurological complications, renal dysfunction, cardiomyopathy, and optic atrophy but are improving with earlier identification and treatment, as well as with liver or liver-kidney transplantation; outcomes in IVA are often normal.

17.1 Background

Organic acidemias are disorders of branched-chain amino metabolism in which non-amino organic acids accumulate in serum and urine.

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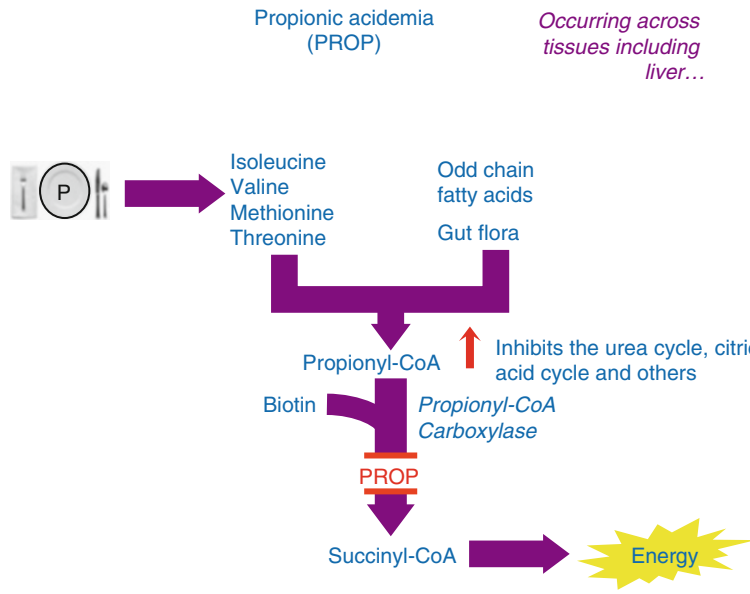

Fig. 17.1 Metabolic pathway of propionic acidemia (PROP)

They are defects in the degradation pathways of leucine, isoleucine, and valine. These conditions are usually diagnosed by examining organic acids in urine with abnormal metabolites also notable on acylcarnitine profile. Organic acidemias comprise a variety of disorders and include methylmalonic acidemia (MMA), propionic acidemia (PROP), isovaleric acidemia (IVA), glutaric acidemia type 1 (GA-1), 3-methylcrotonyl carboxylase deficiency (3-MCC), 3-methylglutaconic acidemia (3-MGA), and vitamin B_{12} uptake, transport, and synthesis defects.

All are autosomal recessive with the exception of the rare, X-linked disorder, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (MHBD) and the newly described cobalamin X (cblX). The two primary disorders of isoleucine and valine catabolism are propionic acidemia (PROP) and methylmalonic acidemia (MMA), and the primary organic acidemia of leucine catabolism is isovaleric acidemia (IVA). These three disorders will be discussed in detail in this chapter. The incidence of MMA ranges from 1:83,000 in Quebec to 1:115,000 in Italy to 1:169,000 in Germany and that of PROP from 1:17,400 in Japan to 1:165,000 in Italy to 1:277,000 in Germany [1–4]. On the basis of newborn screening data, the incidence of IVA has a range of 1:62,500 live births in Germany to ~1:250,000 in the United States [5, 6]. Newborn screening via tandem mass spectrometry has revealed a higher incidence of these disorders than previously noted based on clinical presentation and suggests a broader phenotype with milder and asymptomatic individuals [1, 2, 5, 7–9].

The oxidation of threonine, valine, methionine, and isoleucine results in propionyl-CoA, which propionyl-CoA carboxylase converts into L-methylmalonyl-CoA, which is metabolized through methylmalonyl-CoA mutase to succinyl-CoA. Whereas the breakdown of the above amino acids is felt to contribute to ~50 % of the propionyl-CoA production, gut bacteria and the breakdown of odd-chain-length fatty acids also substantially contribute to propionyl-CoA production (~25 % each), with a minimal contribution by cholesterol metabolism [10–13] (Fig. 17.1).

PROP is caused by a deficiency of the mitochondrial enzyme, propionyl-CoA carboxylase (PCC) [7, 13]. The enzyme is composed of two subunits, an alpha and beta subunit, each encoded by a different gene, *PCCA* and *PCCB*, respectively [7]. The enzyme is biotin dependent with biotin binding to the alpha subunit [13, 14]. Deficiency of the enzyme results in the accumulation of propionyl-CoA and increased concentrations of free propionate in blood and urine. Methylcitrate and 3-hydroxypropionate are the

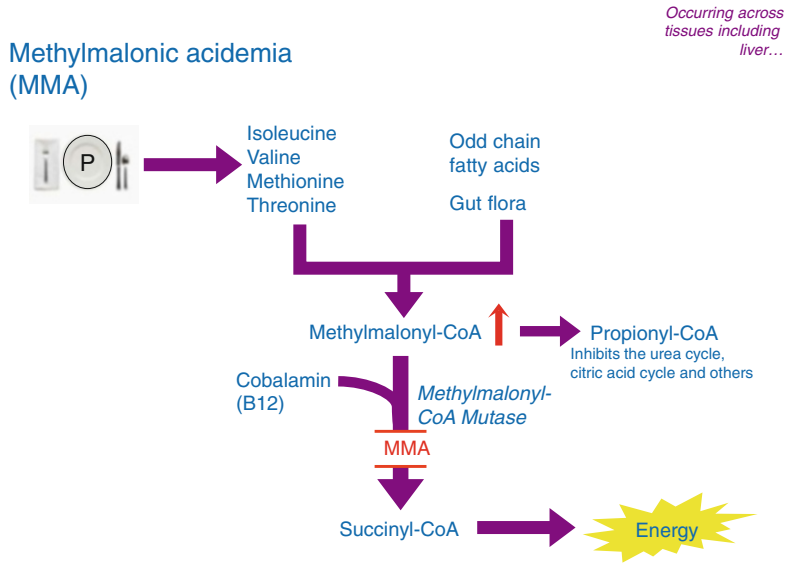

Fig. 17.2 Metabolic pathway of methylmalonic acidemia (MMA)

major diagnostic metabolites seen on organic acid analysis [3, 13]. Elevation of propionylcarnitine (C3) can be seen on acylcarnitine profile [3, 7].

Classic MMA is caused by a deficiency of the enzyme methylmalonyl-CoA mutase, an adenosylcobalamin (AdoCbl)-dependent enzyme consisting of two identical subunits (2α) [3, 7, 13] (Fig. 17.2). About 50 % of cases of MMA are due to a defect in the mutase apoenzyme; in others it is due to a defect in the uptake, transport, or synthesis of its adenosyl-B_{12} coenzyme causing variant forms of MMA that may or may not be associated with homocystinuria. Individuals who are mutase deficient may be further designated as mut^- or mut^0 pending residual enzyme activity [13]. There is good correlation between residual enzymatic activity and severity of the clinical phenotype [3]. Acquired methylmalonic aciduria can also be seen with acquired deficiency of vitamin B_{12}, in pernicious anemia, and in transcobalamin II deficiency [7]. Hence, vitamin B_{12} deficiency must be excluded in all individuals with elevated methylmalonic acid levels [7, 13]. Deficiency of the mutase enzyme results in the accumulation of methylmalonyl-CoA and propionyl-CoA and is reflected in elevations of methylmalonic acid and propionic acid in blood and urine [3, 13]. Methylcitrate, 3-hydroxypropionate, and 3-hydroxyisovalerate are found on urine organic acid analysis [3, 7, 13]. Propionylcarnitine (C3) is also found on acylcarnitine profile in MMA [3, 7].

IVA was initially described in 1966 and became the first organic acidemia described. IVA is caused by a deficiency of the enzyme isovaleryl-CoA dehydrogenase, an enzyme important in leucine catabolism and also important in the transfer of electrons to the respiratory chain [7, 13]. The consequent accumulating metabolites include isovaleric acid, isovalerylglycine, 3-hydroxyisovaleric acid, and isovalerylcarnitine (C5) [7, 13] (Fig. 17.3). These are easily identified on urine organic acid analysis and acylcarnitine profile. The excretion of isovalerylglycine and 3-hydroxyisovaleric acid is diagnostic.

17.2 Clinical Presentation

Organic acidemias may present at any age. In general, they can be divided into two broad groups – a severe, neonatal presentation and a chronic late-onset presentation.

17.2.1 Severe Neonatal-Onset Form

The clinical presentation of the severe, neonatal-onset form of these disorders can be quite similar

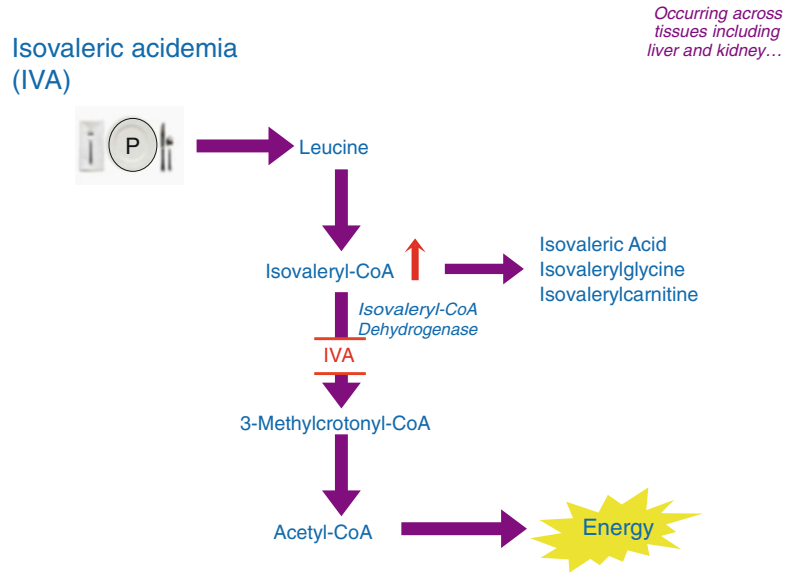

Fig. 17.3 Metabolic pathway of isovaleric acidemia (IVA)

for all three disorders. As is typical with inborn errors of metabolism, the pregnancy and birth history for the child is often unremarkable. Following an initial symptom-free period which may last from hours to weeks, the infant then develops nonspecific symptoms, such as poor feeding, vomiting, dehydration, lethargy, tachypnea, and hypothermia and if unrecognized, quickly progresses to respiratory distress, apnea, bradycardia, coma, cerebral edema, and death [3, 7, 10, 13]. At the time of presentation, the physical examination is primarily one of altered mental status and encephalopathy, but dehydration, hepatomegaly, abnormal tone, and seizure-like activity may also be seen [7, 10, 13]. A sweaty feet or dirty sock smell is classically described for IVA secondary to excretion of 3-hydroxyisovaleric acid [7–9].

17.2.2 Chronic Late-Onset Form

The late-onset form typically presents after a symptom-free period of at least 1 year and maybe much longer (into adolescence or adulthood) [13]. Individuals often present with recurrent attacks of ketoacidosis with coma or lethargy and ataxia during times of catabolic stress such as during an illness or following a high protein meal [7, 13]. The presentation may mimic diabetic ketoacidosis [15–18]. Other individuals may present with acute hemiplegia, hemianopsia, or cerebral edema, or symptoms that mimic a cerebral vascular accident, cerebral tumor, or acute encephalitis [13]. Frequently, symptoms may simulate a neurologic disorder presenting with hypotonia, weakness, ataxia, seizures, abnormal movements, or developmental delay, or symptoms may be misdiagnosed as a gastrointestinal disorder presenting with failure to thrive, anorexia, chronic vomiting, or a Reye-like presentation [3, 7, 13]. Finally, some individuals may present with hematologic manifestations or present with recurrent infections [7, 13].

17.2.3 Laboratory Studies and Diagnosis

Laboratory studies typically reveal a severe metabolic acidosis with an elevated anion gap, ketosis, and hyperammonemia [3, 7, 10, 13]. Hyperuricemia, hyperlactacidemia, and hypocalcemia may also be seen. Blood glucose levels can be reduced, normal, or elevated [10, 13]. Bone marrow involvement as reflected by neutropenia, anemia, thrombocytopenia, or pancytopenia can also be seen and is a rather unique finding of organic acide-

mias [7, 10, 13]. Diagnostic metabolites, as noted above, are seen on urine organic acid analysis and acylcarnitine profile. Quantitative plasma amino acids and urine amino acids are useful to investigate the possibility of combined MMA and homocystinuria due to vitamin B_{12} synthesis defects. Striking elevations of glycine and alanine can be seen in blood and urine and may be an early clue to diagnosis and explains the historical descriptive term of "ketotic hyperglycinemias" [3, 7]. The diagnosis may be confirmed by enzyme assay or molecular studies. Organic acidemias are increasingly identified via newborn screening with elevations in propionylcarnitine (C3) for PROP and MMA and elevated isovalerylcarnitine (C5) for IVA.

Box 17.1: Neurological Complications of Organic Acidemias

- Metabolic stroke with edema evolving into necrosis of the basal ganglia and globus pallidus which leads to disabling movement disorder, motor dysfunction, and hypotonia
- Cerebral atrophy and delayed myelination
- Seizures
- Optic atrophy
- Neuropathy or skeletal myopathy
- Developmental delay; autistic features

17.2.4 Complications

Organic acidemias are multisystem disorders with individuals at risk for a variety of complications. Neurologic complications include metabolic stroke with edema evolving into necrosis of the basal ganglia, especially the globus pallidus, and leading to a disabling movement disorder, motor dysfunction, and hypotonia [3, 13, 19–21]. Basal ganglia lesions, cerebral atrophy, and delayed myelination may be seen on neuroimaging [3, 7, 13, 20, 22]. Clinically, seizures, deafness, optic nerve atrophy, neuropathy, myopathy, developmental delay, and autistic features are seen [19, 21, 23–25] (Box 17.1). Approximately 50 % of affected individuals have an IQ < 80 [19]. The etiology of this neurologic damage is unclear. Theories include direct toxic effects of methylmalonic acid, propionic acid, and methylcitrate, impairment of energy metabolism as mediated by synergistic inhibition of the Krebs cycle and mitochondrial respiratory chain also by the three metabolites, accumulation of decarboxylates in the central nervous system, and/or neuroinflammation [26–32]. Elevations in plasma lactate and ammonia and in CSF lactate, glutamine, glycine, and alanine have also been detected in a patient with neurologic symptoms, but no signs of catabolism suggesting that neurologic abnormalities may be related to localized metabolic derangements [33, 34]. Newer data indicates a synergistic effect of methylmalonic acid and ammonia disturbing the redox homeostasis and causing morphological brain abnormalities, including vacuolization, ischemic neurons, and pericellular edema in a rat model [35]. Propionate accumulation also results in morphological alterations in cerebral cortex astrocytes [21].

Box 17.2: Complications of Organic Acidemias

- Renal tubular acidosis (RTA) with hyperuricemia; chronic renal impairment leading to renal failure
- Superficial desquamation and alopecia due to nutrient or essential amino acid deficiency
- Cardiomyopathy, long QT syndrome
- Pancreatitis – acute or chronic
- Carnitine deficiency
- Osteopenia or osteoporosis
- Immune dysfunction
- Ovarian failure

Another complication of organic acidemias is renal tubular acidosis with hyperuricemia leading to chronic renal impairment and eventually to renal failure [7, 13, 24] (Box 17.2). This appears to be especially prominent in individuals with MMA; however, renal disease and failure has been reported in both newborns and adults with propionic acidemia [36–38]. Renal histology

reveals tubulointerstitial nephritis with fibrosis, tubular atrophy, and mononuclear inflammatory infiltrate [37, 39–41]. Renal disease is found in the majority of individuals with MMA who are over 6 years of age and may be due to chronic glomerular hyperfiltration secondary to excessive methylmalonic acid excretion [8, 13, 20, 27]. The risk of developing renal failure seems to correlate with methylmalonic acid exposure over time and depends on the disease type, with mutase-deficient patients at greater risk than patients with cobalamin defects [40]. Thus, minimizing renal injury may require strict metabolic control to keep methylmalonic acid concentrations as low as possible [13].

Further, cardiomyopathy, more common in PROP than MMA and more commonly dilated than hypertrophic, may occur during acute decompensations or may be the presenting feature and may be rapidly fatal [13, 42–46]. The pathogenesis of cardiomyopathy is unclear – carnitine or micronutrient deficiency, infection, or acute energy deprivation have all been postulated [3, 34, 47]. Cardiomyopathy appears to develop independent of any specific metabolic profile and appears to occur at any age [34, 44, 47]. The mean age of presentation in one series was 7 years [44]. Long QT syndrome (delayed repolarization of the heart) is also reported and may occur in as high as 70 % of patients with PROP [47–51].

A secondary carnitine deficiency due to accumulation of propionyl-CoA and increased concentration of acylcarnitines is also common [13]. Superficial desquamation, alopecia, and corneal ulcerations similar to staphylococcal scalded skin syndrome or acrodermatitis enteropathica-like syndrome, typically associated with diarrhea, may be seen secondary to acute protein malnutrition or essential amino acid deficiency, especially isoleucine deficiency [34, 52–54]. Immune dysfunction has also been suggested with an increased risk for viral or bacterial infections, but good studies are lacking [34]. Chronic moniliasis has been described and reflects the effect of propionyl-CoA and methylmalonate on T-cell number and function [7]. Finally, acute and chronic pancreatitis, osteopenia or osteoporosis, and ovarian failure may also occur; the etiologies of which remain unclear [3, 7, 24, 34, 36, 47, 55–59].

17.3 Pathophysiology

The pathogenesis of the clinical features of organic acidemias remains complex and incompletely understood. Complications arise despite apparent good metabolic control [29, 33, 47]. The metabolic blocks cause metabolite accumulation, triggering an endogenous intoxication. Propionyl-CoA and its metabolites inhibit the Krebs cycle resulting in reduced ATP synthesis and are known to have inhibitory effects on pyruvate dehydrogenase complex, N-acetyl-glutamate synthetase, and on the glycine cleavage system [7, 11, 12]. Further, methylmalonyl-CoA is known to inhibit pyruvate carboxylase [11, 60, 61]. Similarly, isovaleric acid causes marked inhibition of Na(+), K(+) ATPase activity [62]. Thus, there is an energy deficit secondary to substrate insufficiency and toxin accumulation [63]. In addition, these inhibitory effects appear to explain some of the clinical signs seen in MMA and PROP, such as hypoglycemia, lactic acidemia, hyperglycinemia, and hyperammonemia [11].

The etiology of the hyperammonemia seen in organic acidemias is different than that seen in urea cycle disorders. Recall that in the urea cycle, carbamoyl-phosphate synthesis is activated by N-acetylglutamate (NAG) [64]. Propionyl-CoA, which is accumulating in PROP, and isovaleryl-CoA, accumulating in IVA, are potent inhibitors of N-acetylglutamate synthase (NAGS) [9, 65]. Thus, NAG production is reduced, and lack of NAG results in carbamoyl-phosphate synthetase inhibition and elevated ammonia levels [65]. It has also been suggested that hyperammonemia may be related to inability to maintain adequate concentrations of glutamate precursors through a dysfunctional Krebs cycle secondary to accumulating methylcitrate and the decline in citric acid excretion [64].

In addition, it has become increasingly evident that there is significant mitochondrial dysfunction and impairment of the oxidative phosphorylation system [29, 41, 66–69]. This impairment is felt to be secondary to inhibition of the Krebs cycle enzymes citrate synthase, aconitase, and isocitrate dehydrogenase by methylcitrate, inhibition of pyruvate carboxylase by methylmalonic acid, and inhibition of pyruvate dehydrogenase complex,

succinyl-CoA synthetase, and ATP citrate lyase by propionic acid [70]. As a consequence, cellular depletion of available CoA occurs which results in impairment of energy metabolism [29]. Hence, oxidative phosphorylation impairment may be an additional mechanism to explain the late complications seen in organic acidemias [27, 29]. In addition, there are extensive mitochondrial ultrastructural changes in liver and kidney samples from MMA patients providing evidence of mitochondrial dysfunction and respiratory chain impairment [41, 66, 69]. Further, it is postulated that free radical production and oxidative damage may also be involved in the pathophysiology of these disorders [21, 27, 71–73].

17.4 Management

The goal of treatment of an individual with an organic acidemia is to reduce the accumulation of toxic metabolites; maintain normal growth, development, and nutritional status; and prevent catabolism [13]. Therapy is multifaceted and typically involves a diet based on the restriction of propiogenic amino acids combined with medication supplementation. Individualized dietary prescriptions, as prescribed by a metabolic nutritionist, balance the necessary intake of the restricted amino acids, other protein, and energy to provide the recommended daily allowances of nutrients and allow for adequate growth [74]. This is frequently accomplished by the use of special propiogenic amino acid-restricted metabolic formulas combined with a prescribed amount of natural protein provided by breast milk or regular infant formula in infancy and regular solid foods in older children [13]. Provision of protein intake modestly above the recommended daily allowance (RDA) is well tolerated and can provide a buffer against catabolism [47]. Target plasma range for restricted amino acids in PROP and MMA (isoleucine, valine, methionine, threonine) is low normal to normal [75]. In IVA, it is often sufficient to restrict natural protein to the recommended minimum daily requirements without the use of a leucine-free metabolic formula [13, 75]. Goal target plasma range for leucine is 50–180 μM or normal range for the laboratory and 200–400 μM for glycine [75].

For all patients, particular attention must be paid to adequate energy intake. Energy requirements have been reported to be lower than predicted for age and sex during the well-fed state secondary to lower energy expenditure [76–78]. During illness, however, resting energy expenditure increases, requiring increased caloric intake to prevent catabolism and decompensation [13, 74]. These needs may require the use of additional fat and carbohydrate sources or protein-free modules. Catabolism is the major reason for acute decompensation [47]. If individual amino acids are found to be low, supplementation may be required, but no studies prove the efficiency of consistent supplementation of isoleucine and valine [78]. Nutrition management guidelines have been published by Yannicelli and Knerr et al. [74, 75] and are described in Chap. 20.

Therapy of IVA varies slightly from that of PROP and MMA. Isovaleryl-CoA conjugates with glycine via the enzyme glycine-N-acylase, forming isovalerylglycine, and also binds with carnitine, via carnitine N-acylase, to form isovalerylcarnitine [79, 80]. Both products, isovalerylglycine and isovalerylcarnitine, are easily excreted in the urine. This feature is exploited for management. Thus, glycine (150–300 mg/kg/day) and carnitine (50–100 mg/kg/day) are both supplemented in individuals with IVA resulting in excretion of isovaleric acid [7, 9, 10, 13, 60, 61, 75, 79–83]. Subsequently, a strict metabolic diet may not be needed.

Supplementation of carnitine (100–400 mg/kg/day divided two to three times per day) is also an important aspect of the treatment of PROP and MMA [3, 7, 13, 19, 47, 74]. Provision of oral carnitine is effective in preventing carnitine depletion, regenerating the intracellular pool of free coenzyme A (CoA), and allows urinary excretion of propionylcarnitine, thereby reducing propionate toxicity [13, 75]. High doses of carnitine may cause a fishy odor due to overproduction of methylamines and may cause diarrhea [7, 74] but may be particularly helpful in PROP [47].

All patients with MMA should be tested for responsiveness to vitamin B_{12} [7, 13]. Testing regimes vary but responsiveness can be

determined by monitoring quantitative plasma or urine methylmalonic acid levels or by measuring metabolites via urine organic acid analysis. Vitamin B_{12} responsiveness leads to prompt and sustained decrease of propionyl-CoA byproducts [13]. Results should be confirmed by additional studies. Many vitamin B_{12}-responsive patients may need minimal to no protein or amino acid restriction [13]. In responsive patients, vitamin B_{12} is supplemented orally once per day or intramuscularly or subcutaneously daily or weekly with a beginning dose of 1 mg [13, 75]. A biotin-responsive form of PROP has not been seen, but biotin at 5–20 mg/day is sometimes supplemented in PROP [7, 19, 47, 74, 75].

As propionate production may result from gut bacteria, an intermittent antibiotic regime to reduce gut propionate production is sometimes implemented. The antibiotic metronidazole has been effective in reducing urinary excretion of propionate metabolites when used at a dose of 10–20 mg/kg once per day [10, 13, 74, 75]. The regime of therapy varies, but 7–10 consecutive days each month is a common practice [13, 19, 22, 75]. Some care providers prefer neomycin (50 mg/kg) because it is not absorbed [7]. Care must be taken to avoid complications associated with chronic antibiotic use including leukopenia, peripheral neuropathy, and pseudomembranous colitis [13]. Metronidazole may also cause anorexia and dystonia [47, 74]. There are no studies that evaluate the clinical efficacy of metronidazole in improving clinical outcome, reducing ammonia levels, or reducing episodes of acute decompensation [47]. Overall, results of use have been variable as measured by change in metabolite excretion likely reflecting a variable colonization of gut bacteria by organisms which may or may not produce propionate [7].

Supplementation with N-carbamylglutamate (50–100 mg/kg/day) has been suggested to help restore ureagenesis and improve hyperammonemia; however, limited information is available that supports chronic use of N-carbamylglutamate [12, 47, 84]. Similarly, chronic therapy with sodium benzoate (150–250 mg/kg/day) has been proposed to help correct chronic hyperammonemia and hyperglycinemia [19]; however, there is no evidence that supports a role of sodium benzoate in chronic treatment especially given the evidence that higher glycine levels may be indicative of good metabolic control [47, 85]. Multivitamins may be given to reduce the risk of micronutrient deficiency. Citric acid and ornithine alpha-ketoglutarate have also been proposed to help sustain Krebs cycle flux during illness [64, 86]. In addition, coenzyme Q10 and vitamin E have been suggested as possible therapies for MMA-related optic neuropathy or secondary respiratory chain deficiency [27, 87, 88], and angiotensin II inhibition has been suggested to help delay renal disease [89]. The role of growth hormone and supplemental alanine to promote anabolism has been suggested, but experience is limited [7, 47, 90–92]. Glutathione deficiency treated with high doses of ascorbate has also been reported [93].

In addition, prompt treatment of intercurrent illnesses, particularly those placing the individual at risk for catabolism (e.g., vomiting, diarrhea, fever), and avoidance of fasting are paramount to reduce the risk of acute decompensations. Many children develop anorexia and feeding difficulties necessitating the placement of a gastrostomy tube to prevent fasting and ensure adequate dietary intake [19, 55, 94]. Patients and families should be provided an emergency medical letter as well as a sick-day protocol [47]. A MedicAlert bracelet or necklace is also recommended [47].

Management of an acute decompensation involves reduction or discontinuation of protein and provision of calories to stop catabolism and promote anabolism by infusion of glucose and intralipid [3]. Fluid recommendations are standard for age. Using a 10 % dextrose solution at 120–150 mL/kg/day (or 1.5 times maintenance) often can provide the necessary level of glucose delivery [22, 75]. Rehydration should occur over a 48-h period to prevent cerebral edema [75]. Additional calories are added by using intralipid at 1–3 g/kg/day [22, 75]. If hyperglycemia develops, an insulin drip (0.01–0.1 units/kg/h) may be necessary, but dextrose delivery rate or amount should not be decreased [7, 22, 75]. Bicarbonate supplementation (1–2 mEq/kg) may be necessary to help correct acidosis [75]. If severe hyperammonemia is

present, hemodialysis or hemofiltration or ammonia scavenging medications may be necessary [19, 22, 75]. Recently, carbamylglutamate (50–100 mg/kg/day) has been demonstrated to be beneficial in controlling the hyperammonemia associated with an acute decompensation [19, 65, 95–99]. Administration of intravenous L-carnitine in relatively high doses (100–400 mg/kg/day) is used in acute illness [22, 75]. Metabolic decompensation in PROP may be complicated by hyperlacticacidemia due to thiamine deficiency, requiring vitamin supplementation (10 mg/kg/day) [75, 100, 101]. If the illness is prolonged, total parenteral nutrition may be necessary. Otherwise, reintroduction of protein occurs as tolerated, but should be reintroduced within 24–36 h of therapy initiation [22, 75]. Frequent monitoring of laboratory studies and for possible complications is required.

For a fragile, medically intractable individual, liver, renal, or combined liver-kidney transplantation may be considered [102–108]. Transplantation is not a cure as it only partially corrects the enzymatic defect, but may result in improved metabolic stability, neurologic function, and quality of life [19, 47, 63, 109–115]. Liver transplantation has also been shown to improve cardiomyopathy [44, 116]. Dietary therapy, perhaps liberalized, and carnitine supplementation are continued following transplantation [102, 105, 107, 109, 117]. Neurologic dysfunction, including metabolic stroke, and renal disease are not always prevented with transplantation [7, 11, 13, 19, 47, 118, 119]. One-year survival rate following transplant was 72.2 % in a multisite, retrospective study of 12 individuals with PROP [47, 110].

17.5 Monitoring

Monitoring of patients with organic acidemias will vary according to each clinics' practice but should occur with some degree of regularity. Patients should be seen routinely in clinic with routine monitoring of laboratory studies. Quantitative plasma amino acids should be obtained at least monthly in all patients managed with a restricted diet, although this practice varies between clinics. Quantitative methylmalonic acid levels are available in selected laboratories and may be used to follow individuals with MMA [7]. There is no established biomarker for monitoring therapeutic control in IVA [9]. Propionate levels may be difficult to obtain for individuals with PA; some advocate following the citrate-to-methylcitrate ratio via quantitative urine organic acid analysis if available [120]. Propionylcarnitine has not been demonstrated to correlate with severity or level of control [47]. Ammonia, acid-base balance, and anion gap have been demonstrated to be important biochemical parameters in identifying an impending metabolic decompensation and to assess severity of PROP and MMA patients [121, 122]. The frequency of monitoring laboratory studies varies pending the patient's age and clinical stability. Laboratory studies to obtain every 6–12 months include complete blood count, complete metabolic panel (to include electrolytes, renal and liver function studies), carnitine, urinalysis, β-type natriuretic peptide, and calculated glomerular filtration rate as well as annual nutrition monitoring studies to include prealbumin, 25-hydroxy vitamin D, vitamin B_{12}, iron, ferritin, and other micronutrients (thiamine, selenium) [47]. Additional laboratory studies to consider during acute illness include complete blood count, complete metabolic panel (to include electrolytes, renal and liver function studies), amylase, lipase, ammonia, osmolality, lactate, coagulation studies, creatine kinase, and urine ketones. Families can also be taught to test for urine ketones using Ketostix at home as an early warning sign for pending decompensation [7] (Box 17.3).

In addition to laboratory studies, management of an individual with an organic acidemia often requires the involvement of additional subspecialty services including neurology, nephrology, cardiology, neuropsychology, and ophthalmology. The utilization of these subspecialties is individualized to the clinical presentation of the patient. The patient may be seen yearly if only monitoring due to increased risk or may be seen frequently if organ system involvement is already noted [47]. Cardiology evaluation, however, with echocardiogram, ECG, and 24-h Holter monitoring is

Box 17.3: Laboratory Monitoring in Organic Acidemias[a]

Routine:

- Plasma amino acids
- Prealbumin
- Serum methylmalonic acid concentrations (MMA)
- Urine organic acids
  - Urinary citrate-to-methylcitrate (MC) ratio (MC ≤ 2 times citrate in PROP)

Annual

- Complete blood count
- Electrolytes, renal and liver function tests
- Carnitine (total, free, and esterified)
- Nutrient adequacy: (vitamin D, B_{12}, thiamin, iron studies, minerals (zinc, selenium))
- β-type natriuretic peptide
- Urine analysis

Acute illness (additional)

- Amylase, lipase, ammonia, ketones

[a]Frequency depends on age of patient and clinical status, recommended monthly routine laboratory evaluations.

recommended yearly in individuals with PROP [47]. Schreiber et al. also recommended a baseline EEG and repeat studies as clinically indicated in all patients with PROP [21]. Long-term and repeated neuropsychological assessment is an excellent tool for tracking developmental progress or decline over time. Early evaluation and, if necessary, intervention is recommended beginning at a young age. Routine bone densitometry (DEXA scan) is also recommended for all patients typically beginning at age 5 years [123].

17.6 Summary

The outcome of individuals with organic acidemias is quite variable. In general for PROP and MMA, late-onset forms appear to have a better prognosis as compared with early-onset forms, mut^- MMA patients appear to do better than mut^0 patients, and individuals with vitamin B_{12}-responsive MMA appear to have improved outcome over patients with vitamin B_{12}-unresponsive forms [3, 8, 20, 24, 40, 94, 124, 125]. In MMA, an earlier age of onset, the presence of hyperammonemia at diagnosis, and a history of seizures also predicted more severe impairment [126]. Also, in general, individuals with IVA appear to have a better outcome than those with MMA or PROP; however, in contrast to MMA and PROP, the neurocognitive outcome in patients with a neonatal presentation is more favorable than in patients with a late diagnosis [127]. Mortality has been reported to be >80 % in the neonatal-onset form of these disorders and as high as 40 % before 16 years of age in the late-onset forms [19, 128]. Survival has improved [19]. The survival at 1 year of age in patients with mut^0 was 65 % in the 1970s but has increased up to 90 % in the 1990s [11]. Death may be due to cerebral edema, cerebral or cerebellar hemorrhage, infection, renal failure, heart failure, arrhythmias, cardiomyopathy, pancreatitis, or irreversible metabolic decompensation [7, 34, 45, 51, 128–131].

Morbidity is also high with frequent complications, poor growth and nutritional status, poor neurodevelopmental progress with frequent progressive neurocognitive deterioration, abnormal neurologic signs such as chorea and dystonia, and frequent and severe relapses of metabolic decompensation [3, 4, 8, 19, 51]. Overall, developmental outcome is poor in PROP and MMA, with the majority of patients demonstrating developmental delay [4, 19, 34, 51, 55, 63, 127]. Martin-Hernandez et al. reported on the long-term needs of adult patients with organic acidemias [24]. In this series of 15 patients, largely with late-onset disease, two-thirds of the patients had neurologic or visceral complications and three-quarters of them required some kind of social support [24]. In contrast, developmental outcome in IVA is normal in 60 % or more of the patients [63, 127]. In addition, long-term complications and the risk of metabolic decompensations associated with catabolic stress in individuals with IVA are also much less frequent than compared to PROP and MMA [24, 127].

Evaluation of older patients is beginning to suggest a risk of psychiatric disorders [63].

Outcome and prognosis, however, may be changing and improving with early identification via newborn screening. A benign MMA phenotype has been described, and some patients with MMA have remained symptom-free [1, 132, 133]. In addition, infants diagnosed with IVA by newborn screen have also remained asymptomatic with carnitine supplementation and mild or no dietary restriction and retrospectively identified siblings ranging in age from 3 to 11 years were also asymptomatic [5, 9]. Dionisi-Vici et al. compared the outcome of 29 patients with MMA, PROP, or IVA diagnosed clinically to 18 similar patients diagnosed by newborn screening. The newborn-screened population demonstrated an earlier diagnosis, significantly reduced mortality (11 % compared to 51 %), and an increased number of patients with normal development at <1 year of age [8]. A more stable clinical course with less frequent relapses of decompensation was also demonstrated [8]. Similar findings were also found by Grünert et al. in a population of PROP patients; however, they did not demonstrate a reduction of complications in patients diagnosed by newborn screening [134]. Overall, newborn screening and early diagnosis may result in decreased early mortality, decreased severity of initial symptoms, and improved neurodevelopmental outcome [8]. Outcome data, however, is early and limited, and more long-term follow-up studies are needed.

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Glutaric Acidemia Type I: Diagnosis and Management

18

Curtis R. Coughlin II

Contents

Core Messages

- Glutaric acidemia type 1 (GA-1) is an autosomal recessive disorder of lysine, hydroxylysine, and tryptophan metabolism caused by a deficiency of glutaryl-CoA dehydrogenase. It results in the accumulation of 3-hydroxyglutaric and glutaric acid [1].
- Patients can present with brain atrophy and macrocephaly, often with concurrent acute dystonia triggered by an intercurrent childhood infection and often with fever. This can occur anytime during the first 6 years of life, with a vulnerable period between 6 and 18 months of age [2].
- GA-1 is identified by elevated glutarylcarnitine (C5DC) on the newborn screening panel.

18.1 Background

Glutaric acidemia type 1 (also referred to as glutaric aciduria type 1) is a cerebral organic aciduria involved in lysine and tryptophan metabolism. Glutaric acidemia type 1 (GA-1) is characterized by a complex movement disorder, which is the result of an injury to the basal ganglia (striatal necrosis). Striatal damage typically

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occurs during an acute encephalopathic crisis, which is often precipitated by an illness or other catabolic stress.

Treatment for GA-1 involves management of illnesses to avoid an acute encephalopathic crisis, dietary lysine restriction, and carnitine supplementation. Implementation of treatment has vastly reduced the incidence of neurologic sequelae in patients, although this outcome is dependent on an early diagnosis and initiation of treatment to prevent acute crises and subsequent striatal damage.

GA-1 is a rare organic acidemia with a worldwide incidence estimated at 1:100,000 live births. The incidence of GA-1 is significantly higher in genetically isolated communities such as the Amish community, Canadian Oji-Cree natives, and the Irish travelers [3–6]. Screening in these high-risk populations has allowed for prospective treatment of patients prior to neurologic injury and has significantly reduced the risk of striatal injury [4]. As a result, newborn screening (NBS) for GA-1 has been uniformly adopted by public health screening programs (Chap. 2). Prior to screening, patients with GA-1 were identified based on characteristic neurologic findings and often had marked elevations of abnormal metabolites. NBS has identified a number of asymptomatic patients in whom confirming a diagnosis has been problematic.

18.2 Clinical, Genetic, and Biochemical Findings

18.2.1 Phenotype

The initial patients reported with GA-1 were siblings who, after a period of normal development, experienced significant neurological deterioration in the first year of life [7]. As more patients with GA-1 were identified, a distinctive neurologic phenotype began to emerge including a complex movement disorder (dystonia and akinetic-rigid parkinsonism) and acute bilateral striatal injury identified on brain imaging. Prior to initiation of large-scale screening programs, the diagnosis of GA-1 was dependent on the clinician recognizing the distinct phenotype.

If identified through NBS or prior to an encephalopathic crisis, patients are asymptomatic with only macrocephaly as a characteristic physical finding. In one retrospective review, macrocephaly was present in 74 % of patients at birth [8]. The highest risk for encephalopathic crisis occurs during a critical period of brain development (age 3–36 months), and 95 % of affected individuals have an encephalopathic crisis prior to 24 months of age [8–10]. The vulnerable period for neurologic injury is often reported as the first 6 years of life, as the oldest reported patient with an encephalopathic crisis experienced a repeat crisis at 70 months of age [8]. As a result, the benefit of strict dietary treatment has been questioned after the age of 6 years [10].

Dystonia and axial hypotonia are reported to be the predominant neurologic findings following an encephalopathic crisis, although dyskinesia and slight spastic signs have also been reported [11, 12]. The phenotype of GA-1 does evolve with time, and a fixed dystonia and akinetic-rigid parkinsonism have been reported in older patients [13].

Brain imaging (typically through magnetic resonance imaging or computerized tomography) is often performed following an encephalopathic crisis, and certain imaging findings have been suggested as pathognomonic for GA-1. Patients are often noted to have widening of Sylvian fissures and frontotemporal atrophy, and these findings may be evident in the newborn period. A subset of patients have developed an acute subdural hemorrhage, which is an important clinical entity as parents have even been investigated for non-accidental trauma prior to the diagnosis of GA-1 being established [4]. Following an acute crisis, the basal ganglia is affected, and this injury typically affects the putamen and caudate as well as the pallidum [14]. White matter changes are also noted on MRIs of patients as they age including those patients who remain asymptomatic.

18.2.2 Genetics and Biochemistry

GA-1 is an autosomal recessive disorder which results from mutations in the glutaryl-CoA dehydrogenase (*GCD*) gene [15]. Although a few

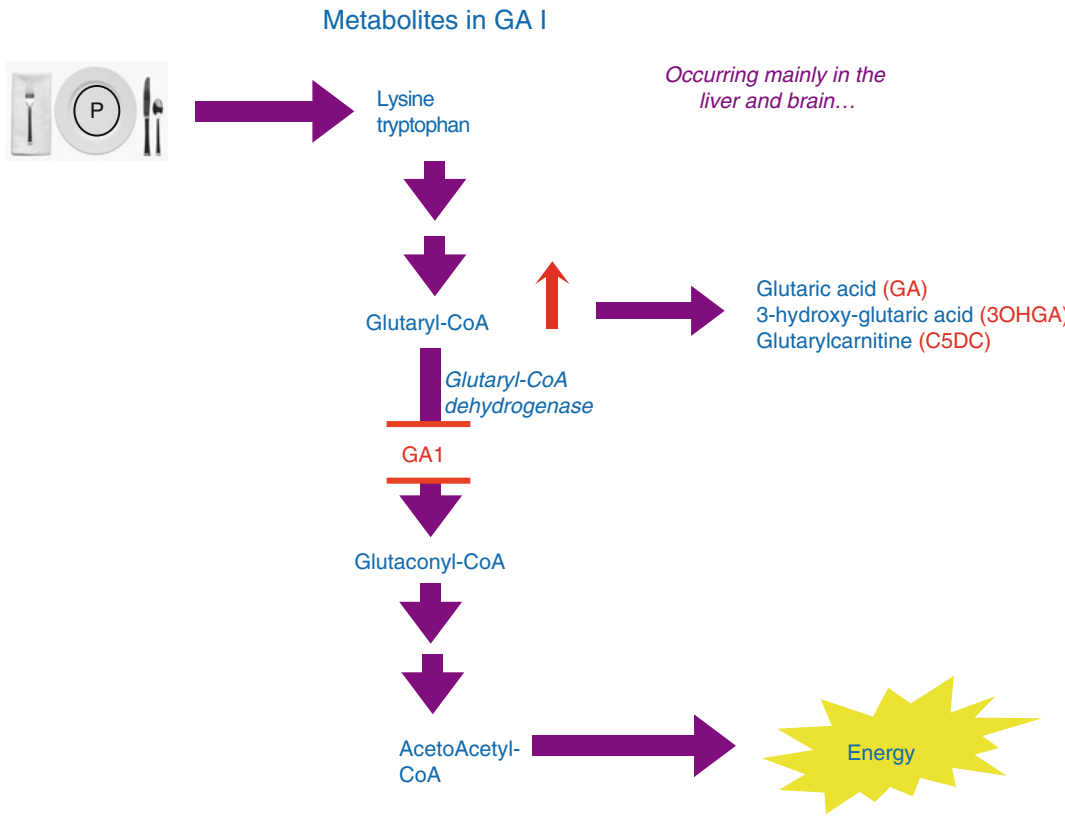

Fig. 18.1 Metabolism of lysine and tryptophan in GA-1. Defects in GCDH result in the accumulation of glutaryl-CoA and subsequently GA, 3OHGA, and C5DC (©2008 by the CHCO IMD Clinic, Aurora Colorado)

recurrent mutations have been identified, especially in isolated genetic populations, the majority of individuals have private or familial mutations. *GCD* encodes the enzyme glutaryl-CoA dehydrogenase (GCDH) that is involved in the degradation of the amino acids lysine, hydroxylysine, and tryptophan. A deficiency of GCDH results in an increased amount of glutaryl-CoA, and subsequently glutaryl-CoA is pushed into alternate metabolic pathways. This leads to the characteristic accumulation of glutaric acid (GA), 3-hydroxyglutaric acid (3OHGA), and glutarylcarnitine (C5DC) (Fig. 18.1).

GA-1 is often referred to as a "cerebral organic aciduria" as the phenotype is isolated to that of a cerebral or neurologic phenotype. Lysine crosses the blood-brain barrier through a specific sodium-independent, facilitative amino acid transporter known as γ+. Lysine competes with arginine, ornithine, and homoarginine for cellular uptake through the transporter. As a result, arginine supplementation has been suggested as possible adjunct therapy in GA-1. Arginine would theoretically reduce the cerebral uptake of lysine by overwhelming the γ+ transporter with arginine [16, 17]. The benefit of arginine supplementation in the treatment of GA-1 is still unclear.

18.3 Diagnosis and Management

Diagnosis: Prior to the initiation of screening programs, the diagnosis of GA-1 was suspected either as a result of characteristic brain imaging or the presence of a movement disorder. Abnormal biochemical findings would then support the already suspected diagnosis of GA-1. As previously discussed, the defect in GCDH subsequently results in the accumulation of GA, 3OHGA, and C5DC. The accumulation of these metabolites is dependent on a number of factors, and two distinct groups of patients have been

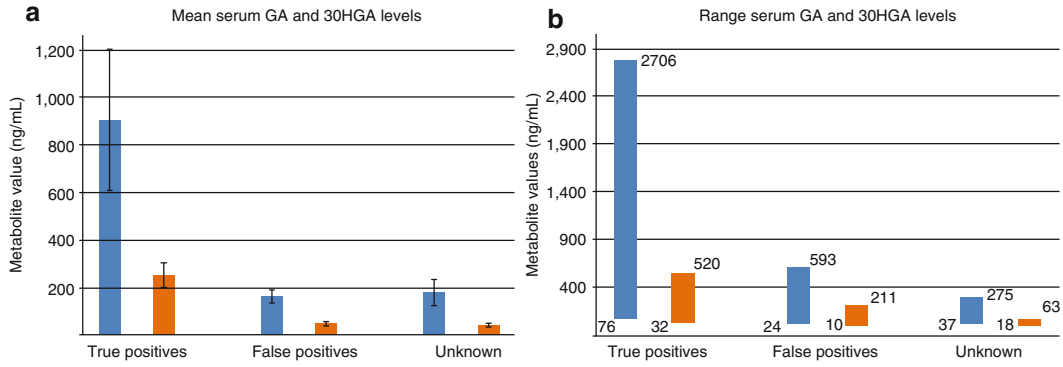

Fig. 18.2 Glutaric acid and 3-hydroxyglutaric acid concentrations in affected and unaffected subjects. (**a**) Mean serum GA (*blue*) and 3OHGA (*orange*) levels are significantly different between affected and unaffected patients. (**b**) There is overlap in the range of GA and 3OHGA levels between affected and unaffected patients often making a single metabolite uninformative. Subjects' affected status was classified as true positive, false positive, or unknown by the referring physician through a retrospective survey. All serum samples were analyzed in the Goodman Biochemical Laboratory (Chap. 8)

reported based on the degree of GA intermediates that are excreted. Patients whose accumulation of GA, 3OHGA, or C5DC is not significantly elevated are referred to as having a "low excretor" phenotype as compared to patients with elevations in these metabolites who are referred to as "high excretors."

As a result of NBS, the majority of individuals with GA-1 are now diagnosed shortly after birth and prior to striatal injury. NBS typically detects elevations of C5DC in dried blood spots, although the efficacy of NBS for GA-1 is still unknown [18]. Individuals with GA-1 have had false-negative NBS [19], and unaffected individuals have had false-positive NBS.

A number of affected patients with GA-1 have had false-negative NBS due to the low excretor phenotype which resulted in minimal elevations of C5DC [20]. In some isolated genetic populations, the low excretor phenotype is more common, and other strategies, such as DNA-based NBS, have been utilized to increase the sensitivity and specificity of NBS [3]. Other congenital or acquired disorders can also result in elevations of C5DC and cause false suspicion for GA-1. Individuals with renal insufficiency have decreased excretion of C5DC, which results in increased retention of C5DC and elevated C5DC in serum [21]. Also other inborn errors of metabolism (IEM) may have elevations of C5DC or elevations of C10-OH (C10-OH cannot easily be differentiated from C5DC) requiring further testing to different GA-1 from other inborn errors of metabolism [22].

In order to correctly diagnose true positives for GA-1 following a positive NBS, often further metabolite testing is recommended. This can include an acylcarnitine profile for repeat measurement of C5DC, urine organic acids, and quantification of GA and 3OHGA. In a retrospective review of serum GA and 3OHGA, there was significant overlap between the metabolite values of those individuals with confirmed GA-1 (true positives) and those who were false positive (Fig. 18.2).

Due to this significant overlap, the value of GA and 3OHGA alone is not suitable to identify affected individuals. Of note, elevations of GA are also common in other IEM as well as acquired disorders, and patients should be evaluated for other conditions when appropriate (Table 18.1).

The diagnosis of GA-1 can only be confirmed by deficient GCDH enzyme activity or the presence of two known disease-causing mutations in *GCD* [10]. This is especially important in cases of non-accidental trauma and in those asymptomatic individuals identified through screening programs.

The normal neurologic outcome in early-treated patients is dramatic as compared to the

natural history of GA-1. Despite its significant benefits, treatment does place a burden on the family. As a result, it is desirable to only treat those individuals who are deemed "at risk" for neurologic injury. Unfortunately no discernable biochemical marker has been associated with neurologic outcome in GA-1. As reported by Christensen et al., the clinical outcome in those patients with a low excretor phenotype is identical to those individuals with a high excretor phenotype (Fig. 18.3) [23]. Similarly, Kolker et al. reported that the age at encephalopathic crisis between high and low excretors is similar (Fig. 18.4) [8]. This suggests that the degree of metabolite excretion does not correlate with the risk for neurologic damage. Even patients with significant residual enzyme activity (up to 30 % of enzyme activity) have had a severe neurologic damage following an encephalopathic crisis [25].

Table 18.1 Known causes of glutaric acid elevations in plasma or urine

Inborn errors of metabolism
Glutaric aciduria type I
Glutaric aciduria type II (multiple acyl-CoA dehydrogenase deficiency)
Glutaric aciduria type III (glutaryl-CoA oxidase deficiency)
Glycerol kinase deficiency
HMG-CoA lyase deficiency
Methylmalonic aciduria
Mitochondrial disorders
2-oxoadipic aciduria
Propionic aciduria
Additional causes
Bacterial production
MCT containing formulas
Riboflavin deficiency (acquired)
Valproic acid

In the absence of a genetic, enzymatic, or biochemical correlation with phenotype, it is important that every patient with GA-1 be treated similarly and that no patient be labeled as having a mild clinical phenotype [10]. Inadequate treatment can lead to irreversible neurologic damage, and, as a result, a marker of disease control would be ideal.

The use of accumulating metabolites for disease control is a common paradigm in the treatment of IEM such as the use of phenylalanine to evaluate the treatment of an individual with phenylketonuria. As already discussed, the degrees to which metabolites accumulate differ significantly between affected individuals as exemplified by the low excretor phenotype. But just as these metabolites are not indicative of overall outcome, there is no evidence that GA, 3OHGA, or C5DC are reliable biochemical markers for the monitoring of nutrition treatment [10].

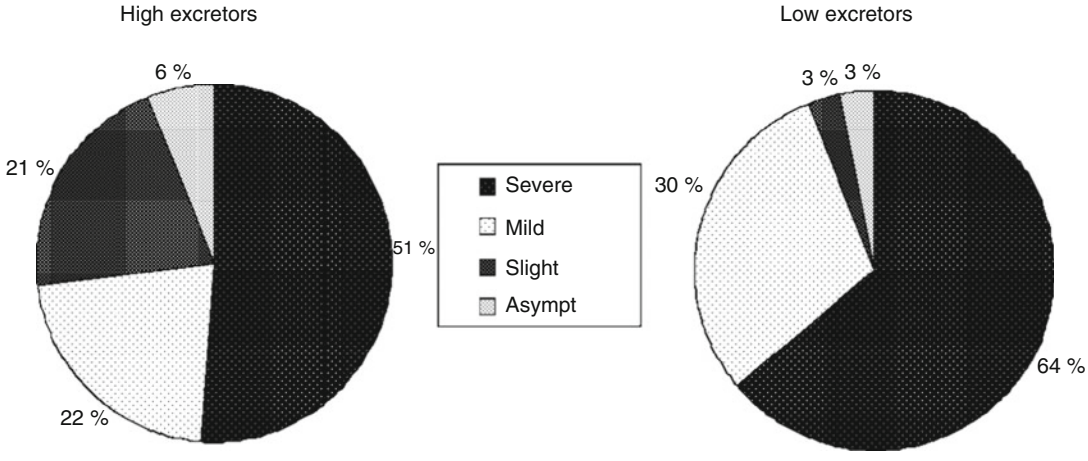

Fig. 18.3 Phenotype of 76 GA-1 patients classified as previously published [23, 24]

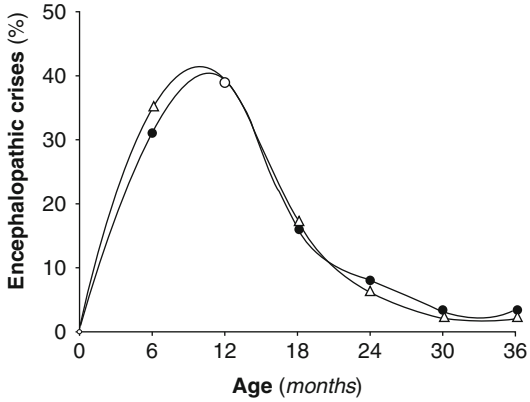

Fig. 18.4 Acute encephalopathic crises. Age at first encephalopathic crisis in high (●) and low excretors (Δ) [8]

18.4 Treatment

Emergency management: In the majority of cases, striatal necrosis occurs during an encephalopathic crisis usually precipitated by an infectious illness, although in 10–20 % of patients, neurologic symptoms are present without a precipitating event [26]. As a result, emergency treatment of infectious illness is paramount to avoiding an encephalopathic crisis and striatal injury. Since it is impossible to determine when an acute crisis may occur, patients should receive emergency management during every illness and surgical intervention. Emergency treatment in GA-1 is similar to that in other IEM and includes avoidance or reversal of the catabolic state by providing a source of energy, reduction of lysine or natural protein, carnitine supplementation for detoxification and treatment of the present illness [27]. Consensus recommendations have developed to implement emergency treatment at the first sign of an intercurrent illness [4, 10].

18.5 Summary

Individuals with GA-1 are at risk for irreversible neurologic damage resulting in a debilitating, complex movement disorder. Without a reliable biomarker to stratify those at risk, treatment should be initiated in all affected patients and emergency management should be implemented at the first sign of illness.

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Nutrition Management of Glutaric Acidemia Type 1

19

Laurie E. Bernstein

Contents

Core Messages

- Glutaric acidemia type 1 (GA-1) is an autosomal recessive disorder of lysine, hydroxylysine, and tryptophan metabolism.
- A defect of glutaryl-CoA dehydrogenase results in the accumulation of 3-hydroxyglutaric acid and glutaric acid.
- Nutrition management of GA-1 consists of restricting lysine and tryptophan, supplementing L-carnitine, and providing sufficient energy to prevent catabolism.
- Patients with GA-1 have a particularly high risk of permanent cerebral damage from a metabolic crisis.

19.1 Background

Glutaric acidemia type 1 (GA-1) is an autosomal recessive disorder of lysine, hydroxylysine, and tryptophan metabolism caused by a deficiency of glutaryl-CoA dehydrogenase (Fig. 19.1).

GA-1 results in the accumulation of 3-hydroxyglutaric acid and glutaric acid in the urine [1, 2], the metabolites most likely associated with the risk of neurological damage (Box 19.1).

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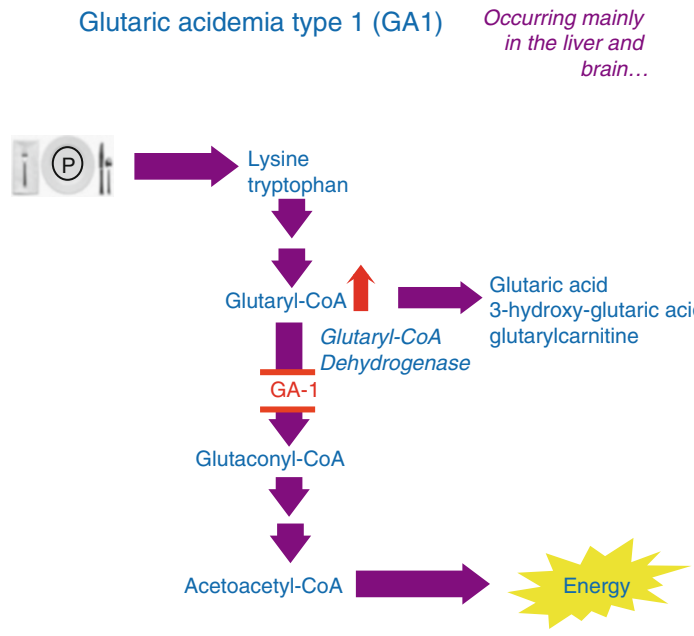

Fig. 19.1 Metabolic pathway of glutaric acidemia type 1 (GA-1)

Box 19.1: Principles of Nutrition Management in GA-1

Restrict: Lysine and tryptophan

Supplement: L-carnitine, riboflavin[a], pantothenic acid[a]

Toxic metabolites: 3-hydroxyglutaric acid and glutaric acid[b]

[a]Practice varies – supplemented in some clinics.

[b]These metabolites accumulate but concentrations are not related to patient outcomes.

However, they have not been reliable when used to assess patient outcomes [3].

The management of GA-1 poses several challenges: (1) metabolic decompensations are associated with a very high risk of permanent neurological insult in GA-1; (2) good biomarkers to guide therapy have not been identified; and (3) there is a lack of agreement about how long strict dietary treatment is necessary. The risk for neurological damage appears to be greatest in newborns and early childhood, when cerebral lysine uptake is the highest [1, 2]. There are no published reports of acute encephalopathic crises in children over age 6 years, but there are documented cases of chronic neurological deterioration in patients with late-onset disease without crises [4]. Diet management using medical food and protein restriction rather than treatment with a protein restriction alone may be advisable [4, 5]. Many clinics continue to recommend a less stringent, but lifelong, dietary treatment despite the current understanding of the efficacy of the diet after age 6 years. A further description of the diagnosis and management of GA-1 is described in Chap. 18.

19.2 Nutrition Management

19.2.1 Chronic Nutrition Management

Expanded newborn screening allows for early diagnosis of GA-1 that leads to timely, preventive

Table 19.1
Recommended daily intake for a patient with GA-1 [1, 4, 8]

Age	Protein[a] (g/kg)	Lysine[a] (mg/kg)	Tryptophan[a] (mg/kg)
Birth to 6 months	2.75–3.5	65–100	10–20
6 months to 1 year	2.5–3.25	55–90	10–12
1 year to 4 years	1.8–2.6	50–80	8–12
4 years to 7 years	1.6–2.0	40–70	7–11

Energy, vitamin,* and mineral intakes should meet the DRI and normal fluid requirements
*Some clinics recommend supplemental riboflavin (100 mg/day) and pantothenic acid (400–600 ug/kg/day)
[a]These are average ranges. Adjustments should be made based on growth, laboratory findings and health status

Box 19.2: Initiating Nutrition Management in an Asymptomatic Infant with GA-1

Goal: Prevent neurological insult associated with metabolic crises.

Step-by-step:

1. Establish intake goals based on clinical status and laboratory values.

Intake goals

Age (days)	Protein [1] (g/kg)	Lys [1, 8] (mg/kg)	Lysine/arginine ratio [8]	Carnitine [8] (mg/kg)	Riboflavin[a] [10] (mg)	Pantothenate[b] [8] (μg/kg)
5	2.75–3.5	65–100	1:1.5–1:2	75–100	100	400–600

2. Calculate the amount of infant formula/breast milk[c] needed to meet lysine needs.
3. Determine the amount of protein provided by the whole protein source.
4. Calculate the amount of medical food required to meet remaining total protein needs (Fig. 19.2).
5. Calculate arginine provided by whole protein and GA-1 medical food to ensure lysine-to-arginine ratio is correct. Supplement arginine if needed.
6. Determine the calories provided by both the whole protein source and GA-1 medical food. Provide the remaining calories from a protein-free medical food.
7. Determine the amount of fluid required to make a formula that provides 20–25 kcal/oz (depending on energy needs and volume tolerated).

[a]Some clinics supplement riboflavin. Administer 15–25 mg mixed into 3–4 feedings per day for maximum absorption [10].
[b]May not be supplemented by all clinics. Check individual clinical protocols for guidance.
[c]In severe forms, expressed breast milk is recommended.

management, thereby reducing the risk of acute neurological damage associated with untreated GA-1 [3]. Minimizing the risk of cerebral damage and maintaining normal development and growth are the overarching goals of the nutrition management of GA-1 [6, 7]. The most critical component of nutrition management in patients with GA-1 is the prompt treatment of intercurrent illnesses. L-carnitine supplementation is also an integral component of management.

The diet for a patient with GA-1 is restricted in the amino acids lysine and tryptophan. The goals for nutrient intake are provided in Table 19.1. When well, the patient with GA-1 has normal requirements for most other nutrients, including energy, vitamins, and minerals (Box 19.2). Medical foods free of lysine and tryptophan are used to meet protein goals (Fig. 19.2). These medical foods provide varying amounts of essential amino acids, fat,

	Arginine (mg)	Tryptophan (mg)	Carnitine (mg)	Energy (kcals)
GlutarAde™ Essential[a]	1082	60	10	154
GlutarAde™ Junior[a]	1080	60	30	410
GlutarAde™ AA Blend[a]	1072	60	30	40
Glutarex®-1[b]	1033	0	600	320
Glutarex®-2[b]	1033	0	600	136.6
GA-1 Anamix® Early Years[a]	874	0	0.01	350
XLysXTrpMaxamaid®[a]	890	0	0	130
XLysXTrpMaxamum®[a]	880	0	0	76
GA Gel™[c]	830	60	11	81
GA Express™[c]	815	63	11	49.5
GA™[d]	688	0	0	331

[a]Nutricia North America (Rockville MD; nutricia-na.com)
[b]Abbott Nutrition (Columbus OH; abbottnutrition.com)
[c]Vitaflo USA (Alexandria,VA; vitaflousa.com)
[d]Mead Johnson Nutrition (Evansville IN; meadjohnson.com)

Fig. 19.2 Comparison of medical foods for GA-1 (per 10 g protein)

carbohydrate, vitamins, and minerals as well as L-arginine. In the dietary management of GA-1, it is important to note that there is less tryptophan in whole protein than lysine (on a molar basis); therefore, restricting lysine may cause an over-restriction of tryptophan. Blood concentrations of both amino acids require close monitoring (Sect. 19.3).

Arginine competes with lysine for uptake across the blood-brain barrier and should be provided at 1.5–2 times that of dietary lysine. Strauss et al. [8] recommend providing measured amounts of both arginine and lysine at the same time as the key to efficacy [8]. Reports of improved outcomes in patients consuming a diet providing the recommended lysine-to-arginine ratio have been published, although brain concentrations of these amino acids have not been quantified [8].

Standard infant formula or breast milk provides lysine and tryptophan during infancy. Due to the risk of neurological consequences associated with energy deprivation and catabolism, close monitoring of intake and appropriate weight gain is crucial in all infants. In breastfed infants, weight gain is the primary measure of caloric adequacy. Solid foods that are naturally low in protein (lysine) may be introduced when developmentally appropriate for the child and specialty low protein foods may be used to provide sufficient energy and variety to the diet. Providing sufficient energy can be challenging in patients with GA-1. Those who have sustained cerebral damage usually present with severe dystonia and choreoathetosis, interfering with the patient's ability to eat normally. If severe enough, the patient may require a gastrostomy tube [7]. Energy needs may be increased in patients with dystonia [9] or decreased in patients who are nonambulatory [7].

Monitoring the ratio of lysine to arginine concentrations in the blood, as well as in the diet, may also prove to be a useful strategy when treating patients with GA-1.

L-carnitine supplementation is routinely provided to patients with GA-1 as a way to reduce intramitochondrial glutaryl-CoA and provide extracellular release without the synthesis of glutaric acid and 3-hydroxyglutaric acid. L-carnitine conjugates with coenzyme A esters to form acylcarnitines. The typical L-carnitine dose is 75–100 mg/kg/day or sufficient quantities to maintain free L-carnitine concentrations within the normal range [4]. Large doses of enteral L-carnitine may cause loose stools or diarrhea [11]. In the hospitalized patient with acute illness, a continuous infusion of intravenous L-carnitine is preferably provided.

Glutaryl-CoA dehydrogenase is a riboflavin-dependent enzyme that converts glutaryl-CoA to glutaconyl-CoA. Once the diagnosis is confirmed, a trial of pharmacological doses of riboflavin (100–200 mg/day) may be successful in lowering glutaric acid or 3-hydroxyglutaric acid in some patients with specific responsive mutations [12]. Results range from neurological improvement and reduced urinary glutaric acid excretion in one patient [12] to a reported 20 % increase in residual activity of glutaryl-CoA dehydrogenase [13]. Some centers recommend routine riboflavin supplementation regardless of response. Many preparations of riboflavin are distasteful and cause staining due to the bright orange color of the vitamin [8, 13]. High doses of riboflavin have been reported to cause gastric distress. The recommended regimen is to provide 15–25 mg of riboflavin three to four times per day with food for maximum absorption [10], but some have reported starting patients on 50–100 mg, twice per day.

19.2.2 Acute Nutrition Management

Sick-day protocols for home use are used extensively for many inherited metabolic diseases, including organic acidemias, but the practice is different with GA-1. The risk for neurological injury is highest during illnesses with reduced energy intake, fever, and associated catabolism. Very aggressive treatment and a zero tolerance with regard to hospital admission during any of these presentations can help prevent permanent neurological damage. Thus, if a patient has an illness in which he or she is not consuming adequate energy due to vomiting, poor intake, or diarrhea and/or if the patient has a fever (>38.5 °C), it is considered a medical emergency and the patient must be seen in the emergency department immediately. The consequences of an acute metabolic crisis are dire and include irreversible neurologic sequalae involving damage to the basal ganglia (striatal necrosis), which can cause a normally developing infant or child to have a lifetime of severe physical and developmental disabilities. During an illness that is associated with catabolism, maintaining usual therapy ("well-day" diet) and supplementing L-carnitine is NOT sufficient to prevent an acute crisis; additional nonprotein energy sources must be provided. Management of a sick-day diet at home must be done with the guidance of the metabolic physician, and the threshold for seeking emergency treatment is very low, even for relatively minor illnesses, particularly during the first 6 years of vulnerability. Sick-day management includes reducing natural protein intake, continuing consumption of a lysine and tryptophan-free medical food, and providing extra sources of protein-free energy (e.g., Pro-Phree®, Duocal®, SolCarb®, Polycal®) (Box 19.3). The L-carnitine dose is often increased as well.

Sick-day management is difficult to do at home; the key is in reducing whole protein intake, providing sufficient L-carnitine, and consuming enough energy to prevent catabolism. All patients with GA-1 should have a written emergency department protocol that can be referenced if the patient is seen at a hospital unfamiliar with the management of GA-1. In such cases, the patient's metabolic physician should be contacted and consulted regarding management. Acute medical management must

Box 19.3: "Sick-day" Nutrition Management of a Patient with GA-1

This diet may be used at home for minor illnesses. If the patient does not improve over a relatively short period of time or if energy intake is inadequate, it is considered a medical emergency in GA-1.

	Children's Hospital Colorado[a]	Strauss [8]
Energy	110–120 % of usual intake	95–115 kcal/kg
Whole (natural) protein (g/kg/day)	0.6–0.7	0.5
Lysine-free medical food (g/kg/day)	Maintain current	1.5–2.0
L-carnitine[b] (mg/kg/day)	50	100

[a]Children's Hospital Colorado, GA-1 IMD Clinic protocol; please check your clinic's protocol.

[b]Use caution in diarrheal illnesses.

Box 19.4: Transitioning a Hospitalized Patient with GA-1 from a "Sick-Day" to a "Well-Day" Diet

1. Introduce the sick-day diet (Box 19.3) as soon as the child can tolerate feedings, initially given in combination with IV dextrose to meet energy goals.
2. Wean IV dextrose[a] as formula intake approaches maintenance well-day diet volume.
3. Transition gradually to well-day formula to provide at least half of the protein/lysine intake, starting within 24–36 h after admission.
4. Gradually transition to full intake of the well-day formula prescription before discharge.

[a]See Appendix J

commence quickly to avoid catabolism and includes discontinuing protein feeds for 24–36 h, providing sufficient energy intake, and managing the underlying illness. When admitted to the emergency room, intravenous glucose such as glucose 10 % at 1.5 times maintenance and Intralipid® (Baxter Healthcare, Deerfield, IL) at 2 g/kg/day are often rapidly added, particularly for children with compromised oral intake due to illness. Cessation of the essential amino acid lysine should be limited in duration since its deficiency will induce catabolism resulting in adverse effect. Thus, usually within 24–36 h of cessation of protein feeds, the patient can begin to transition whole protein feeds back to his or her usual diet (Box 19.4).

19.3 Monitoring

Laboratory markers that are good indicators of the clinical status of patients with GA-1 are lacking. While there is no direct relationship between excretion of glutaric acid and 3-hydroxyglutaric acid and patient outcomes, some clinics may consider increases in the concentration of 3-hydroxyglutaric acid or glutaric acid as warning signs of changes in health status and may elect ongoing monitoring of such metabolites.

Nutritional monitoring includes ensuring adequate growth and nutrient intake, especially markers of protein status (Chap. 7). Monitoring plasma amino acids is necessary to ensure that concentrations of the essential amino acids,

Box 19.5: Nutrition Monitoring of a Patient with GA-1

- Routine assessments including anthropometrics, dietary intake, and physical findings (Appendix F)
- Laboratory monitoring
  - Diagnosis specific
    - 3-hydroxyglutaric acid (urine)
    - Glutaric acid (urine)
    - Carnitine (total, free, esterified)
    - Plasma amino acids, including:
      - Lysine
      - Arginine
      - Tryptophan
- Nutrition laboratory monitoring of patients on lysine and/or protein-restricted diets may include markers of:
  - Protein sufficiency[a]
  - Nutritional anemia (hemoglobin, hematocrit, MCV, serum vitamin B_{12} and/or methylmalonic acid (MMA), total homocysteine, ferritin, iron, folate, total iron binding capacity)
  - Vitamin and mineral status (25-hydroxyvitamin D, zinc, trace minerals)
  - Others as clinically indicated

[a]Further described in Chap. 7.

lysine and tryptophan, are maintained within the normal range for age (based on the metabolic laboratory's reference ranges) (Table 19.2).

Care must be taken to avoid essential amino acid deficiencies, particularly tryptophan. Tryptophan is difficult to quantify using certain methodologies [14]. Serum albumin binds tryptophan, with one binding site per albumin molecule. Variable albumin binding may make free tryptophan concentrations variable [14]. The frequency of monitoring depends on the patient's age and health status. During early infancy, many clinics measure plasma amino acids along with anthropometrics each week (Box 19.5).

19.4 Summary

The nutrition management of GA-1 presents a clinical challenge because the benefit of a lifelong lysine and tryptophan-restricted diet is not established; there are no good biomarkers to guide treatment decisions; and acute metabolic crises can result in striatal damage causing irreversible neurological sequelae. Preventing acute metabolic crisis is the primary goal of treatment. Lysine-restricted, arginine-supplemented diets are believed to offer some benefit perhaps by altering the flux of lysine and arginine across the blood-brain barrier. L-carnitine conjugates and removes glutaric acid and 3-hydroxyglutaric acid from the body and is a key part of therapy.

Table 19.2 Plasma concentration reference ranges for arginine, lysine, and tryptophan for patients with GA-1

Amino acid	0–1 month	1–24 months	2–18 years	Adult
Arginine (μmol/L)	6–140	12–133	10–140	15–128
Lysine (μmol/L)	92–325	52–196	48–284	100–250
Tryptophan (μmol/L)	–	5–60	34–47	42–106

Reference ranges for Children's Hospital Colorado; check your laboratory for reference ranges

19.5 Diet Calculation Example for an Infant with GA-1

Example: Infant with GA-1

Patient information	Nutrient intake goals (per day)
Ten (10) day old infant male weighing 3.2 kg who was diagnosed with GA-1 based on elevated 3-OH glutaric acid concentrations. Patient is asymptomatic and eating well. Current intake is 19 oz of Enfamil per day	**Lysine:** 70 mg/kg (range: 65–100 mg/kg) **Tryptophan:** 10–20 mg/kg **Protein:** 3.0 g/kg **Energy:** 125 kcal/kg (range: 95–145 kcal/kg) Recommended caloric density of formula: 20–22 kcal/oz

Select nutrient composition of products used in GA-1 diet calculation example (using standard infant formula as the source of whole protein)

Medical food/formula	Amount (g)	LYS (mg)	ARG (mg)	TRP (mg)	Protein (g)	Energy (kcal)
GA-1 Anamix® Early Years[a]	100	–	1,180	–	13.5	473
Enfamil® Premium Powder[b]	100	750	240	163	10.6	510

[a]Nutricia North America (Rockville, MD)
[b]Mead Johnson Nutrition (Evansville, IN)

Diet prescription summary for diet calculation example (using standard infant formula as the source of whole protein)

Medical food/formula	Amount (g)	LYS (mg)	ARG (mg)	TRP (mg)	Protein (g)	Energy (kcal)
GA-1 Anamix® Early Years[a]	47	–	555	-	6.3	222
Enfamil® Premium Powder[b]	30	224	72	49	3.2	153
Total per day		224	627	49	9.5	375
Total per kg		70	196	15	3.0 (1.0 g/kg of natural protein)	117

[a]Nutricia North America (Rockville, MD)
[b]Mead Johnson Nutrition (Evansville, IN)
Values rounded to nearest whole number for amount of formula powder, Lysine, Arginine, Tryptophan, and Energy. Values rounded to the nearest 0.1 g for protein

Step-by-Step Diet Calculation

Step 1. Calculate the amount of Lys required each day.

Lys goal × Infant Weight = mg Lys per day

70 mg/kg Lys × 3.2 kg = 224 mg/day Lys

Step 2. Calculate the amount of standard infant formula needed to meet the daily Lys requirement.

Amount of Lys required per day ÷ mg of Lys in standard infant formula.

224 mg/day ÷ 750 mg Lys = 0.30

0.30 × 100 g = 30 g standard infant formula needed to meet daily Lys requirement

Step 3. Calculate protein and energy provided from standard infant formula.

Amount of standard infant formula × protein provided in 100 g of standard infant formula.

0.30 × 10.6 g protein = 3.2 g protein in standard infant formula

Step 4. Calculate amount of protein to fill the diet prescription.

Protein goal × Infant weight = daily protein requirement

3.0 g protein × 3.2 kg = 9.6 g daily protein requirement

Daily protein requirement – protein provided by standard infant formula

9.6 g − 3.2 g = 6.4 g protein needed from Lys/Trp-free medical food to fill in the diet prescription.

Step 5. Calculate the amount of Lys/Trp-free medical food required to fill protein requirement.

Protein needed from Lys/Trp-free medical food ÷ protein in 100 g of medical food.

6.4 g ÷ 13.5 g protein in Lys/Trp-free medical food = 0.47 g

0.47 g × 100 g = 47 g Lys/Trp-free medical food required to fill the diet prescription.

Step 6. Calculate the total energy provided from standard infant formula and Lys/Trp-free medial food.

Amount of standard infant formula × kcal in 100 g of standard formula.

0.30 g × 510 kcal = 153 kcal

Amount of Lys/Trp-free medical food × kcal of 100 g of Lys/Trp-free medical food.

0.47 g × 473 kcal = 222 kcals

Add standard infant formula + Lys/Trp free medical food for total kcal provided in diet prescription.

153 kcal + 222 kcal = 375 total kcal

375 kcal ÷ 3.2 kg = 117 kcal/kg

Step 7. Calculate the final volume of the formula to make a concentration of approximately 20–22 kcal per ounce.

Amount of total calories provided by diet prescription ÷ 20 fluid ounces = number of ounces of formula needed to provide caloric concentration of 20 kcal/oz.

375 kcal ÷ 20 kcal/oz = 18.75 oz of formula

(Note: If final volume prescribed is 19 oz, caloric concentration will be 19.7 kcal/oz; if final volume prescribed is 18 oz caloric concentration will be 20.8 kcal/oz- either is acceptable)

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20 Nutrition Management of Propionic Acidemia and Methylmalonic Acidemia

Sandy van Calcar

Contents

Core Messages

- Infants with propionic acidemia (PROP) or methylmalonic acidemia (MMA) can be identified by newborn screening, although those with severe phenotypes may present with symptoms of metabolic ketoacidosis before screening results are available.
- Nutrition management of PROP or MMA involves limiting intact protein and providing a medical food free of the propiogenic amino acids methionine, threonine, valine, and isoleucine.
- Providing sufficient energy to prevent catabolism, especially during periods of illness, is a key component of therapy.
- Individualized therapy is based on the severity of disease as well as monitoring of metabolic and nutrition parameters.
- Treatment often includes L-carnitine supplementation for both disorders and hydroxocobalamin in MMA.

20.1 Background

Propionic acidemia (PROP) and methylmalonic acidemia (MMA) are inherited disorders of the metabolism of the propiogenic amino acids valine, isoleucine, threonine, and methionine and odd-chain fatty acids (Figs. 20.1 and 20.2,

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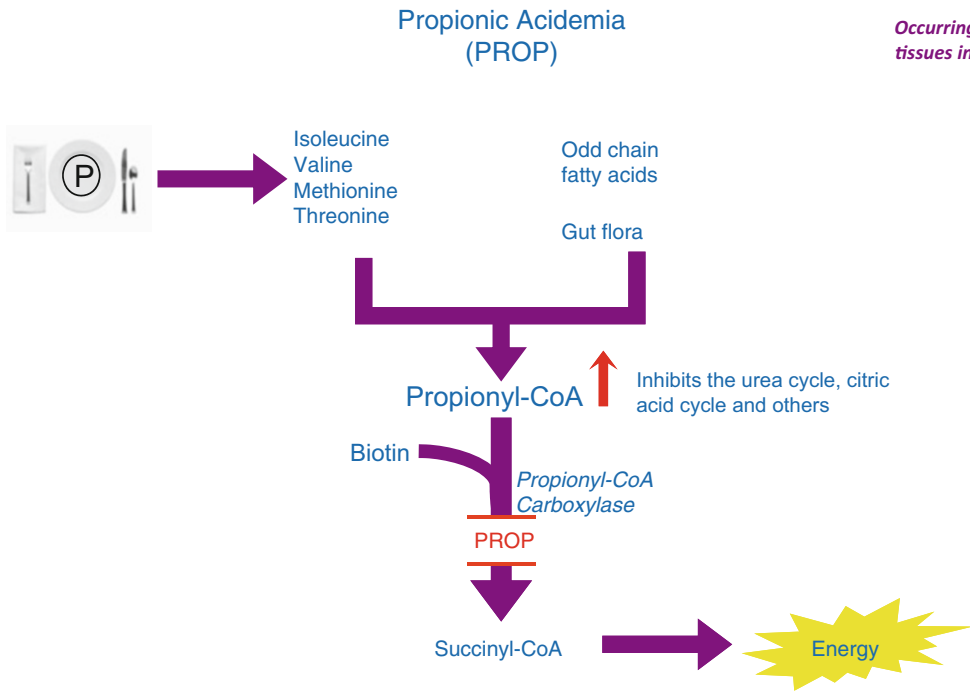

Fig. 20.1 Metabolic pathway of propionic acidemia

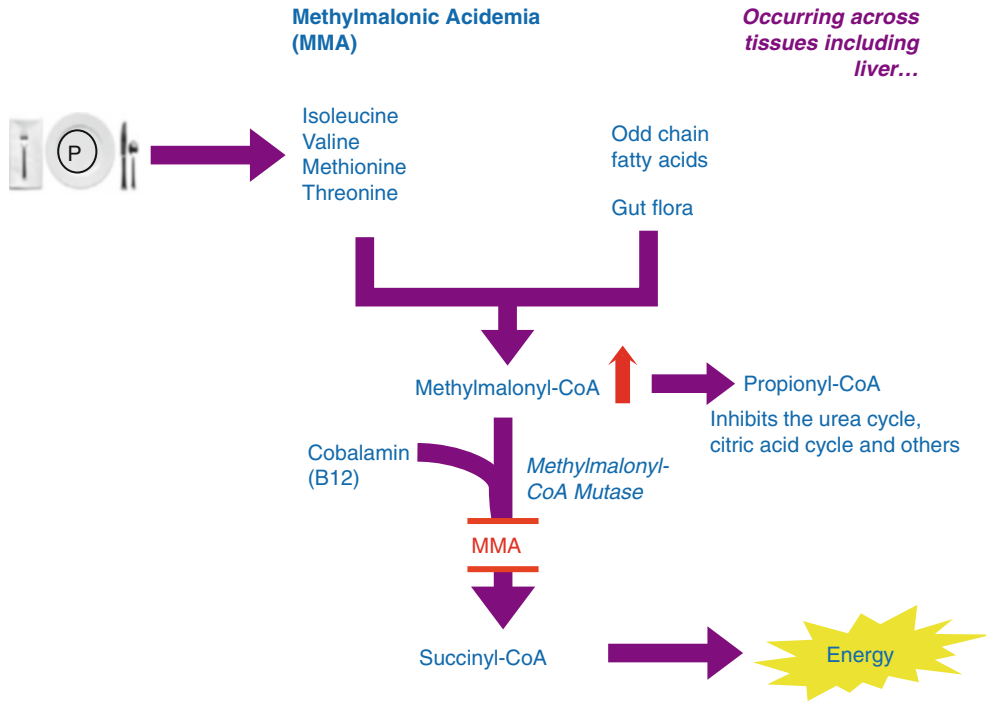

Fig. 20.2 Metabolic pathway of methylmalonic acidemia

respectively). Either disorder can be identified by newborn screening with elevated propionyl (C3) carnitine. Distinction between these two disorders requires additional confirmatory laboratory testing including urine organic acids (Chap. 17). Molecular testing confirms the disorder [2].

Patients with MMA can either have a deficiency of the mutase enzyme (mut^0 or mut^-) or a defect in cobalamin synthesis or utilization (e.g., cobalamin A or cobalamin B deficiency). Cobalamin defects are not covered in this chapter. Those with the mut^0 form of MMA have absent mutase activity and often develop a more severe phenotype than those with the mut^- deficiency who have some residual enzyme activity [2]. There is a wide range of clinical severity in both PROP and MMA and nutrition management needs to be individualized (Box 20.1). Further discussion of organic acidemias, including PROP and MMA, is found in Chap. 17.

Long-term clinical complications include poor growth, cognitive delay, pancreatitis, seizures, and optic nerve atrophy. Cardiomyopathy is common in PROP, and chronic renal disease can develop in MMA caused by mutase deficiency [2, 5, 10].

Box 20.1: Principles of Nutrition Management for PROP and MMA

Restrict: Propiogenic amino acids (valine, isoleucine, methionine, threonine)

Supplement: L-carnitine

Trial of intramuscular hydroxocobalamin in MMA[1]

Trial of biotin in PROP[1]

[1]Conducted in some clinics. See chapter for typical doses.

20.2 Nutrition Management

20.2.1 Chronic Management

Infants with severe forms of either PROP or MMA may present in metabolic ketoacidosis with vomiting, lethargy, and coma before newborn screening results are available. In these cases, elimination of protein and the provision of energy from glucose and lipid by peripheral or central access are necessary to slow protein catabolism [3, 7]. A propiogenic amino acid-free medical food, devoid of valine, isoleucine, methionine, and threonine, and an intact protein source from regular infant formula or breast milk are gradually introduced, often initially by nasogastric feedings (Box 20.2). In some infants, the use of a standard parenteral source of essential amino acids and a parenteral amino acid solution without the propiogenic amino acids may be needed. Frequent monitoring of clinical status and both metabolic and critical labs is required [11].

For typically developing infants with PROP or MMA, solids can be started at the same time as their unaffected peers, usually at 4–6 months of age [12]. Some clinics initially recommend counting milligrams of valine to monitor intake. However, lists of valine content of foods are limited. Thus, counting grams of protein is appropriate for this population. The "Low Protein List for PKU" contains protein content for a wide range of foods [13]. Using protein content from food labels is less accurate but can be used for those with a higher protein tolerance or in older patients. In addition to protein restriction, food sources of odd-chain fatty acids should be avoided. These include milk fat, butter, cream, lard, and some marine oils. Over-restriction of intact protein sources needs to be avoided as poor growth, weight gain, and wound healing have been reported [11].

Infants and young children need a complete propiogenic amino acid-free medical food with carbohydrate, fat, and all micronutrients. For older individuals, medical foods that are concentrated in protein or low in fat may allow the patient to meet his/her protein requirement with a lower volume of medical food (Table 20.1). These are appropriate for overweight patients or those who are able to consume the majority of their energy from foods and require fewer kilocalories from medical food.

Box 20.2: Initiating Nutrition Management for an Asymptomatic Infant with PROP/MMA

Goal: Normalize plasma concentrations of isoleucine (ILE), valine (VAL), threonine (THR), and methionine (MET).

Reduce production of abnormal metabolites.

Provide sufficient energy to prevent catabolism.

Step-by-step:

1. Establish intake goals based on the infant's diagnosis, phenotype (classical vs. mild), clinical status, and laboratory values. Daily nutrient intake goals [7]:

Age (months)	ILE mg/kg	MET mg/kg	THR mg/kg	VAL mg/kg	Protein g/kg	Energy kcal/kg
0–6	60–110	20–50	50–125	60–105	2.75–3.5	125–145
7–12	40–90	15–40	20–75	40–80	2.5–3.25	115–140

2. Determine the amount of intact protein from either a standard infant formula or breast milk[a] required to provide 50 % of the infant's total protein needs.
3. Calculate the amount of the propiogenic amino acids (MET, THR, VAL, ILE) provided by this intact protein source. Intakes should be within the recommended ranges for these four amino acids.

   Alternatively, use the recommended range for VAL to determine the needed amount of infant formula or breast milk. Then, check that intake of ILE, MET, and THR provided by this intact protein source meets recommendations. This method may be most appropriate for infants with more classical forms of PROP or MMA requiring intakes of these amino acids at the lower end of the recommended range.
4. Determine the amount of PROP/MMA medical food required to provide the remainder of the infant's total protein needs.
5. Determine the calories provided by both the intact protein source and PROP/MMA medical food. Provide the remaining calories from a protein-free medical food. These formulas contain only carbohydrate, fat, and micronutrients.
6. Determine the amount of fluid to add to make a final formula concentration of 20–25 kcal/oz, depending on energy needs and volume tolerated.
7. If the child is neurologically intact with an appropriate suck, feed ad lib. Some infants may be unable to ingest adequate volumes to meet their needs and may require tube feedings [3, 5].

[a]In severe PROP or MMA, expressed breast milk is recommended.

Table 20.1 Medical foods for the treatment of PROP/MMA

Infant/toddler	Older–complete	No fat	Different forms
Propimex-1®[a]	Propimex-2®[a]	Maxamaid/Maxamum® X-MTVI[b]	Vitaflo Coolers[d]
MMA/PA Anamix® Early Years[b]	OA-2[c]	MMA/PA Express and Gel[d]	Camino Pro MSUD®[e]
OA-1[c]		Milupa OS-2®[b]	

[a]Abbott Nutrition (Columbus, OH; abbottnutrition.com)
[b]Nutricia North America (Rockville, MD; nutricia-na.com)
[c]Mead Johnson Nutrition (Evansville, IN; meadjohnson.com)
[d]Vitaflo USA (Alexandria, VA; vitaflousa.com)
[e]Cambrooke Therapeutics (Ayer, MA; cambrookefoods.com)

20.3 Adjunct Treatments for Propionic Acidemia and Methylmalonic Acidemia

Since L-carnitine conjugates with toxic acyl-CoA metabolites produced in PROP and MMA, patients often develop a secondary carnitine deficiency. To prevent this, L-carnitine in doses of 100–300 mg/kg/day is prescribed on a routine basis [5, 11]. Some medical foods designed for PROP and MMA contain L-carnitine, and this needs to be considered when determining the amount of supplement to prescribe. The IV form of L-carnitine is often used during hospitalization for acute illness [3].

Some forms of MMA may be responsive to vitamin B_{12}. Responsiveness can be determined by administration of 1.0 mg hydroxocobalamin (IM or IV) for 5 days. A reduction in serum methylmalonic acid concentrations of 50 % or greater suggests responsiveness [14]. For those with cobalamin-responsive forms of MMA, intramuscular (IM) hydroxocobalamin injections of 1.0–2.0 mg are often administered daily. A decreased frequency of IM injections or use of oral supplements may be appropriate for older individuals. The hydroxocobalamin form rather than the standard cyanocobalamin form must be used [15].

In PROP deficiency, a trial of oral biotin (5–20 mg/d) is often given [3, 5, 7]. The benefit of supplemental biotin has been debated, and if an improvement in metabolic parameters is not observed with supplementation, discontinuation is suggested [5]. Other therapies, such as metronidazole and citrate solutions for PROP, may also be prescribed [5, 7].

Box 20.3: Amino Acid Profiles in PROP and MMA

- *Goal*: Maintain the concentration of the propiogenic amino acids MET, THR, VAL, and ILE in the normal range.
- Low concentrations of BCAA (VAL, ILE, and LEU) have been associated with over-restriction of intact protein [4]. If low, incrementally increase the intact protein.
- ILE or VAL supplementation may be necessary if the intake of intact protein has been optimized, but concentrations of these two amino acids remain below the normal range [7].
- Glycine is often elevated in PROP, but not MMA. This is caused by propionic acid inhibition of the glycine cleavage system. How to interpret abnormal glycine concentrations is not well established. Glycine concentrations may be associated with adequate energy intake, but not protein intake [8, 9].

20.4 Monitoring

20.4.1 Growth

Monitoring a patient's growth is critical in PROP and MMA. Failure to thrive is a common finding in patients with PROP and MMA. The patients may have oral motor delays and anorexia secondary to elevated metabolites. When adjusting energy and protein prescriptions, use the patient's ideal weight rather than actual weight to prevent underestimation of needs (Chap. 7). However, because of lower lean body mass in patients with PROP and MMA, the resting energy expenditure for these disorders may be lower than predicted by standard equations [16, 17].

20.4.2 Laboratory Monitoring

Unlike in PKU and MSUD, there are no clear laboratory parameters associated with good metabolic control in PROP and MMA. Monitoring goals need to be individualized based on the patient's phenotype and clinical status. Typically, plasma amino acid profiles are routinely evaluated in patients with PROP and MMA with the goal of preventing deficiency of the restricted amino acids valine, isoleucine, threonine and methionine (Box 20.3).

In addition to plasma amino acids, albumin and prealbumin (also called transthyretin) con-

centrations can be used to assess protein status. Albumin reflects a longer period of time with a half-life of 18–20 days. Prealbumin is a more acute marker with a 2–3 day half-life. In addition, ammonia concentrations may be elevated in a catabolic state and can be useful to monitor for some patients [5].

Most patients with PROP and MMA are prescribed L-carnitine supplements to prevent a secondary carnitine deficiency, and routine measurement of plasma or serum carnitine is recommended [5]. This analysis will include total carnitine, carnitine esters (esterified), and free carnitine concentrations. Low concentrations of free carnitine suggest a need to increase the supplementation dose. With supplementation, the total and ester fractions are often elevated.

There is little standardization for assessing other metabolic parameters for routine monitoring of PROP and MMA. Biomarkers that the author has found helpful include serum concentrations of 2-methylcitric acid in PROP and methylmalonic acid concentrations in MMA. Others suggest plasma C3 acylcarnitine profiles or urine organic acid concentrations of propionate-derived metabolites [4, 5]. However, the excretion of organic acids is affected by water balance, renal status, and other factors making interpretation difficult for routine diet monitoring [18, 19]. Since normalization of these metabolites is not possible in PROP or MMA, these markers are often used to measure relative metabolic control for a patient rather than aiming for an absolute value.

20.4.3 Monitoring Nutrition Status in PROP and MMA

Periodic evaluation of nutrient intake using a 3-day diet record collected prior to a blood draw is recommended. Particular attention to the composition of medical food is necessary since this is often the primary source of both macro- and micronutrients for a patient.

Lab monitoring of nutrition status often includes iron indices since anemia of chronic disease is common in these disorders. Total 25-hydroxyvitamin D is the best marker of vitamin D status. An essential fatty acid profile is helpful, especially if the patient is on a low-fat medical food (Box 20.4).

Box 20.4: Nutrition Monitoring of a Patient with MMA or PROP

- Routine assessments include anthropometrics, dietary intake, and physical findings (Appendix F)
- Laboratory monitoring [5, 7]
  - Diagnosis specific
    - Plasma amino acids
      - Propiogenic (VAL, ILE, MET, THR)
      - Glycine
    - Serum methylmalonic acid (MMA)
    - Serum 2-methylcitrate (PROP)
    - Carnitine (total, free, esterified)
  - Nutrition-related laboratory monitoring of patients on amino acid-restricted diets may include markers of:
    - Protein sufficiency[a] (plasma amino acids, albumin, prealbumin)
    - Nutritional anemia (hemoglobin, hematocrit, MCV, serum vitamin B_{12} and/or methylmalonic acid, total homocysteine, ferritin, iron, folate, total iron-binding capacity)
    - Vitamin and mineral status (total 25-hydroxy vitamin D, zinc, trace minerals)
    - Others as clinically indicated

[a]Further described in Chap. 7.

20.5 Acute Nutrition Management

Patients with PROP and MMA are at risk for metabolic decompensation with any intercurrent illness, serious injury, or surgery [11]. The goal of treatment is to reduce or prevent catabolism by providing sufficient energy. A "sick-day" protocol can be provided at home with minor illnesses (Box 20.5).

Box 20.5: "Sick-Day" Diet for PROP/MMA

Prescribe calories to meet the patient's estimated resting energy requirement, plus additional needs associated with illness. Additional needs vary, but a 20 % increase is a goal (based on author experience and stress factors added to calculations using the Harris-Benedict equation) [1].

- Continue medical food, if tolerated.
- Provide protein-free, carbohydrate-based fluids [6].
- Reduce intact protein by 50–100 % of usual intake for up to 24 h, depending on degree of illness.
- Emphasis should be placed on increasing energy and fluid needs rather than prolonged protein restriction that can exacerbate catabolism [3].

Box 20.6: Management in Acute Illness for a Patient with PROP or MMA[a]

Within 24–36 h of initiating treatment:

- Restart a source of protein [3].
- Medical food is typically initiated first and then intact protein sources are incrementally added. Start with one-fourth to one-half usual tolerance and work-up.
- If oral intake is not possible or not adequate, NG feedings are initiated.
- Provide L-carnitine. If oral L-carnitine is not tolerated, IV L-carnitine (100–200 mg/kg) may be prescribed.

[a]Refer to Chap. 5 for acute medical management.

For those with PROP, home monitoring of urine ketones can be used as an indicator of the degree of illness. Moderate to large ketones indicate compromised metabolic control. The age of the child, severity of illness, and comfort level of caregivers dictates the length of time that home treatment can be continued; however, caretakers need to be instructed to contact the metabolic team if the child is unable to tolerate oral feeds or, if tolerating feeds, has been following a sick-day plan for >24 h.

If hospitalization is necessary, protein feeds are often discontinued for 24–36 h, and extra energy is provided to reverse catabolism (Box 20.6).

20.6 Transplantation

Severe forms of MMA and PROP are associated with significant complications including cardiomyopathy, renal disease, and developmental delay. Thus, liver transplantation becomes an option, especially for those patients with recurrent metabolic episodes that are not adequately controlled using conventional strategies [5]. Liver transplantation improves the quality of life in patients with MMA and PROP and reduces the likelihood of a metabolic crisis and progressive cardiac and neurologic disability [20]. However, in PROP and MMA, transplantation is less curative than in some metabolic disorders, and problems in other organ systems can persist. Metabolic stroke has been reported after liver transplantation [20, 21]. Patients who have undergone liver transplantation for PROP have improved measures of metabolic control, but some metabolites, such as urine methylcitrate and propionylcarnitine, do not normalize [20, 21]. Patients with MMA can still develop renal disease and optic atrophy despite transplantation. Combined liver-kidney transplantation (LKT) is an option, especially if renal disease is already present [22]. However, plasma MMA does not normalize, even after LKT and patients remain at risk for neurological complications and optic atrophy [23].

After liver transplantation, continued restriction of intact protein may be necessary in both MMA and PROP, but the amount of protein recommended varies. Some clinics recommend avoidance of high protein foods but not a specific protein restriction [24]. In other cases, medical food may need to be continued if protein tolerance is less than the DRI. It is unknown if the degree of protein restriction required after transplantation correlates with long-term clinical out-

comes. The goal is to limit protein to the extent necessary to normalize biochemical markers as much as possible.

20.7 Summary

The clinical outcome and lifespan of patients with PROP and MMA have improved with advances in nutrition management, adjunct therapies, and aggressive treatment during metabolic crises. However, many questions remain about optimal treatment in both chronic and acute settings. Research is needed to better determine the nutritional needs of this group and improve methods to monitor treatment decisions. For patients undergoing transplantation, continued support from a metabolic team is needed to assure optimal long-term outcomes.

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21 Nutrition Management During Pregnancy: Maple Syrup Urine Disease, Propionic Acidemia, Methylmalonic Acidemia, and Urea Cycle Disorders

Sandy van Calcar

Contents

Core Messages

- In inherited metabolic diseases other than phenylketonuria (PKU), the mother is at risk of metabolic decompensation during periods of catabolism, but the infant does not appear to be at risk of adverse outcomes.
- Adjustments in protein and energy intakes are needed throughout pregnancy based on frequent monitoring of plasma amino acids and other metabolic labs.
- The postpartum period is an especially dangerous time for women with intoxication disorders, and close monitoring is needed for at least 6 weeks after delivery.

21.1 Background

There has been extensive experience in nutrition management of pregnancy in women with phenylketonuria (PKU). In PKU pregnancies, high phenylalanine concentrations can affect the developing fetus causing microcephaly, developmental delay, and congenital anomalies, including cardiac defects (Chap. 13). With the

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improvement in treatment, women with urea cycle disorders (UCD), maple syrup urine disease (MSUD), and the organic acidemias, propionic acidemia (PROP), and methylmalonic acidemia (MMA), are now of child-bearing age.

Although there is limited experience in managing pregnancies in these disorders, it has become apparent that the risk for metabolic decompensation increases for the mother, especially during the postpartum period when protein catabolism is greatest. However, unlike in PKU, it appears that the infant may not be at increased risk of adverse outcomes in these other protein metabolism disorders. This chapter will review what has been learned about managing pregnancies in these intoxication disorders.

21.2 Pregnancy in Maple Syrup Urine Disease

Pregnancies in women with classical MSUD require close monitoring throughout pregnancy, delivery, and the postpartum period. There are six published cases [1–5] and experience with five additional pregnancies at the University of Wisconsin-Madison. In 6 of these 11 pregnancies, elevated leucine concentrations were noted in the postpartum period as protein catabolism increases after delivery with the rapid involution of the uterus [6]. One of these women was noncompliant with post-pregnancy recommendations and died 51 days after delivery [3], emphasizing the importance of continued monitoring and treatment after delivery. In these cases, normal infant outcomes were reported, even for an infant born to a woman with poor leucine control throughout pregnancy [3]. However, there is little long-term follow-up of these infants reported in the literature. In addition, successful breastfeeding while maintaining maternal metabolic control is also possible [4].

21.3 Pregnancies in Propionic Acidemia

There are only two published reports of successful pregnancy in women with propionic acidemia [1, 7]. The clinic at the University of Wisconsin-Madison has followed three additional pregnancies in two women with mild propionic acidemia (7 % and 9 % residual propionyl-CoA carboxylase activity). Frequent monitoring to adjust both diet treatment and carnitine supplementation was necessary throughout pregnancy. In all five pregnancies, a dextrose infusion was provided by peripheral IV during delivery and the immediate postpartum period. Complications included placenta previa in one pregnancy [7] and preeclampsia requiring early Cesarean section in both pregnancies from one woman (Case Example 2). Severe metabolic decompensation was not reported during pregnancy, delivery, or the postpartum period. None of the infants showed congenital anomalies, and normal developmental outcomes have been reported.

21.4 Pregnancies in Methylmalonic Acidemia

There have been several reports in the literature of pregnancies in women with various forms of methylmalonic acidemia, including mutase$^-$, cobalamin A, and mild cobalamin C defects; both cobalamin-responsive and cobalamin-nonresponsive phenotypes are included in these reports [7–13]. A recent summary of 13 completed pregnancies in women with MMA found a wide range of treatment regimens including diet, L-carnitine supplementation, and/or intramuscular (IM) hydroxocobalamin injection [12]. Five of 13 completed pregnancies resulted in preterm deliveries (32–36 weeks) with a majority of these pregnancies requiring Cesarean section (C-section) delivery, often because of fetal distress [12]. At delivery, all women were treated with IV dextrose (+/–IV carnitine) up to 8 days postpartum. However, no malformations or adverse outcomes were reported for the infants, despite elevated serum methylmalonic acid concentrations throughout pregnancy.

21.5 Pregnancies in Urea Cycle Disorders

Numerous cases in the literature describe pregnancy and fetal outcome in women with various urea cycle disorders [14–21]. Like in

pregnancies in MSUD and organic acidemias, women with UCD are especially at risk for metabolic decompensation during the first trimester when poor energy intake is common, during any intercurrent illness, with prolonged delivery, and in the postpartum period. Women are especially vulnerable to hyperammonemia during the postpartum period where severe mental status changes, coma, and death have been reported after delivery, even in women with mild forms of the disorder [14, 15]. In some reports, the patient was not diagnosed with a UCD until she developed symptoms during the postpartum period [15, 16].

21.6 Nutrition Management During Pregnancy

Based on published cases and the author's experience, there are some general recommendations that apply to all pregnancies in these disorders:

21.6.1 Maintain Normal Maternal Weight Gain During Pregnancy

Weight gain goals are the same for pregnancies when the mother has an inborn error of metabolism as for the general population (Table 21.1). Weight loss should be avoided since this can cause protein catabolism and elevated amino acid concentrations (based on author's experience). Energy needs increase as pregnancy progresses, especially in late pregnancy when fetal growth is the greatest [22].

21.6.2 Maintain Adequate Energy and Protein Nutriture Throughout Pregnancy

Both energy and protein needs increase as pregnancy progresses to allow for increased maternal requirements and adequate fetal growth [22] (Fig. 21.1). To prevent protein deficiency, any woman requiring a medical food prior to pregnancy will need to continue this throughout pregnancy. Even if a woman has a milder form of a disorder and has not required medical food as an adult, reintroduction of a medical food may be needed during pregnancy, as illustrated in the propionic acidemia case study in Sect. 21.7. Protein needs are also higher when consuming an amino acid-based medical food compared with a diet exclusively of intact protein sources (Chap. 7).

21.6.3 Maintain Plasma Amino Acid Concentrations Within the Normal Range and Anticipate a Higher Intact Protein Tolerance as Pregnancy Progresses

Blood concentrations of many amino acids decrease as pregnancy progresses with the increase in placental uptake and other changes in maternal/fetal metabolism [23]. Normal values for pregnancy need to be considered in interpretation of plasma amino acid profiles. Plasma amino acids need to be monitored frequently, and, if low, an increase in the amount of intact protein is prescribed to maintain the restricted amino acids in the normal range.

As with total protein, the needs for individual amino acids increase as pregnancy progresses,

Table 21.1 Recommendations for total and rate of weight gain during pregnancy based on prepregnancy BMI [26]

Prepregnancy BMI	BMI[a] (kg/m^2)	Total weight gain (pounds)	Rates of weight gain in 2nd and 3rd trimester[b] (pounds/week)
Underweight	<18.5	28–40	1 (1–1.3)
Normal weight	18.5–24.9	25–35	1 (0.8–1)
Overweight	25.0–29.9	15–25	0.6 (0.5–0.7)
Obese (includes all classes)	>30.0	11–20	0.5 (0.4–0.6)

[a]To calculate BMI, go to www.nhlbisupport.com/bmi/

[b]Calculations assume a 0.5–2 kg (1.1–4.4 lbs) weight gain in the first trimester (based on [27–29])

Energy = $EER_{nonpregnant}$ + additional energy for pregnancy + energy deposition

Trimester 1: EER + 0 + 0 kcals
Trimester 2: EER + 160 + 180 kcals
Trimester 3: EER + 272 + 180 kcals

Protein

DRI = 0.88 g/kg/d or +21 g/d
RDA = 1.1 g/kg/d or +25 g/d

Fig. 21.1 Estimated energy and protein requirements for each trimester of pregnancy [22]

especially in the late second and third trimesters when fetal growth is the greatest [1, 22]. Even for patients with classic phenotypes, high-protein foods may be needed toward the end of pregnancy in order to maintain plasma amino acids in the normal range. Adding milk to the medical food, if tolerated, is a good option.

Overrestriction of amino acids may contribute to the poor fetal growth that has been noted in the second and third trimester in MSUD and MMA pregnancies [1, 12]. If a single amino acid is supplemented as part of treatment, additional supplementation may be required to prevent low plasma concentrations, even with the increase in intact protein tolerance as pregnancy progresses.

In the author's experience with MSUD pregnancies, supplementation of valine and isoleucine may be needed, even for women who did not require supplementation to maintain normal plasma concentrations before pregnancy. If higher concentrations of valine and/or isoleucine are noted, the amount of the supplements is decreased rather than reducing intake of intact protein. The primary biomarker in a MSUD pregnancy is leucine; if the plasma leucine concentration is within goal range, then the amount of intact protein prescribed should not be reduced in an attempt to reduce isoleucine and valine concentrations. Although the teratogenicity of the branched-chain amino acids remains uncertain, in our limited experience, moderate elevations in valine and isoleucine have not seemed to pose harm to the mother or infant.

21.6.4 Plan Ahead for Intercurrent Illness and Complications Affecting Dietary Intake

As with any pregnancy, persistent nausea and vomiting and intercurrent illness can occur. For women with intoxication disorders, these catabolic events need to be aggressively addressed to prevent increasing concentrations of amino acids and associated toxic metabolites. Antiemetics can be prescribed. For women who have a difficult time taking medical food, a gastrostomy tube may need to be considered [24]. A plan for any needed admissions should be established ahead of time and emergency protocols updated [25].

21.6.5 Refer to an Obstetric Clinic Specializing in High-Risk Pregnancy

Given the risk of metabolic decompensation during pregnancy and postpartum period, women with amino acidopathies or urea cycle defects should be followed by an obstetric clinic specializing in high-risk pregnancies [1]. Frequent assessment of fetal growth is also needed. For successful maternal and fetal outcomes, a multidisciplinary approach is required with input from both the obstetric and metabolic teams [4, 18, 21].

21.6.6 Anticipate Postpartum Catabolism

Delivery and the postpartum period are catabolic processes, and women with amino acidopathies or UCD are at high risk for metabolic decompensation during these times. The risk may be greatest for women with classical forms of these disorders, although severe decompensation has been reported in women considered to have milder phenotypes [14, 19]. The risk for decompensation increases if delivery is prolonged and/

or a sufficient source of calories and protein equivalents is not provided during delivery and the postpartum period.

Postpartum catabolism is caused by rapid protein turnover associated with hormonal changes and the involution of the uterus. Uterine mass decreases approximately 50 % during the first 10 days after delivery [6]. In the author's experience with MSUD pregnancies, the greatest risk for decompensation occurred between Day 3 and Day 14 after delivery. Many of the cases reported in the literature note an increase in metabolites during this time frame. Even after a woman is discharged, frequent monitoring and contact is needed to assure adequate energy intake and to assess for signs of decompensation. Catabolism gradually slows, but it may take 6–8 weeks after delivery for protein metabolism to return to a pre-pregnancy state [6, 25] (Box 21.1).

Box 21.1: Nutrition Interventions for a Pregnant Woman with MSUD, PROP, MMA, or UCD

- Promote normal maternal weight gain during pregnancy.
- Provide adequate energy and protein nutriture throughout pregnancy.
- Maintain plasma amino acid concentrations within the normal range.
- Anticipate a higher intact protein tolerance as pregnancy progresses.
- Plan ahead for intercurrent illness and complications affecting dietary intake.
- Refer to an obstetric clinic specializing in high-risk pregnancy.
- Anticipate postpartum catabolism and plan to provide adequate nutrition.

21.7 Summary

Although experience is still limited, it appears that women with maple syrup urine disease, propionic acidemia, methylmalonic acidemia, or a urea cycle disorder are at greater risk for adverse outcomes than their infants. The postpartum period is of particular concern for metabolic decompensation in these women. Infant outcomes appear normal, although most reports do not follow the children beyond toddler years and formal developmental testing has not been completed. However, despite overall poor control in some of the reported pregnancies, the infants do not have the dysmorphology, microcephaly, cardiac defects, or developmental delays that have been described in infants born to women with poorly controlled PKU. Systematic collection of data from more pregnancies is needed before definitive conclusions and standardized recommendations can be provided.

21.8 Case Examples

To illustrate these principles, the following are examples of pregnancy in a woman with classical MSUD and a woman with mild propionic acidemia followed at the University of Wisconsin-Madison.

Case Report 1: Pregnancy in Maple Syrup Urine Disease

A 22-year-old woman homozygous for the classical Y393N mutation found in the Mennonite population presented to the clinic at approximately 4 weeks gestation in good metabolic control. Her history included a severe neonatal presentation at 4 days of age with numerous admissions for illness as a child. However, as an adolescent and adult, she was able to manage the majority of illnesses at home. She maintained excellent metabolic control throughout her life and has no evidence of developmental delay or other symptoms associated with poorly treated MSUD.

Plasma amino acid concentrations were monitored one to two times per week. Goals for the pregnancy included maintaining leucine and isoleucine concentrations between 100 and 300 μmol/L and valine concentrations between 200 and 400 μmol/L. Prealbumin, albumin, and

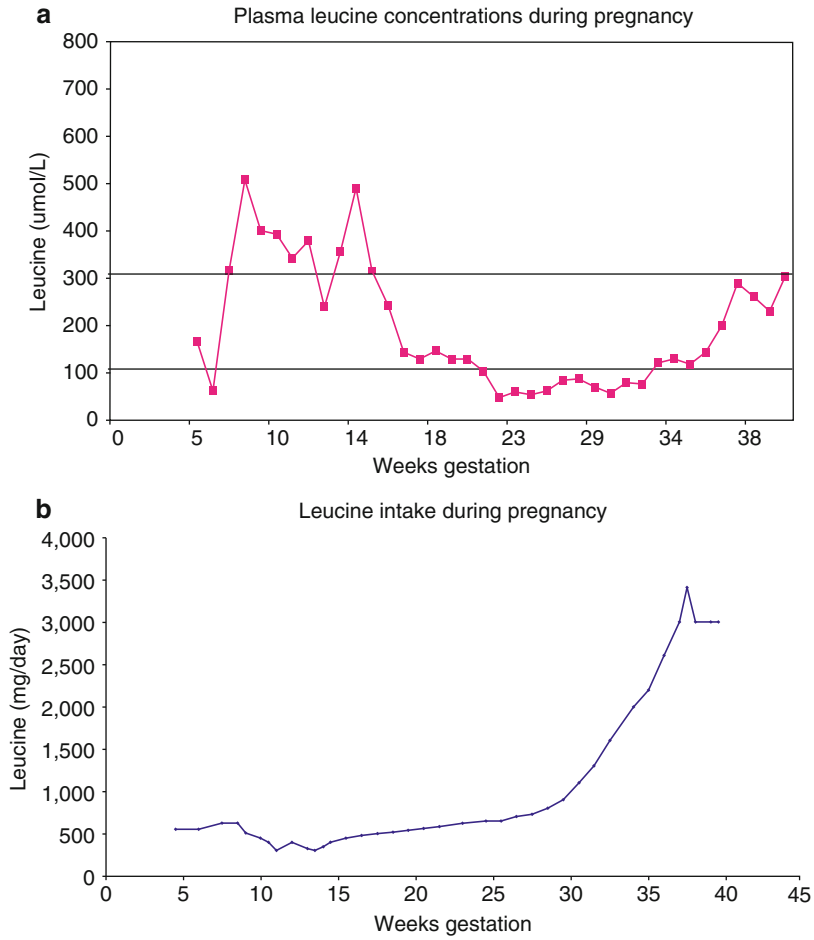

Fig. 21.2 (**a**, **b**) A marked increase in dietary leucine was required to maintain plasma leucine concentrations between 100 and 300 μmol/L after 25 weeks gestation in a woman with classical MSUD

other nutrition markers were monitored monthly. She was referred to a high-risk obstetric clinic, and a fetal ultrasound was completed monthly after the first trimester. Maternal weight gain and fetal growth were normal throughout pregnancy.

During the first trimester, the patient struggled with morning sickness and required antiemetic medication. Her leucine tolerance remained essentially unchanged during the first trimester but increased rapidly during the second and third trimesters (Fig. 21.2b). Her initial leucine prescription was 550 mg/day and increased to 3,400 mg/day prior to delivery. Weekly increases of >100 mg/day were required to prevent low leucine concentrations after 25 weeks gestation (Fig. 21.2a, b).

A vaginal delivery was planned, but the fetus was in a breech position, and a C-section was performed at 39 weeks gestation. Since delivery and the postpartum period are catabolic processes, a central PICC line was placed prior to delivery to administer branched-chain amino acid (BCAA)-free IV solution with dextrose and lipid for energy (Box 21.2). Isoleucine and valine supplements were given orally. To reduce postpartum catabolism, the treatment plan included maintaining the same prescription of energy and protein that she tolerated at the end of pregnancy. Plasma amino acids were measured daily, and reintroduction of dietary leucine was based on the plasma leucine concentration.

Case Report 2: Pregnancy in Propionic Acidemia

This is the second pregnancy for a 28-year-old woman with mutations in the β-subunit of the propionyl-CoA carboxylase enzyme. She was diagnosed at 4 years of age in a metabolic coma. She has a history of seizures and a cardiac complication of long QT syndrome. As an adult,

Box 21.2: Example of Delivery and Postpartum Nutrition in a Patient with Classical MSUD

Nutrition Plan

Breech position: C-section required

- Central PICC line placed with maintenance fluids:
  7 percent BCAA-free amino acid solution in normal saline (NS) at 50 ml/h
  20 percent dextrose at 35 ml/h
  20 percent Intralipid at 15 ml/h
  This provides 2,300 kcals, 4.5 mg/kg/min glucose, 1 g/kg lipid
- Monitor electrolytes and glucose; insulin if needed.
- Gradual decrease in IV sources as oral intake improves.
- Breastfeeding is planned.

Case Report

The patient was able to restart medical food by 12 h after delivery, and by postpartum day 2, she was consuming as much medical food as she consumed at the end of pregnancy. Leucine levels remained within the normal range. Thus, she was weaned off of parenteral solutions over a 2-day period, and her leucine prescription was incrementally increased to her prepregnancy leucine prescription of 550 mg/day. However, her plasma leucine began to increase on Day 5 after delivery, so intact protein was removed from the diet, and additional energy was provided by reintroduction of IV dextrose and lipid solutions. However, the plasma leucine continued to increase. It was only after reintroduction of protein from the BCAA-free parenteral amino acid solution that the plasma leucine concentration decreased. On Day 6, she was consuming 3.0 g/kg of protein (50 % formula, 50 % IV) and 4,500 kcals from both oral and IV sources. Plasma leucine decreased rapidly on this regimen.

To prevent another spike in the leucine concentration, IV energy and protein sources were reduced gradually over a 4-day period. She was discharged on Day 11 after delivery. After discharge, plasma amino acids were checked two times/week for 2 weeks and then weekly. Her dietary leucine tolerance increased slowly, and it was not until 30 days after delivery that she tolerated her prepregnancy leucine intake of 550 mg/ day.

The infant had normal APGAR scores at birth with weight at 25 percentile and length at 50 percentile. The mother attempted to breastfeed, but her milk supply remained poor even with pumping. It is unclear if MSUD contributed to this; however, a recent report describes a woman with classical MSUD who was able to breastfeed successfully [4]. The child at 3 years of age continued normal growth and development.

This woman's second pregnancy progressed similarly to her first with a dramatic increase in BCAA tolerance as the pregnancy progressed. To avoid the increase in leucine concentrations during the postpartum period as seen in her first pregnancy, the reduction in energy and protein from TPN sources was more gradual over a 7-day period, and leucine from oral sources was introduced more gradually. At her discharge 10 days after delivery, her leucine intake was only 60 % of her prepregnancy prescription. Her leucine tolerance did not return to her prepregnancy tolerance until 6 weeks after delivery.

she did not take a medical food but self-restricted her protein intake to 0.6–0.8 g/kg prior to pregnancy. Her first pregnancy was complicated by preeclampsia requiring a C-section delivery at 31 weeks gestation. The infant showed slowed fetal growth by ultrasound. Despite complications of prematurity, this child at 10 years of age shows no cognitive delays.

Unlike her first pregnancy where a medical food was not started until 14 weeks gestation, a medical food was started prior to pregnancy to assure better protein nutriture during her second pregnancy. Maternal weight gain was normal. To maintain normal plasma concentrations of valine, isoleucine, methionine, and threonine, her intake of intact protein increased as pregnancy

a

	1st pregnancy (◆)	2nd pregnancy (■)
Pre-pregnancy total protein intake	**0.7 g/kg**	**1.0 g/kg**
Total protein intake at 20 weeks	**1.1 g/kg**	**1.3 g/kg**
Total protein intake at delivery	**1.4 g/kg**	**1.6 g/kg**
Initiation of medical food	**14 weeks**	**Pre-Pregnancy**
Total material weight gain	**15 kg (33 lbs)**	**13 kg (28 lbs)**
Carnitine dose at delivery	**150 mg/kg**	**100 mg/kg**
Gestational age at delivery	**31 1/7 weeks**	**32 0/7 weeks**
Birth weight	**1170 g**	**1826 g**

b

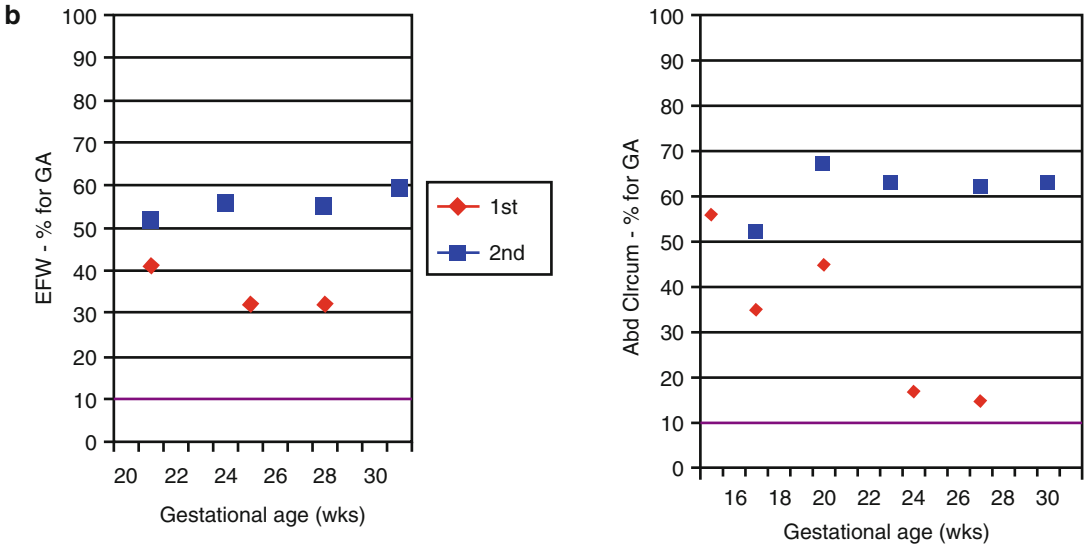

Fig. 21.3 (**a**)Comparison of energy and protein intake, maternal and birth weight, and carnitine supplementation in two pregnancies in a woman with mild propionic acidemia. (**b**) Estimated fetal weight (*EFW*) and abdominal circumference (*Abd Circum*) measured by ultrasound in the two pregnancies. Ultrasound measurements are reported as percentiles based on gestational age

progressed. Even with the increased intake of intact protein, valine and isoleucine supplements were added to achieve normal concentrations of these two amino acids. She continued biotin (10 mg/day) and carnitine supplementation. Plasma carnitine concentrations were frequently monitored, and her carnitine dose increased from 50 to 150 mg/kg prepregnancy weight.

Despite a more aggressive treatment regimen, she again developed preeclampsia and delivered at 32 weeks gestation by C-section. A 10 % dextrose solution was provided by peripheral line during delivery and for 3 days postpartum. Despite prematurity complications, this child at 7 years of age shows no cognitive delays. Improved energy and protein nutriture may have played a

role in better fetal growth measured by ultrasound during the second pregnancy. Figure 21.3 shows total protein intake, maternal weight gain, and fetal growth measurements during both pregnancies.

It is unknown if propionic acidemia played a role in the development of preeclampsia for this woman. The other three known pregnancies to women with propionic acidemia delivered at term. In all five pregnancies, there have been no developmental delays or other complications reported in the children.

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Part IV

Fatty Acid Oxidation Disorders

Fatty Acid Oxidation Defects

22

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Contents

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Core Messages

- Fatty acid oxidation disorders often present with intermittent symptoms triggered by prolonged fasting.
- Avoidance of fasting is a key component of treatment.
- Symptoms of cardiac or skeletal muscle dysfunction pose problems, particularly for patients with long-chain fatty acid oxidation disorders.

22.1 Biochemistry [1–9]

Fatty acids are long alkanes with a single carboxylic acid. The most common fatty acids in our body are palmitic acid with 16 carbons and stearic acid with 18 carbons. Unsaturated fatty acids have a single unsaturated bond as in oleic acid with 18 carbons and a single cis-unsaturated bond at position 9 designated as C18:1 (the number behind the colon denominating the number of unsaturated bonds). We obtain fatty acids with more than one unsaturated bond from our food, mostly from vegetable oils. The most common polyunsaturated fatty acids are linoleic acid C18:2 and α-linolenic C18:3. Fatty acids are stored in fat tissue as triglycerides. They are released by lipolysis to free fatty acids and glycerol and transported in the blood to the tissues that use them.

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Fatty acids usually contain an even number of atoms and are catabolized in sequential cycles to acetyl-coenzyme A. This process is called β-oxidation and occurs inside the mitochondria. First, fatty acids need to enter the mitochondria, a process which occurs via the carnitine cycle.

22.1.1 The Carnitine Cycle

In a first step after entering the cells, fatty acids move to the outer mitochondrial membrane bound to fatty acid-binding proteins. At the outer mitochondrial membrane, they are converted into the respective acyl-CoA (a coenzyme A ester of a fatty acid) by acyl-CoA synthases with the use of ATP. Acyl-coenzyme A esters are then converted into acylcarnitines by transferring the acyl moiety from coenzyme A to carnitine through the action of carnitine palmitoyltransferase I (CPT-I). This first step is the regulatory step of fatty acid oxidation. The acylcarnitine then enters the mitochondria in exchange for carnitine coming outside by the action of the carnitine-acylcarnitine translocase (CACT). Once inside the mitochondria, the acylcarnitine is exchanged for coenzyme A to an acyl-CoA ester through the action of carnitine palmitoyltransferase II (CPT-II). This process is reversible, and acyl-CoA that accumulates in the mitochondria can exit the mitochondria as acylcarnitine esters (Fig. 22.1). This process is taken advantage of in acylcarnitine analysis that evaluates which acylcarnitines are accumulating in the mitochondria.

22.1.2 Fatty Acid β-oxidation

Fatty acid beta-oxidation is depicted in Fig. 22.2. In the first step, the acyl-CoA is oxidized by acyl-CoA dehydrogenase removing two hydrogens and transferring them to FAD (flavin adenine dinucleotide) resulting in 2,3-enoyl-CoA. The unsaturated bond is between atoms 2 and 3 on the beta-carbon. There are several different acyl-CoA dehydrogenases, which differ in the chain length specificity of their substrates. Short-chain acyl-CoA dehydrogenase (SCAD) acts on fatty acids with four to six carbons. Medium-chain acyl-CoA dehydrogenase (MCAD) acts on fatty acids with six to ten carbons. Long-chain acyl-CoA dehydrogenase (LCAD) acts on fatty acids with 10–18 carbons but has limited activity in fatty acid oxidation. These three enzymes (LCAD, MCAD, and SCAD) occur in the mitochondrial matrix. Very long-chain acyl-CoA dehydrogenase (VLCAD) acts on fatty acids of 10–20 carbons and acts in the inner mitochondrial membrane. VLCAD is the enzyme that first starts the β-oxidation for normal dietary fatty acids of 16 and 18 carbons.

The second step then uses water and places one hydrogen on carbon 2 and a hydroxyl group on carbon 3. This hydratase enzyme thus makes a 3-hydroxyacyl-CoA. There are at least two hydratases: long-chain enoyl-CoA hydratase, present in the inner mitochondrial membrane, and short-chain enoyl-CoA hydratase, present in the matrix. The next step removes two hydrogens from the hydroxyl group on carbon 3 and transfers them to NAD making NADH and H+ by the enzyme 3-hydroxyacyl-CoA dehydrogenase. There are two enzymes, a long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) present in the inner mitochondrial membrane and a short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) present in the mitochondrial matrix. In the last step, the thiolase enzyme cleaves the carbon chain between carbons 2 and 3 and transfers the shortened carbon chain onto coenzyme A making a new acyl-CoA of two carbons shorter and releasing acetyl-CoA. There is a long-chain enolase in the inner mitochondrial membrane and a short-chain thiolase in the mitochondrial matrix. The three long-chain enzymes, long-chain hydratase, long-chain 3-hydroxyacyl-CoA dehydrogenase, and long-chain thiolase, are part of the same single trifunctional enzyme, which consists of an α- and a β-chain and resides in the inner mitochondrial membrane close to the VLCAD enzyme. The VLCAD and the trifunctional enzyme are responsible for β-oxidation of acyl-CoAs from 12 to 20 carbons, before releasing them to the enzymes in the mitochondrial matrix.

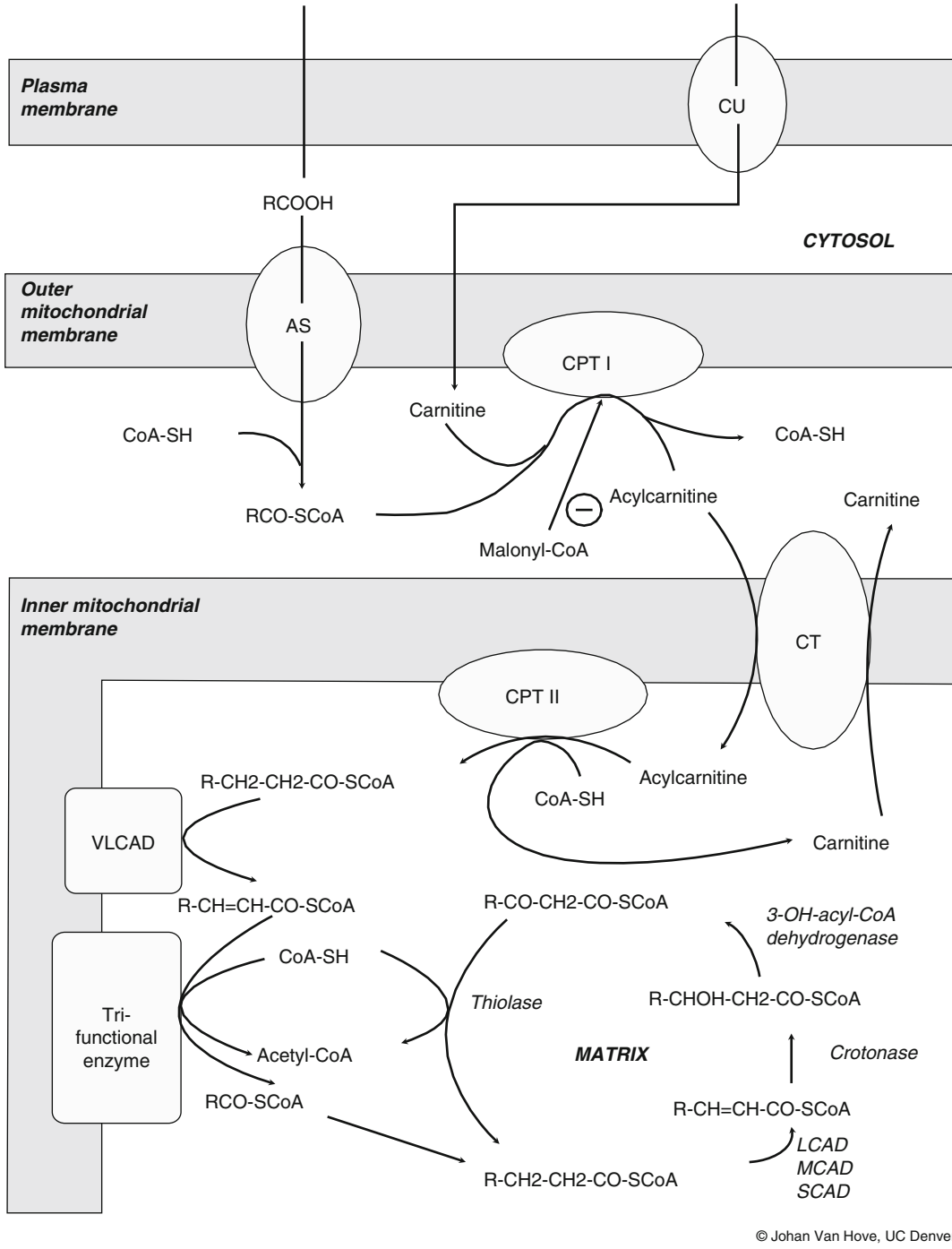

Fig. 22.1 The carnitine cycle and fatty acid oxidation

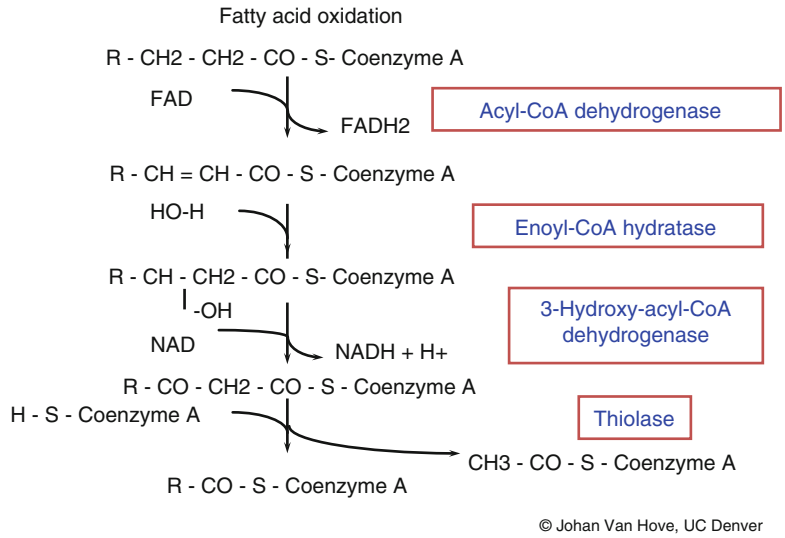

Fig. 22.2 Beta-oxidation of fatty acids

22.1.3 Ketogenesis and Ketone Utilization

When fatty acid oxidation occurs at a high rate, the liver will generate excess acetyl-CoA molecules that are used in ketogenesis. Ketones are generated by first making 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by HMG-CoA synthase, which is then converted to acetoacetate by HMG-CoA lyase. The free acetoacetate is in equilibrium with 3-hydroxybutyrate with a usual 3-hydroxybutyrate-acetoacetate ratio of 3:1. Other tissues can use ketones by three steps: in a first step, acetoacetate enters the mitochondria by the monocarboxylate transporter MCT1, it is then activated by coenzyme A transfer from succinyl-CoA by succinyl-CoA:3-oxoacid transferase (SCOT), and then acetoacetyl-CoA is cleaved by acetoacetyl-CoA thiolase, of which there are two, α- and β-ketothiolase. Lack of one of the enzymes in ketone generation will result in insufficient ketogenesis, whereas a lack of an enzyme in ketone utilization will result in excessive ketosis.

22.1.4 Regulation of Fatty Acid Oxidation and Ketone Metabolism

The rate at which fatty acid β-oxidation occurs is related to the availability of its substrates, the free fatty acids. Free fatty acids are released from fat tissue when glucose and insulin concentrations are low. The amount of ketones produced is directly related to the amount of free fatty acids available, and a ratio of ketones to free fatty acids can be used for assessment of the efficacy of this process. This ratio is the best measure of overall intactness of the process, and free fatty acids, 3-hydroxybutyrate, and acetoacetate should be measured in case of hypoglycemia. Insufficient levels of ketones for the amount of free fatty acids indicate a defect in β-oxidation or in ketogenesis, and excessive ketones for the amount of free fatty acids indicate a defect in ketone utilization. There is a relationship between low glucose concentrations and the release of free fatty acids and hence of ketone generation, which can also be used as an indicator of a ketone disorder but is more indirect. After a meal, the preferred substrate is carbohydrate with very limited fatty acid oxidation, less than 10 % of total capacity. Hence, only patients with severe disorders of fatty acid oxidation will be symptomatic at this stage. During short-term fasting, primarily glucose from glycogen breakdown is used. Only after a prolonged fast of at least 12–15 h, for children over 1 year of age, will lipolysis occur and fatty acid oxidation commence at full rate. The highest rates of ketogenesis occur in children ages 1 to 3 years, decreasing thereafter. After 12–15 h of fasting, even patients with mild fatty acid oxidation

defects will be unable to keep up with the high rate of fatty acid β-oxidation and can become symptomatic. The fasting associated with illness is a common trigger for fatty acid oxidation defects. The cytokines and hormones released with the stress of infection further promote lipolysis and hence shorten the duration of fasting, and stimulate high rates of β-oxidation occur and symptoms can be expected in fatty acid oxidation defects. Most fatty acid oxidation defects thus typically occur with intermittent symptoms. These patients tend to be asymptomatic between episodes but have intermittent problems usually elicited by fasting and infections or other illnesses. Only patients with severe fatty acid oxidation defects who have no residual enzyme activity can present at any time with symptoms related to their fatty acid oxidation disorder. Finally, muscle cells use fatty acid β-oxidation during prolonged exercise. During short exercise, muscles will use blood glucose and endogenous glycogen stores. During prolonged exercise, muscles will switch to the use of fat as an energy substrate. At that time, patients with fatty acid oxidation defects can exhibit muscle symptoms, including weakness, cramps, and lysis of muscle cells called rhabdomyolysis.

22.2 Symptoms of Fatty Acid Oxidation Defects

Fatty acids are an important fuel for the liver, heart, and for muscle during long exercise. Symptoms of fatty acid oxidation defects therefore can include elements of liver, cardiac, or skeletal muscle dysfunction. In the liver, symptoms occur intermittently, mainly during episodes of fasting, even more so during infection. The common symptoms constitute a Reye-like syndrome of hypoglycemia, elevated transaminases, mild hyperammonemia, and brain edema with lethargy and coma. Uric acid is usually elevated due to energy failure. All fatty acid oxidation defects can present with Reye-like syndrome. In fact, most cases of idiopathic Reye syndrome diagnosed before 1990 were patients with an unrecognized fatty acid oxidation defect. Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is exceptional in that it causes chronic cholestasis. In addition, carrier women during pregnancy with a fetus affected with LCHAD deficiency have a high rate of acute fatty liver of pregnancy.

In the heart, fatty acid oxidation defects can cause cardiomyopathy. The cardiomyopathy is usually associated with a degree of hypertrophy. Cardiomyopathy is typical for severe fatty acid oxidation defects of long-chain fatty acids. Cardiomyopathy in those with carnitine transporter defect is typically dilated in nature without hypertrophy. Severe ventricular arrhythmias (ventricular tachycardia, ventricular fibrillation, torsades de pointes) occur in fatty acid oxidation defects. They are frequent in severe fatty acid oxidation defects of long-chain fatty acids and particularly prominent in carnitine-acylcarnitine translocase deficiency but can also occur in MCAD deficiency during decompensation. Atrioventricular block can occur but is rare.

In skeletal muscle, symptoms are usually triggered by prolonged exercise. They most often consist of acute cramping followed by rhabdomyolysis. Muscle weakness can be a feature, but is not common. Rhabdomyolysis results in the large release of muscle cell proteins in blood and can result in precipitation of small proteins such as myoglobin in the renal tubules resulting in acute renal blockade and renal insufficiency. Prevention of acute renal failure thus requires hyperhydration, often needing large amounts of intravenous fluids. Some patients exhibit recurrent rhabdomyolysis, whereby any excessive exercise will immediately result in symptomatic rhabdomyolysis. Finally, even fatty acid oxidation defects such as MCAD can have elevated creatine kinase concentrations with metabolic decompensations without muscle symptoms.

22.3 Diagnostic Testing

The diagnosis of fatty acid oxidation defects can be made by recognition of typical metabolites, by functional assays, by specific enzyme activity assays, and by molecular analysis. A primary test

is the recognition of insufficient ketone concentrations for the duration of fasting and the amount of its precursor free fatty acids. During the acute presentation, quantitative ketones in serum (3-hydroxybutyrate and acetoacetate) should be measured together with free fatty acids. The first-line test for fatty acid oxidation defects is usually measurement of the amounts of carnitine (total, free, and esterified carnitine) and an analysis of the specific fatty acyl esters attached to carnitine in the acylcarnitine profile. The normal acylcarnitine profile should show high concentrations of the natural precursors to fatty acid oxidation palmitoyl-CoA and oleyl-CoA as C16 or C18:1 carnitine esters and of its end product acetyl-CoA as acetylcarnitine (C2). All intermediates should be low (<1 μM). An increase in a specific acylcarnitine species can indicate a blockage at the level of fatty acid oxidation and is often very specific and diagnostic. Most disorders are best diagnosed by acylcarnitines in plasma. The exception is carnitine palmitoyltransferase I (CPT-I), where a decrease in palmitoylcarnitine is looked for, which is more readily diagnosed in blood spots. Fasting results in a mild increase in C14 acylcarnitines, and normal values associated with fasting have to be used in this setting, which is often relevant for samples taken during episodes of symptoms. Acylcarnitine analysis is used in newborn screening for fatty acid oxidation defects. Urine organic acids can show increased dicarboxylic acids, but these have very limited specificity. Medium-chain fatty acid oxidation defects (MCAD and MADD (multiple acyl-CoA dehydrogenase deficiency)) have increased acylglycine esters (hexanoylglycine and suberylglycine) that can be recognized on urine organic acids analysis or can be quantified in specific acylglycine analysis. SCAD deficiency and MADD can have increased ethylmalonic acid. A profile of free fatty acids in serum can be diagnostic, for instance, by showing increased cis-4-decenoic acid in MCAD deficiency, but is nowadays rarely used. The sensitivity of acylcarnitine analysis can be increased by first loading with fatty acids. This is typically done *in vitro* by incubating fibroblasts with both fatty acids and carnitine and measuring acylcarnitines in the medium and the cell pellet in a test called the fatty acid oxidation probe analysis. This test allows for the diagnosis of even mild fatty acid oxidation defects. Enzyme assays exist for most fatty acid oxidation defects in either fibroblasts or in leukocytes. This is most often used in VLCAD deficiency. However, the large spectrum of mutations in VLCAD deficiency often including many mild mutations with substantial residual activity results in an overlap between the enzyme activities seen in carriers and those in patients affected with mild mutations, making diagnosis imperfect. Molecular analysis of all fatty acid oxidation disorders is now readily available. Sequencing should be complemented with exonic deletion and duplication analysis and is frequently used as the first confirmatory test following recognition by metabolite analysis.

22.4 Overview of Selected Fatty Acid Oxidation Disorders

22.4.1 Medium-Chain Acyl-Coenzyme A Dehydrogenase (MCAD) Deficiency [10–25]

MCAD deficiency is the most common fatty acid oxidation defect in North America and Northern Europe. The medium-chain acyl-CoA dehydrogenase has substrate specificity for fatty acyl-CoAs with chain lengths of six, eight, and ten carbons, including the unsaturated C10:1 acyl-CoA derived from unsaturated fatty acids. There are overlapping substrate specificities at each chain length with other acyl-CoA dehydrogenases such as SCAD for shorter chain lengths and LCAD for longer chain lengths. As a result, even in patients with two severe mutations with no residual activity of the MCAD enzyme, there is still about 20 % of residual enzyme activity. Thus, during the normal fed state, there is sufficient capacity of fatty acid oxidation that a normal flux of fatty acid metabolism is measured. As a result, outside of acute episodes, patients with MCAD deficiency are asymptomatic. Only when the demand for fatty acid oxidation is increased, such as during prolonged fasting, will the

Box 22.1: Clinical Presentation of MCAD Deficiency

- Age:
  - Most common: infancy up to 2 years
  - Neonatal period: 5 % of MCAD patients present in first 3 days of life; usually breastfed
  - Can occur at any age under stress
- Triggering factors: fasting and infections
- Symptoms
  - Reye-like syndrome
  - Hypoglycemia
  - Heart: arrhythmia, sudden death
  - SIDS-like
  - Autopsy: fatty liver and heart, brain edema

reduction in fatty acid oxidation capacity become evident and will symptoms occur.

Clinical symptoms usually present during the first 2 years of life but can occur at any age (Box 22.1). Up to 5 % of patients present in the first 3 days of life, often with fatal consequences. These patients have all been exclusively breastfed, where breast milk production was somewhat delayed and the infant was starving. Triggering factors are always prolonged fasting and often infections. Typical symptoms involve a Reye-like syndrome with hypoglycemia, elevated transaminases with mild hepatomegaly, elevated uric acid, and mild hyperammonemia (with values usually in the hundreds). There is brain edema resulting in lethargy and coma. Cardiac arrhythmias have been recognized as a terminal event in many children. Elevated creatine kinase is seen in half the children, but frank rhabdomyolysis is very rare. There is hypoketosis for the duration of fasting, the level of free fatty acids, and the degree of hypoglycemia (hypoketotic hypoglycemia). The unrecognized condition has a mortality of 20 % and a high morbidity with resulting learning problems. Children who become ill in the evening and do not receive typical treatment can be found dead in their crib by sudden infant death syndrome (SIDS). At autopsy, fatty infiltration of the liver and heart is found as well as brain edema, distinguishing it on pathology from SIDS. Postmortem analysis of acylcarnitine in a blood spot or in bile can be diagnostic, or molecular analysis can be pursued.

The condition is autosomal recessive and is caused by mutations in the *ACADM* gene. The most common mutation is c.985A>G resulting in p.K329E. The mutation is present in 90 % of symptomatic cases and in about 50 % of cases identified on newborn screening. The mutation is most prevalent in Northern European populations. The incidence of MCAD deficiency ranges from 1:24,000 to 1:17,000. Diagnosis is usually made by acylcarnitine analysis showing elevated C6, C8, C10, and C10:1 acylcarnitines with an increase in C8 greater than the increase in C10. Other diagnostic metabolites are increased concentrations of the acylglycines, suberylglycine, and hexanoylglycine in urine. Total carnitine concentrations are often decreased. Sequencing the *ACADM* gene is used as a confirmatory test. Given the high frequency of MCAD deficiency and the intermittent symptomatology, it is necessary to screen family members including not only siblings but also parents, as asymptomatic affected status of parents has been observed (pseudodominant inheritance). This condition requires not only a conducive genotype, but also a strong environmental stress factor, most importantly a long duration of fasting before becoming symptomatic. This makes it also well amenable to treatment. This condition satisfies all criteria for newborn screening and has been added in most countries to the universal newborn screening programs.

Foremost in the treatment of MCAD deficiency is the avoidance of long fasting at all times (Box 22.2). Families are instructed in counting fasting time. They are told to review back to the last meal that provided good caloric intake and to count the time of fasting since then. They are given a maximal time of fasting that can be tolerated before they must seek medical help. For children over 1 year of age, the maximal fasting time is 15–18 h. The shortest fasting time at which MCAD-deficient children were observed to exhibit hypoglycemia was 12 h. The duration of routine fasting should be maximally 12 h, thus

Box 22.2: Treatment of MCAD Deficiency

- Avoid prolonged fasting under any circumstances.
  - Infections, medical procedures, alcohol consumption.
  - Provide caloric support in illness.
- Provide L-carnitine in acute episodes – consider utility in chronic management.
- Avoid medium-chain triglycerides (MCT).
- Rarely supplement uncooked cornstarch at night.

leaving families a few hours in which to try different strategies to provide calories before requiring intravenous support.

During clinic visits, scenarios where long fasting can occur are reviewed such as illness in the morning after an overnight physiologic fast of sleep. With an illness in the evening with reduced oral intake or vomiting, long fasting will occur overnight. Parents are then instructed to wake up their child and to try to feed the child and, if unsuccessful, to bring the child to the emergency room and not to wait until the morning. All caregivers in the family must be educated, and families that do not attend clinic and show noncompliance must be particularly paid attention to as this is a risk factor for metabolic decompensation and a risk of fatality during illness. Notes must be given to other medical professionals to avoid long fasting associated with medical or dental procedures, and prophylactic intravenous glucose support instituted in case of long procedure-associated fasting. Enteral intake of sufficient calories must be ensured before patients can be released to home after an anesthesia or dental extraction without complications of nausea, vomiting, or trismus. Fat restriction is not needed except to avoid very high-fat diets such as Atkins diet or ketogenic diet. Valproate interferes with fatty acid oxidation and should be avoided. Low levels of carnitine are common in patients with MCAD deficiency. The degree of carnitine deficiency outside of acute episodes is mild for most children and does not interfere with fatty acid oxidation. During acute episodes, carnitine levels can fall precipitously worsening the fatty acid oxidation flux, and carnitine supplementation is then indicated. Some children, particularly young infants, have limited capacity to make carnitine and can have very low concentrations, in which case carnitine supplementation can be indicated as a routine daily dosing. Additional risk factors for low carnitine include very young age such as infancy, heterozygosity for carnitine transporter defect, vegetarian diet, renal disease, or pregnancy. Adults have a longer fasting tolerance but can still develop a fatal Reye-like syndrome. Care should be taken for the consumption of alcohol, whereby use of excessive amounts of alcohol both interferes with fatty acid oxidation and liver function and creates a risk for hypoglycemia, prolonged fasting (e.g., vomiting during hangover), and decreased consciousness.

Outcome of treatment is excellent. Patients with MCAD have become Olympic athletes or attained professions of the highest education. With careful management, particularly of fasting avoidance, we have instituted prophylactic admissions but have avoided any episode of metabolic decompensation. Both mortality and morbidity are strongly reduced.

22.4.2 Very Long-Chain Acyl-Coenzyme A Dehydrogenase (VLCAD) Deficiency [26–38]

22.4.2.1 Biochemistry and Symptoms

The very long-chain acyl-coenzyme A dehydrogenase (VLCAD) enzyme has substrate specificity for long-chain acyl-CoAs of 10–20 carbons. VLCAD is the main enzyme for acyl-CoAs of this chain length, and there is very little other enzyme activity. Therefore, mutations that completely abolish residual activity will result in a near complete block of fatty acid oxidation flux and can cause symptoms outside of fasting, often in the neonatal period. The majority of mutations of patients identified with VLCAD on newborn screening are mild and leave residual activity. These patients are still at risk for intermittent symptoms at times of increased fatty

Box 22.3: Clinical Presentation of VLCAD Deficiency

- Cardiac (severe form): acute cardiomyopathy with severe ventricular arrhythmia risk
- Liver: Reye-like syndrome (hypoglycemia, elevated transaminases, mild hyperammonemia, and brain edema with lethargy and coma)
- Muscle: myopathy, rhabdomyolysis
  - Often associated with prolonged exercise, fasting, stress, or exposure to cold

acid oxidation flux such as during fasting or in the muscle with prolonged exercise.

There are three clinical presentations for VLCAD deficiency (Box 22.3). All patients, regardless of genotype, are at risk of developing Reye-like syndrome identical to that of MCAD deficiency. The triggers are similarly prolonged fasting and infection. All patients are also at risk of myopathic symptoms with intermittent episodes of rhabdomyolysis, sometimes of painful cramping, and rarely of weakness. Some patients have frequent recurrent episodes of severe rhabdomyolysis. These can be triggered by prolonged exercise, fasting, stress, or exposure to cold. Even patients with mild mutations have experienced recurrent rhabdomyolysis. Only patients with severe VLCAD deficiency develop cardiomyopathy. The cardiomyopathy is usually hypertrophic. It can range from mild hypertrophy without involvement of cardiac contractility to severe dysfunction requiring transplantation. Cardiac ventricular arrhythmias are also possible with prolonged QT, ventricular tachycardia, torsades de pointes, and ventricular fibrillation. Patients who present in the neonatal period with ventricular arrhythmias and who have a structurally normal heart often have severe fatty acid oxidation defects.

22.4.2.2 Diagnosis

VLCAD deficiency is identified on acylcarnitine profile by elevations of long-chain acylcarnitines C12, C12:1, C14, C14:1, C14:2, C16, and C18:1. Characteristic is the pronounced elevation of C14:1 higher than C14 and C14:2. Care must be taken to use fasting-appropriate normal values, as fasting causes a mild elevation of C14:1 and C14 in normal individuals reflecting the greater fatty acid oxidation flux. In mild patients, the acylcarnitine profile can be abnormal on the first newborn screening at 24 h of life and can then normalize in the next days, while the patient can still be vulnerable to life-threatening Reye-like syndrome in the right context of illness. Molecular analysis of the *ACADVL* gene with both sequencing and exonic deletion and duplication analysis is the next step. Deletions or duplications are rare (<1 % of alleles). Enzyme activity assays are possible in leukocytes, but there is some overlap between the affected patients with residual activity and carriers, leaving room for diagnostic uncertainty in select cases. VLCAD is frequently identified on newborn screening with an incidence of 1:20,000 to 1:40,000. Most patients diagnosed on newborn screening carry at least one mutation.

22.4.2.3 Treatment

The primary treatment is the avoidance of long-duration fasting, similar to that of MCAD deficiency (Box 22.4). For patients with severe VLCAD deficiency (with the cardiac phenotype), the fasting duration is shorter than for MCAD and must be strictly adhered to. Extra energy can be provided as medium-chain triglycerides. For patients with the severe form, fat intake must be severely restricted to about 10 % of caloric intake, and up to 20 % of calories must be provided as medium-chain triglycerides. During such severe fat restriction, care must be taken to provide sufficient essential fatty acids. This improves cardiomyopathy, reduces long-chain acylcarnitines, and reduces the frequency of rhabdomyolysis episodes but does not prevent them. Odd chain length medium-chain triglycerides such as triheptanoin oil have similar results, with comparative studies with regular medium-chain triglycerides pending. For the prevention of rhabdomyolysis, prolonged exercise must be avoided with breaks of 15–30 min instituted after 30–45 min of exercise. Carbohydrates and medium-chain triglyc-

Box 22.4: Treatment for VLCAD Deficiency

Treatment of mild VLCAD:

- Avoid long fasting, see MCAD Box 22.2
- Diet: The use of diet in this setting is not consistent, and in the case of diet, the ratio of medium chain triglycerides to long chain triglycerides varies by clinic.
- Rhabdomyolysis
  - Prevention: avoid prolonged exercise of more than 45 min; add alternative energy source such as MCT or extra protein before scheduled exercise
  - Treatment: hyperhydration, alkalinization, analgesia, rest

Treatment of severe VLCAD:

- Same as mild VLCAD plus:
- Avoid long fasting: use shorter fasting duration, include uncooked cornstarch at bedtime
- Diet:
  - Diet very low in long chain fat 10 % of calories, ensure sufficient amounts of essential fatty acids
  - Additional energy source: add MCT at 20 % of calories, high protein intake (in particular if muscle or cardiac symptoms)
- Monitor cardiac function:
  - Contractility for hypertrophic cardiomyopathy, which may even sometimes require transplantation
  - Arrhythmias, severe ventricular, which may sometimes require a cardioconverter

eride supplements can be taken prior to exercise and reduce the incidence of rhabdomyolysis and muscle cramps, as well as improve exercise capacity of medium-intensity exercise. These interventions are effective at improving the cardiac symptoms, at avoiding Reye-like syndrome, and at reducing but not preventing rhabdomyolytic episodes. The cardiomyopathy responds to dietary intervention but can be so severe as to require cardiac transplantation.

22.4.3 Long-Chain 3-Hydroxyacyl-Coenzyme A Dehydrogenase (LCHAD) Deficiency [39–49]

22.4.3.1 Biochemistry and symptoms

The trifunctional protein is a mitochondrial inner membrane enzyme that consists of an α-chain which has the 3-hydroxyacyl-CoA dehydrogenase enzyme activity and a β-chain which has the hydratase and thiolase activity. A common mutation c.1528G>C, comprising 60 % of the alleles, specifically impairs the 3-hydroxyacyl-CoA dehydrogenase enzyme activity while leaving the other two enzyme activities intact. Other mutations impair all three enzyme activities and are called trifunctional protein deficiency. Patients with the c.1528G>C mutation therefore tend to have higher concentrations of 3-hydroxyacylcarnitines and a greater incidence of symptoms such as retinal dysfunction. No other enzyme has overlapping enzyme activity, and patients with two severe mutations without residual activity can have severe symptoms such as cardiomyopathy similar to that of the severe cardiac phenotype of VLCAD deficiency.

Patients with LCHAD deficiency can have Reye syndrome, cardiomyopathy, and myopathic symptoms of recurrent rhabdomyolysis similar to the phenotype of VLCAD deficiency. Patients with LCHAD deficiency have additional symptoms that are specific to this fatty acid oxidation disorder. Infants can present with prolonged cholestasis with fibrosis leading to liver insufficiency. They tend to have chronic lactic acidosis that can be confused with respiratory chain enzyme deficiency and can have decreased activities of respiratory chain enzymes on biopsy. In childhood, patients can develop a retinal dystrophy with retinitis pigmentosa leading to loss of vision. The incidence and progression of retinitis pigmentosa is related to the elevation of 3-hydroxyacylcarnitines and is more common in isolated LCHAD deficiency with c.1528G>C mutation carriers. Patients can develop disabling and painful peripheral neuropathies. Finally, during pregnancy of a fetus affected with LCHAD deficiency, the mother who is an obligate heterozygote can develop acute fatty liver of pregnancy or hemolysis, elevated liver enzymes, and low platelets (HELLP)

syndrome. This complication is particularly prevalent in fetuses affected with the 1528G>C mutation, more so than in trifunctional protein-deficient patients. However, the incidence of LCHAD in the common HELLP syndrome is low (<1 %).

22.4.3.2 Diagnosis

Acylcarnitine profiles indicate elevated long-chain acylcarnitines C12, C14, C18, and C18:1, but they also show elevated 3-hydroxyacylcarnitines hydroxy-C14, hydroxy-C16, and hydroxy-C18:1. Urine organic acid analysis shows dicarboxylic acids and 3-hydroxydicarboxylic acids. These latter metabolites can rarely also be observed in certain patients with respiratory chain enzyme deficiencies. Lactate and the lactate to pyruvate ratio are often elevated. The incidence of LCHAD deficiency on newborn screening is estimated at 1:60,000. The diagnosis is usually confirmed by mutation analysis of the genes for the α-chain *HADHA* and the β-chain *HADHB*. Enzyme assays are nowadays rarely available.

22.4.3.3 Treatment

The treatment of LCHAD deficiency is similar to that of severe VLCAD deficiency. Strict treatment is necessary in order to reduce the development of long-term complications such as retinal dystrophy. Treatment consists of avoidance of fasting, severe reduction of dietary fat, and provision of medium-chain triglycerides, while guarding for sufficient amounts of polyunsaturated fatty acids, in particular docosahexaenoic acid (DHA). Fat restriction and particularly sufficient MCT oil provision are associated with reduced concentrations of 3-hydroxyacylcarnitines.

22.5 Ketogenesis Defects

3-Hydroxy-3-methylglutaryl-coenzyme A synthase is the first step in the synthesis of ketones. Patients present with hypoketotic hypoglycemia and hepatomegaly. They respond promptly to treatment with glucose. No other metabolites accumulate, and urine organic acids and plasma acylcarnitines are normal. The primary diagnostic sign is the low ketones to free fatty acids ratio, in the presence of hypoglycemia. If not identified on acute presentation, then a careful fasting test can help establish the diagnosis. Confirmatory testing is best done by molecular analysis by sequencing the *HMGCS2* gene. Treatment is by avoidance of long-term fasting.

22.6 Ketolysis Defects [50–60]

After the activation to its coenzyme A ester of acetoacetate by succinyl-CoA:3-oxoacid transferase, the acetoacetyl-coenzyme A must be cleaved into acetyl-CoA by thiolase. There are three thiolase enzymes: one cytosolic enzyme and two mitochondrial enzymes (distinguished by the laboratory property of one being activated by potassium and the other is not). The main enzyme only cleaves acetoacetyl-CoA and provides a baseline thiolase activity. The other enzyme cleaves both acetoacetyl-CoA and 2-methylacetoacetyl-CoA derived from isoleucine metabolism. The added capacity of this latter enzyme is required to metabolize the high flux of ketones generated during fasting and full ketogenesis. In its absence, insufficient capacity exists to metabolize the large flux of ketones and they accumulate. The accumulation of keto acids causes an anion gap metabolic ketoacidosis. Clinical symptoms usually reflect the metabolic acidosis with Kussmaul breathing and vomiting. Unresolved, the accumulation of keto acids can result in brain damage including basal ganglia stroke and developmental delays. Treatment consists of reducing ketosis by providing glucose, and preventive treatment consists of avoidance of long-duration fasting. β-Ketothiolase also metabolizes branched methylketones such as methylacetoacetyl-CoA derived from isoleucine. Rarely, metabolic decompensation can be triggered by excessive protein intake, and a modest reduction in protein intake to a maximum of 2 g/kg/day is usually indicated. Diagnosis is made by the recognition of hyperketosis (>6 mM ketones in blood) and the presence of methylketones in urine organic acids (2-methylacetoacetate, 2-methyl-3-hydroxybutyrate). During the fed state, these metabolites can be very low, and it can be difficult to establish a diagnosis without confirmatory test-

ing. Confirmatory testing is done by measuring the enzyme activity of β-ketothiolase in fibroblasts or by sequencing the *ACAT1* gene.

Succinyl-CoA:3-oxoacid transferase (SCOT)-deficient patients have mutations that abolish the function of the enzyme completely. They cannot metabolize even the low flux of normal ketone body generation and metabolism, resulting in metabolic ketoacidosis occurring in the neonatal period. Patients with SCOT have excessive ketones even in the fed state, resulting in neonatal acidosis, tachypnea, hypotonia, vomiting, obtundation, and coma. A few rare patients have been reported to have mutations that leave some residual activity. These patients present with hyperketosis upon fasting similar to β-ketothiolase deficiency. Treatment is difficult. Diagnosis is made by enzyme assay in fibroblasts or by mutation analysis of the *OXCT1* gene.

Patients with mutations in the monocarboxylate carrier exhibit intermittent hyperketotic metabolic acidosis. Patients with biallelic mutations have more profound acidosis than patients with a mutation on a single allele. The primary diagnostic method is sequencing of the *MCT1* gene.

22.7 Summary

Fatty acid oxidation disorders and ketone metabolism disorders often present with intermittent symptoms triggered by prolonged fasting. Avoidance of fasting is a key component of treatment, and its practical application requires continued education of parents to maintain vigilance. Symptoms of cardiac and skeletal muscle pose important problems particularly in patients with long-chain disorders, for which current treatment is only partially effective.

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23 Nutrition Studies in Long-Chain Fatty Acid Oxidation Disorders: Diet Composition and Monitoring

Melanie Boyd Gillingham

Contents

Core Messages

In patients with long-chain fatty acid oxidation disorders (LCFAOD):

- Energy requirements may differ from that of the normal population.
- Diets high in lean sources of protein appear to increase lean body mass and decrease liver lipid content.
- Plasma acylcarnitine profiles improve when dietary long-chain fat is restricted and MCT is supplemented.
- MCT improves exercise tolerance.
- Low-fat diets may cause deficiencies of essential fatty acids, DHA, and/or fat-soluble vitamins.
- DHA supplementation improves retinal function in patients with LCHAD.
- Lowering potentially toxic metabolites, the hydroxyacylcarnitines, in plasma slows the progression of retinopathy.

23.1 Background

Disorders of fatty acid oxidation (FAODs) are relative newcomers to the arena of inborn errors of metabolism. The first well-documented disorders were described in the early 1970s in patients with skeletal muscle weakness or exercise-induced rhabdomyolysis [1–4]. As such, there is

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a dearth of evidence on which to base nutrition recommendations and determine appropriate biomarkers to monitor over time. This chapter will address some of the common difficulties with monitoring patients with FAOD and summarize the currently available evidence to support a rational treatment approach to nutrition therapy and monitoring. The focus of this particular chapter is long-chain FAOD, including carnitine palmitoyltransferase (CPT2), very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, and trifunctional protein (TFP) deficiency.

23.2 Estimating Total Energy Needs in Patients with FAOD

Providing adequate energy and carbohydrates is a key component of the nutritional management of FAOD. Maintaining anabolism suppresses fat oxidation and lowers circulating acylcarnitines. However, it is unclear if patients with FAOD have similar energy expenditure as individuals without FAOD. Estimates of energy needs are based on normal populations with a standard body composition, energy expenditure, and substrate oxidation.

Body composition and energy expenditure were recently compared between nine adolescent subjects with LCHAD deficiency and age-, sex-, and BMI-matched controls [5]. Patients with LCHAD deficiency tended to have a higher percent of fat mass and lower percent of fat-free mass than the control subjects (Fig. 23.1a). This difference in body composition appears to be due to both a slight increase in fat mass and a decrease in lean mass, as suggested by similar fat mass and lean mass indexed to height between groups (Fig. 23.1b). The fat content was distributed equally between the trunk and extremities in the subjects. There was no difference in intrahepatic lipid content between patients with LCHAD deficiency and control subjects (Fig. 23.1c, d) as measured by magnetic resonance spectroscopy (MRS). LCHAD-deficient patients exhibited a trend toward more lipid deposition in the extramyocellular space but no difference in intramyocellular lipid deposition of the soleus muscle compared to control subjects (Fig. 23.1c, e). The lean mass compartment of the body is metabolically active tissue and the most tightly associated with total energy expenditure; therefore, patients with lower lean body mass may have less metabolically active tissue and lower energy needs than the normal population.

Subjects with LCHAD deficiency, and perhaps other FAODs, have lower total lean mass. Thus, traditional energy estimates may overestimate their total energy needs. We measured energy expenditure in this group of subjects to determine if their energy expenditure differs from the normal population without a FAOD. Resting energy expenditure was not significantly different between LCHAD-deficient patients and control subjects (Fig. 23.2a, b). Respiratory quotient (RQ) was significantly higher in patients with LCHAD deficiency after an overnight fast compared to control subjects (Fig. 23.2c). There was no significant difference in protein oxidation between the groups, but, as expected, patients with LCHAD deficiency oxidized more carbohydrate and less fat at rest than controls (Fig. 23.2d). We used doubly labeled water to measure total energy expenditure in subjects and controls. Total energy expenditure was approximately 15 % lower in LCHAD-deficient patients in comparison to control subjects (Fig. 23.2e, f). We suspect that the similar resting energy expenditure and lower total energy expenditure is due to lower physical activity among subjects with LCHAD deficiency.

If there are differences in both body composition and total energy expenditure among subjects with LCHAD deficiency, these need to be taken into account when estimating total energy requirements (Box 23.1).

Normal assumptions about body composition and activity may not be true for subjects with a FAOD. Further research on energy balance in a wider patient population is needed. For clinical

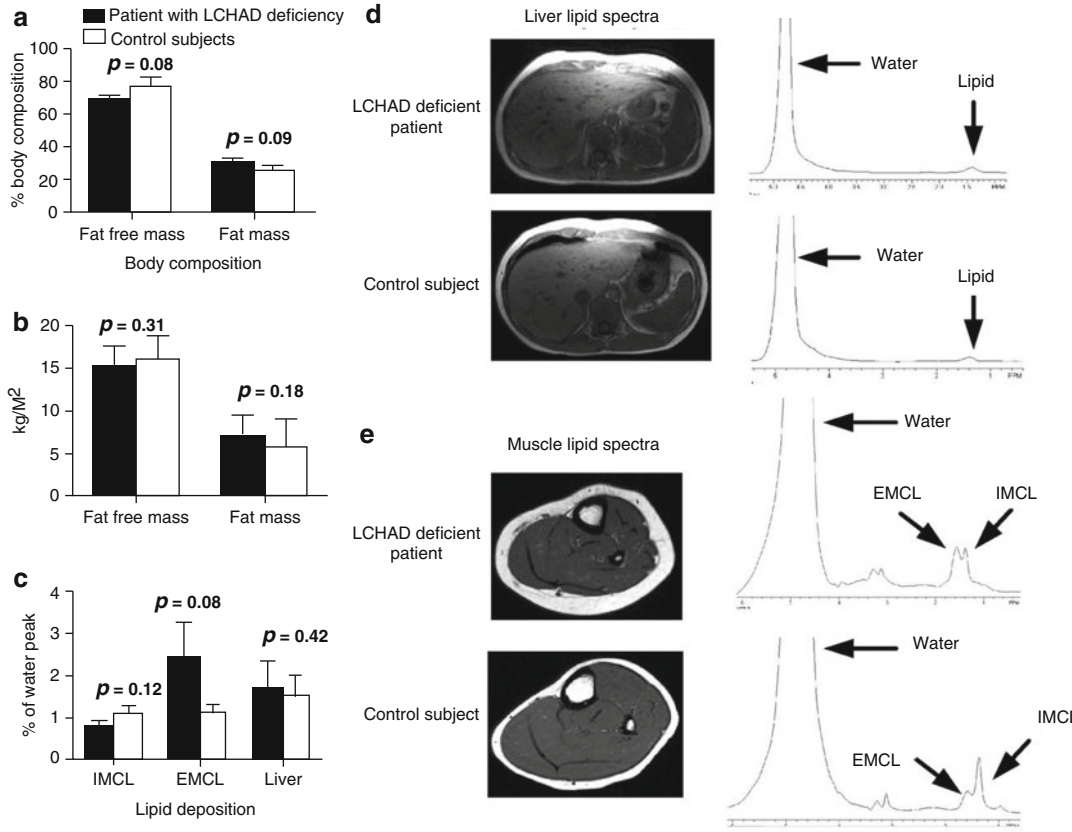

Fig. 23.1 Body composition and lipid deposition. Data are presented as means ± SD. (**a**) There is a trend for long-chain 3-hydroxy acyl-CoA dehydrogenase (*LCHAD*)-deficient patients ($n = 9$; *closed bars*) to have less fat-free mass and more fat mass compared with control subjects ($n = 9$; *open bars*) when expressed as %body mass. (**b**) There was no difference in fat-free or fat mass expressed as mass/surface area between groups. (**c**) There was a trend for LCHAD-deficient patients ($n = 9$; *closed bars*) to have more extramyocellular lipid (*EMCL*) but no difference in intramyocellular lipid (*IMCL*) compared with control subjects ($n = 9$; *open bars*). Liver lipid content was not significantly different between groups. (**d**) A representative magnetic resonance imaging (*MRI*) image of the abdomen and proton spectra of the liver for one LCHAD-deficient patient and the matched control subject are presented. The lipid peak is expressed as a percent of the water peak. (**e**) A representative MRI image of the calf and proton spectra for one LCHAD-deficient patient and the matched control subject are presented. The lipid peak is expressed as a percent of the water peak

practice today, there are two potential approaches to this conundrum. One approach would be to use a lower activity factor when calculating total energy needs such as 1.2 or 1.3 after calculating basal resting energy requirements (REE) with standard equations. Another would be to use novel total energy expenditure equations published in the Institute of Medicine's (IOM's) Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids [6]. The IOM collected all available doubly labeled water data and derived formulas for various population groups. The formulas derived for overweight and obese boys and girls most closely matched our data for patients with fatty acid oxidation disorders even though these subjects are not overweight or obese. This formula takes into account the difference between

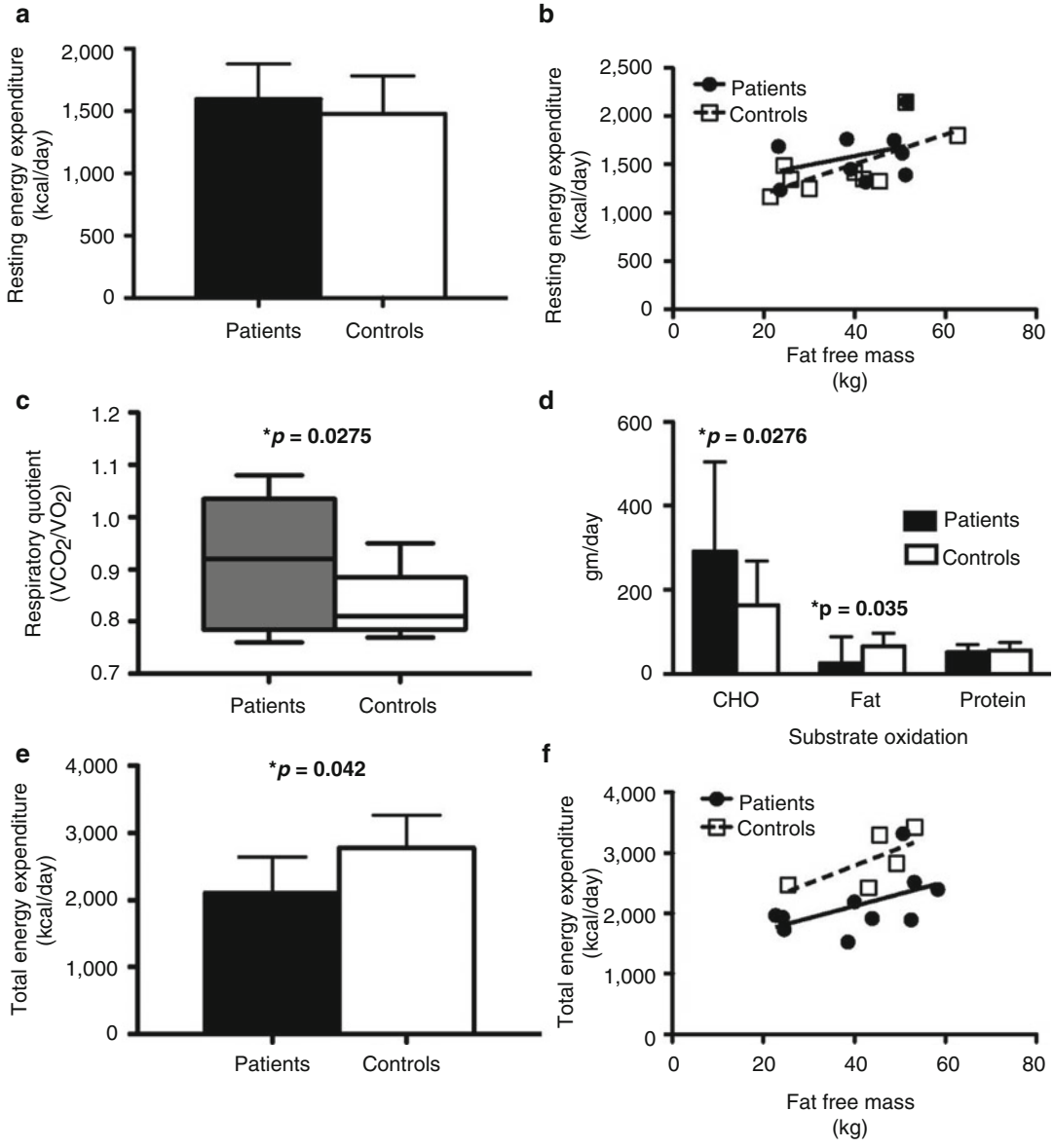

Fig. 23.2 Energy expenditure and substrate oxidation. Data are presented as means ± SD. Indirect calorimetry was measured after a 10-h overnight fast. LCHAD-deficient patients (n = 9; *closed bars* and *closed circles*) have a similar resting energy expenditure as control subjects (n = 9; *white bars* and *open squares*) expressed as mean kcal/day (**a**) or kcal/kg of fat-free mass (**b**). Resting respiratory quotient was significantly higher in the LCHAD-deficient patients (n = 9; *gray box plot*) compared with control subjects (n = 9; *white box plot*). (**c**) LCHAD-deficient patients (n = 8; *closed bars*) oxidized more carbohydrate and less fat than controls (n = 8; *white bars*). Total energy expenditure was lower in LCHAD-deficient patients (n = 9; *closed bars* and *closed circles*) compared with control subjects (n = 6; *white bars* and *open squares*) expressed as mean kcal/day (**e**) or as kcal/kg of fat-free mass (**f**)

lean mass and fat mass and perhaps a lower activity. It is possible that these formulas better estimate energy needs in patients with FAOD to recommend adequate energy for anabolism but at the same time not promoting overfeeding or weight gain (Table 23.1).

Box 23.1: Estimating Energy Requirements in the Normal Population Compared to Patients with FAOD

Standard equations assume the individual has normal:

- Energy expenditure
- Substrate oxidation
- Body composition

Patients with FAOD have been shown to have:

- Lower energy expenditure
- Higher carbohydrate oxidation and lower fat oxidation
- Lower lean body mass and higher fat mass

Table 23.1 Calculating estimated energy needs for FAOD using the IOM equation for obese children

Weight maintenance TEE[a] in overweight boys ages 3–18 years
$\text{TEE} = 114 - (50.9 \times \text{age[year]}) + \text{PA} \times (19.5 \times \text{weight[kg]} + 1161.4 \times \text{height[m]})$
Where PA is the physical activity coefficient:
PA = 1.00 if PAL is estimated to be ≥1.0 <4.0 (sedentary)
PA = 1.12 if PAL is estimated to be ≥1.4 <1.6 (low active)
PA = 1.24 if PAL is estimated to be ≥1.6 <1.9 (active)
PA = 1.45 if PAL is estimated to be ≥1.9 <2.5 (very active)
Weight maintenance TEE[a] in overweight girls ages 3–18 years
$\text{TEE} = 389 - (41.2 \times \text{age[year]}) + \text{PA} \times (15.0 \times \text{weight[kg]} + 701.6 \times \text{height}[m])$
Where PA is the physical activity coefficient:
PA = 1.00 if PAL is estimated to be ≥1.0 <1.4 (sedentary)
PA = 1.18 if PAL is estimated to be ≥1.4 <1.6 (low active)
PA = 1.35 if PAL is estimated to be ≥1.6 <1.9 (active)
PA = 1.60 if PAL is estimated to be ≥1.9 <2.5 (very active)

[a]Total energy expenditure

23.3 High-Protein Diet

Subjects with a FAOD are instructed to limit long-chain fat that can potentially lower the protein content of the diet unless low-fat, high-protein foods (e.g., lean meats, nonfat dairy products) are specifically incorporated into the diet plan. We have found that LCHAD-deficient subjects consume a relatively low-protein diet with 7–11 % of calories from dietary protein [7]. We investigated the effects of a standard high-carbohydrate diet or a high-protein diet for 4 months among subjects with CPT2, VLCAD, or LCHAD deficiency. The standard diet consisted of 10 % long-chain fats, 10 % medium-chain triglycerides (MCT), 12 % protein, and 68 % carbohydrate. The high-protein diet consisted of 10 % long-chain fats, 10 % MCT, 25 % protein, and 55 % total carbohydrate intake. Protein content was increased by using very lean boneless, skinless chicken breasts or lean turkey, fat-free dairy, and Beneprotein® (Nestle Nutrition Science, Florham Park, NJ), a whey protein. Six subjects were randomized to the high-carbohydrate diet, and seven subjects were randomized to the high-protein diet.

At the end of 4 months, subjects on the high-protein diet had a significant increase in lean body mass compared to those on the high-carbohydrate diet, but there was no significant difference in the change in fat mass over that same period of time. The high-protein diet resulted in a significant drop (about a 25 %) in liver lipid content compared to the standard diet. This was consistent across all patients. There was a significant decrease in liver lipid content. The high-protein diet improved overall body composition compared to the high-carbohydrate diet.

Despite the lower total carbohydrate intake, subjects on the high-protein diet had similar fat

oxidation as measured by a stable isotope tracer, 1-C^{13}-oleic acid. There was no difference in whole body fat oxidation between the high-carbohydrate diet and high-protein diet. Blood acylcarnitines were also measured before and after the breakfast meal. There was no significant difference in acylcarnitines between subjects on the high-protein diet compared to the high-carbohydrate diet. The high-protein diet with fewer carbohydrates did not increase fat oxidation or decrease metabolic control compared to the high-carbohydrate diet. Encouraging patients to have good sources of lean protein appears beneficial especially for body composition and liver lipid content. One caveat to this data is that children under the age of 7 years were not studied. Younger children depend on carbohydrate as an energy source and have a higher incidence of hypoglycemia. It is unknown if a slightly higher protein intake with a lower carbohydrate intake in younger children would increase the incidence of hypoglycemia.

23.4 LCHAD Retinopathy

A progressive chorioretinopathy is frequently observed in patients with LCHAD or TFP deficiency but not among any of the other FAODs [8–12]. Retinopathy occurs in up to 70 % of patients with LCHAD deficiency [13]. The progressive chorioretinopathy can lead to significant visual impairment and disability [7, 13–16]. The chorioretinopathy associated with LCHAD deficiency is reported to begin with peppery pigment clumping in the macula, which can appear early in life (Fig. 23.3) [13, 17].

The disease later progresses to atrophy of the posterior choroid, affecting the blood supply to the retina, the rods and cones, and the photoreceptors of the retina. As the retinopathy progresses, patients begin to lose both color and night vision followed by loss of central vision resulting in legal blindness. The etiology of the chorioretinopathy of LCHAD deficiency is unknown but may relate to toxic effects of accumulating metabolites or deficiency of the long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA), which has an important role in retinal function [7, 13–15]. Treatment with DHA has been reported to stabilize but not reverse eye findings [15]. In a study following 14 patients over 5 years, the elevated hydroxyacylcarnitines was the factor most associated with decreased retinal function and vision loss suggesting that they are potentially toxic to the retina although direct evidence of toxicity is lacking (Fig. 23.4) [15].

If the accumulation of hydroxyacylcarnitines is toxic to the retina, then lowering plasma metabolites should slow progression of the retinopathy and be a key goal of nutrition therapy. Restricting dietary fat intake decreases the accumulation of potentially toxic metabolites such as long-chain acylcarnitines in patients with LCHAD, TFP, or VLCAD deficiency [7, 18, 19]. In addition, supplemental MCT provides an alternate energy source downstream of the enzymatic block and decreases long-chain fatty acid (LCFA) oxidation [7, 14, 20–22]. There is a linear relationship between lower LCFA and increased MCT with hydroxyacylcarnitines in patients with LCHAD deficiency. When we separated the cohort into two groups, low- and high-MCT consumers, patients with LCHAD or TFP deficiency consuming 10 % of energy from LCFA and 10–20 % energy from MCT have significantly lower plasma hydroxyacylcarnitines than subjects consuming more LCFA or less MCT (Fig. 23.5) [7].

Subjects with LCHAD and TFP deficiency who maintained lower hydroxyacylcarnitines had significantly better vision and slower progression of chorioretinopathy over 5 years of follow-up [15]. In addition, patients who had fewer episodes of metabolic decompensation and fewer hospitalizations also had better vision and slower progression of retinopathy [23].

Diet may not be the only factor that determines the amount of accumulating hydroxyacylcarnitines; genotype may also play a significant role in metabolite concentrations. There is a common point mutation in TFP of European origin where there is a g to c transition at nucleotide 1,528 or c.1528G(arrow)C . In the US population,

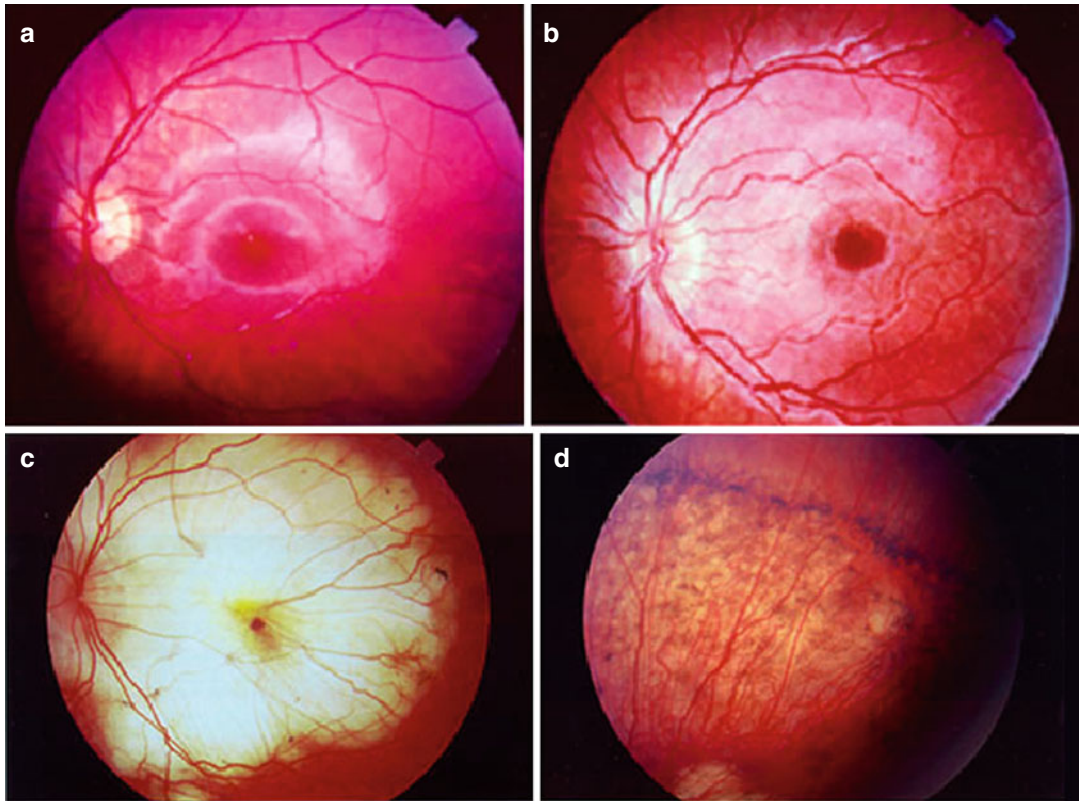

Fig. 23.3 Chorioretinopathy associated with LCHAD deficiency. (**a**) Retinal photo of a normal eye; (**b**) retinal photo of a child with early LCHADD retinopathy. Note the *dark* peppery clumping in the center of the eye and the *white area* to the *left*; (**c**) Retinal photo of a child with late-stage LCHADD retinopathy. The *white areas* represent part of the retina where the photoreceptors and underlying support cells have been lost and central vision is gone. (**d**) Retinal photo of the same eye as observed in panel (**c**) looking at the peripheral retina. The pigment that started centrally has moved outward toward the periphery as illustrated by the dark peppery clumps seen in the peripheral view

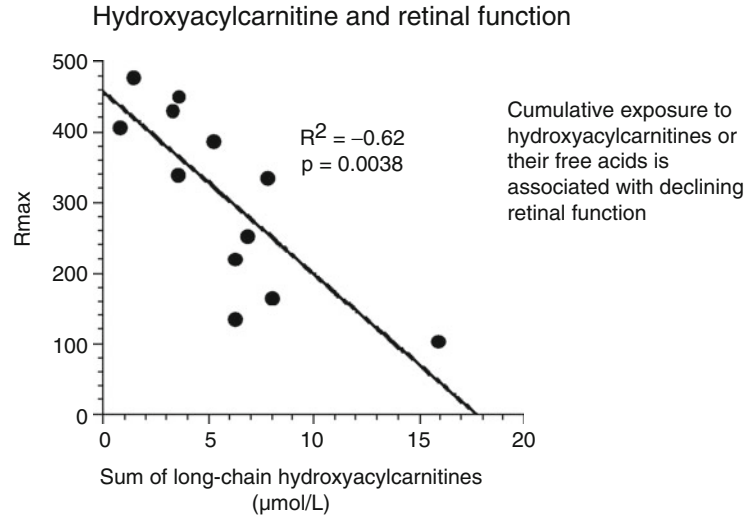

Fig. 23.4 Declining retinal function with increasing long-chain hydroxyacylcarnitines. Rmax, the maximal response of the retina to a bright flash of light measured during an electroretinogram (ERG), is lower in patients with higher circulating hydroxyacylcarnitines

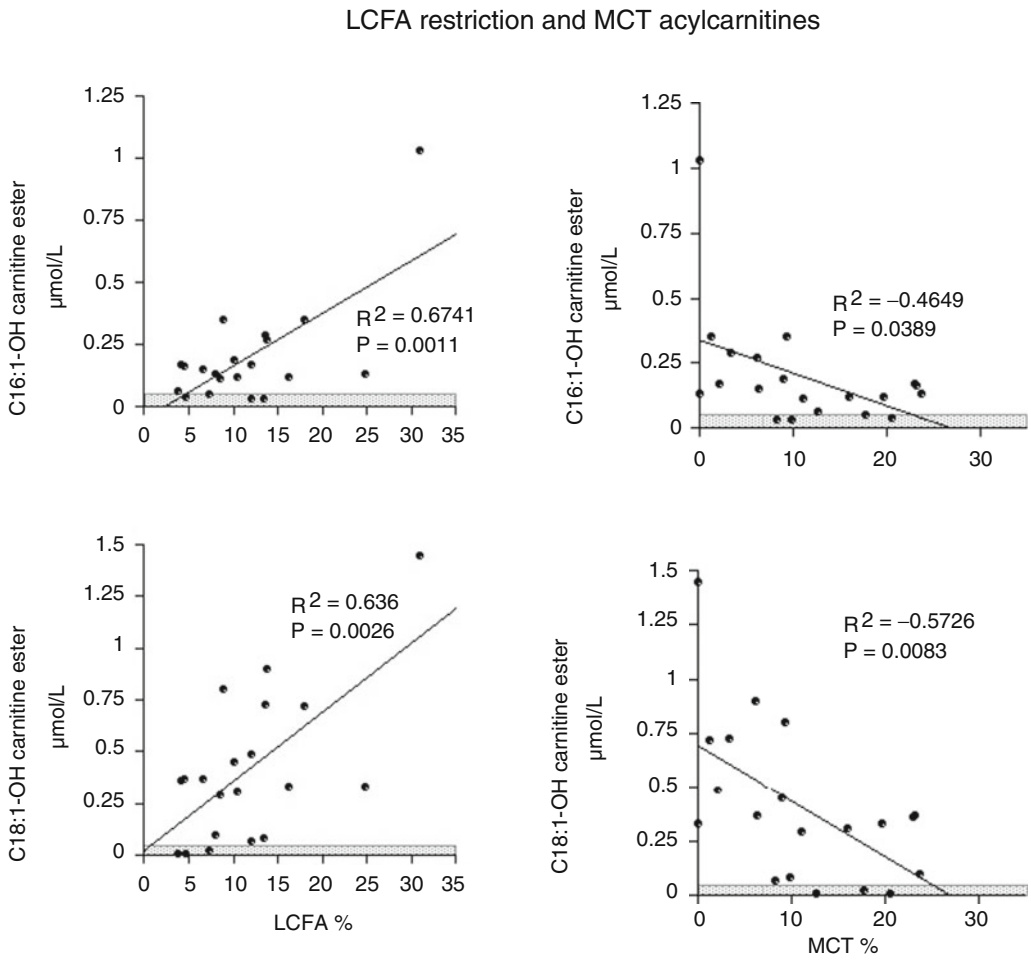

Fig. 23.5 Positive impact of a long-chain fatty acid restriction and supplementation of MCT on acylcarnitines in patients with LCHAD or TFP deficiency

about 80 % of the mutant alleles in patients with TFP deficiency carry this common mutation, and patients can be either heterozygous (meaning they carry only one copy of the common mutation) or homozygous (meaning they have two copies of the common mutation). There are a few patients who carry different mutations on the allele and do not have the common mutation; these patients have TFP deficiency. We examined the accumulation of hydroxyacylcarnitines after fasting and exercise by genotype [24]. The TFP-deficient patients have significantly less accumulated hydroxyacylcarnitines compared to subjects with the common mutation (Fig. 23.6).

For the RD working with patients with LCHAD and TFP deficiency, the patient who is homozygote or compound heterozygote for the common mutation will tend to have higher hydroxyacylcarnitines on a fairly low-fat diet compared to the later-onset TFP-deficient patient. It will most likely not be possible to normalize their hydroxyacylcarnitines. One goal would be to strive to keep the sum of the long-chain hydroxylated species <2.5 mM. Low-fat diet in patients with TFP deficiency may in fact normalize or nearly normalize their acylcarnitine profile. The effects of fat restriction on laboratory values will vary by genotype.

23.5 MCT and Exercise

At rest, skeletal muscle burns free fatty acids almost exclusively (85–90 % of total energy) [25]. During exercise, muscle glycogen stores

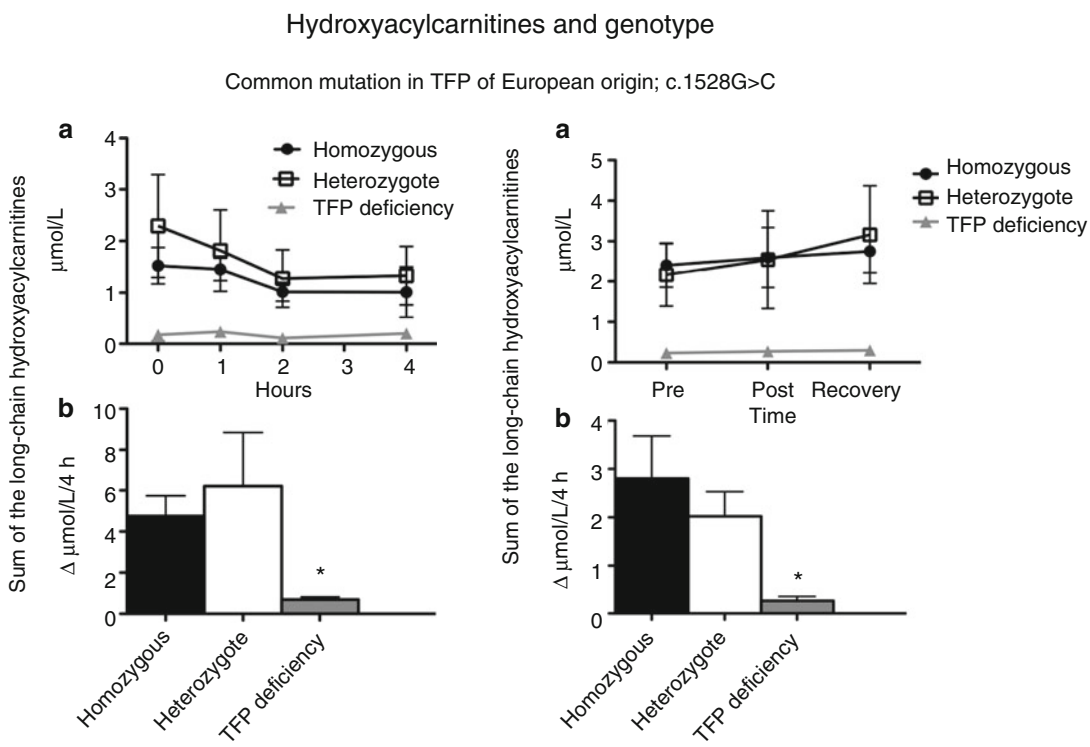

Fig. 23.6 Differences in hydroxyacylcarnitines between TFP and common mutation. (**a**) Post-prandial change in the sum of long-chain hydroxyacylcarnitines among subjects who are homozygous ● or heterozygous □ for c.1528G>C mutation and subjects who have TFP deficiency Δ. There was no difference in long-chain hydroxyacylcarnitinesbetween homozygous and heterozygous for c.1528G>C mutation at any timepoint. Long-chain hydroxyacylcarnitines were significantly lower among the siblings with TFP deficiency at all timepoints. (**b**) Total long-chain hydroxyacylcarnitines area under the curve (AUC) was significantly lower among the siblings with TFP deficiency compared to the other two groups. *indicates $p<0.05$ [24]

provide the majority of energy during the initial 20 min. Thereafter, the ratio of energy that comes from stored carbohydrates and fatty acids is dependent on the intensity of the exercise. At low or moderate exercise intensity, fatty acids provide as much as 60 % of the required energy for exercise [26]. Patients with long-chain FAOD often have recurrent episodes of exercise-induced rhabdomyolysis. Rhabdomyolysis may be related to an energy deficit in skeletal muscle resulting from the inability to oxidize LCFA. The standard treatment for rhabdomyolysis has been a low-fat, high-carbohydrate diet designed to maximize energy production from glucose oxidation. Subjects with CPT2 deficiency have been shown to have a lower heart rate and perceived exertion (Borg scale) with an increased duration of exercise when given carbohydrates intravenously or orally prior to exercise [27, 28]. Simple, easy to digest carbohydrates before and during exercise may help prevent the onset of exercise-induced rhabdomyolysis.

Alternatively, fatty acid supplements that bypass the block in long-chain FAOD may prove to be beneficial. The use of MCT as a performance-enhancing energy substrate has been studied in athletes. Trained adult athletes given MCT while exercising oxidized 72 % of the MCT dose during that bout of exercise [29]. Oral MCT is rapidly absorbed into the circulatory system (<20 min) and preferentially oxidized by the liver and mus-

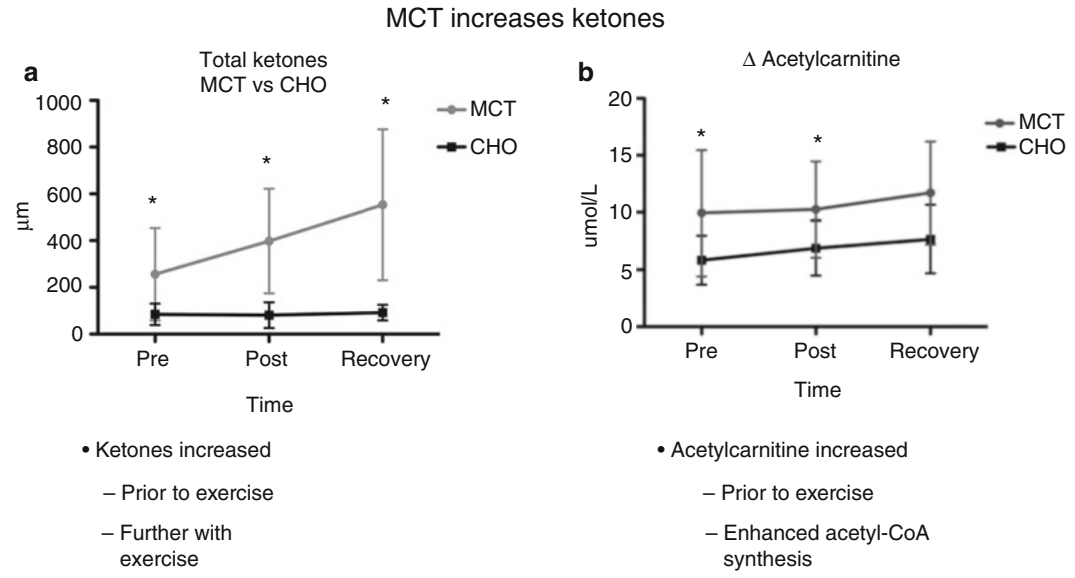

Fig. 23.7 Patients with FAOD had greater ketone production when given MCT compared to carbohydrates prior to exercise. (**a**) Change in total serum ketone bodies and (**b**) acetylcarnitines with and without MCT, measured immediately before, immediately after, and 20 minutes post exercise (*indicates significant difference) [33]

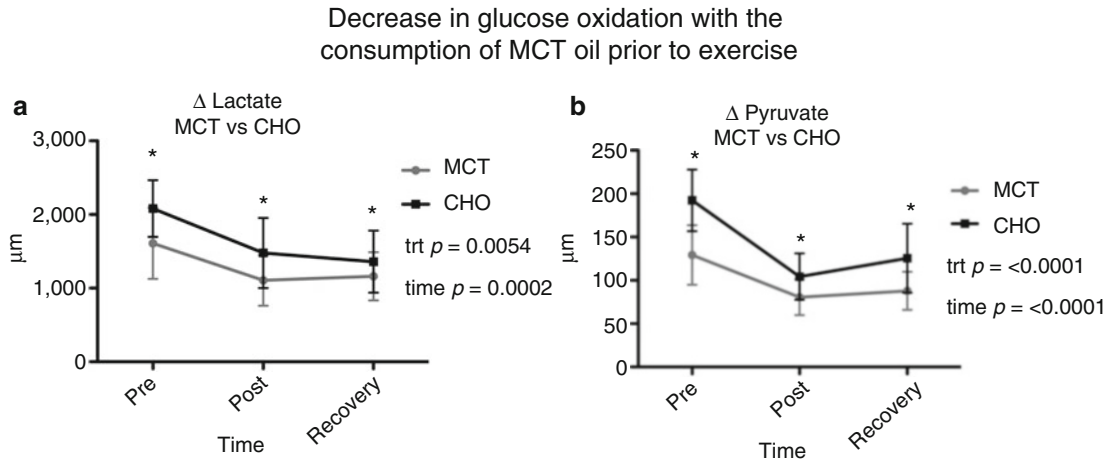

Fig. 23.8 A decrease in lactate and pyruvate production suggests that MCT oil decreases glucose oxidation during exercise when taken immediately prior to and during exercise. (**a**) change serum lactate, (**b**) change in serum pyruvate, measured immediately before, immediately after, and 20 minutes post exercise (*indicates significant difference)

cle [30, 31]. MCT given immediately prior to exercise improved exercise tolerance in subjects with FAOD (Figs. 23.7, 23.8, and 23.9) [32, 33].

Patients had significantly lower heart rates with higher ketone synthesis when given MCT prior to a moderate-intensity treadmill exercise test. Similarly, a recent case report found that MCT prior to exercise lowered muscle pain and the incidence of rhabdomyolysis in a patient with VLCAD deficiency [34]. Oral MCT supplementation of 0.3 g per kg lean body mass or 0.15–0.2 g per kg total body weight, with or without carbohydrates, immediately prior to exercise may improve exercise tolerance among patients with CPT2, VLCAD, and LCHAD/TFP deficiency.

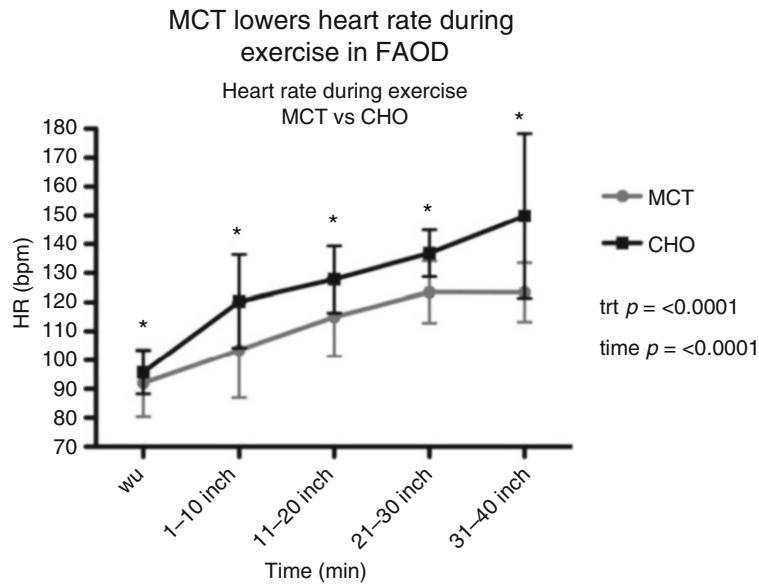

Fig. 23.9 MCT lowers heart rate during exercise and lowers the cardiac workload. Cardiac output and decreased oxygen utilization may also be improved with the administration of MCT oil during exercise

23.6 Essential Fatty Acids

Biochemical evidence of essential fatty acid deficiency has been diagnosed in treated patients with LCHAD, TFP, and VLCAD deficiency although overt clinical symptoms of deficiency are rarely documented [7, 14, 35]. Patients with long-chain FAOD on low-fat diets are at high risk for essential fatty acid deficiency, and plasma fatty acids should be monitored annually, preferably by a quantitative method [36]. Providing 4 % of energy as linoleic acid and 0.6 % as α-linolenic acid normalized plasma levels of essential fatty acids in two children with VLCADD [35]. However, providing >5 % of energy from essential fatty acids in the context of a diet low in total fat (10–20 % of energy) means other fats must be severely restricted from the diet. Thus, saturated long-chain fat intake from prepared foods should be minimized, and the majority of the long-chain fat intake should be provided by oils rich in essential fatty acids (Chap. 24: Nutrition Management of Fatty Acid Oxidation Disorders). In addition to preventing EFA deficiency, consuming more polyunsaturated fatty acids and decreasing consumption of saturated fat may lower plasma acylcarnitine concentrations. A study in cultured fibroblasts of patients with VLCAD, LCHAD, and TFP deficiency suggested linoleic (18:2) and linolenic (18:3) produced significantly fewer acylcarnitines than oleic (18:1) and palmitic (C16:0) acids [37].

Essential fatty acids include both omega-6 and omega-3 fatty acids because mammals cannot insert double bonds in those positions and must acquire these fatty acids in their food [6]. The omega-6 fatty acid linoleic acid (C18:2n-6) is the parent fatty acid and is required to maintain a normal skin water barrier and serves as a precursor for the endogenous synthesis of arachidonic acid, C20:4n-6. Arachidonic acid is required for normal growth and immune and reproductive functions. Linoleic acid is found in abundance in cooking oils such as sunflower, corn, and canola oil. Arachidonic acid is found in animal products such as eggs and meats. The intake of arachidonic acid among patients with FAOD may be lower than the normal population because foods high in arachidonic acid are also typically high in fat and may be avoided. The omega-3 fatty acid α-linolenic acid (C18:3n-3) is the parent fatty acid, but its primary function is to serve as a precursor for the endogenous synthesis of elongated products like eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) [6]. EPA and

DHA are involved in normal brain, visual, and immune functions. The conversion of linolenic acid to EPA and DHA is minimal and inefficient. In patients consuming very low-fat diets, it is often difficult to increase plasma DHA concentrations by providing α-linolenic acid from dietary sources such as flaxseed or walnut oil [14]. EPA and DHA are found preformed in fatty fish such as salmon or halibut. Due to the high fat content of these fish, they are extremely difficult to incorporate into a very low-total fat diet. Therefore, in patients with FAOD, supplementation specifically with DHA and EPA, rather than providing dietary precursors, may be needed to maintain plasma DHA within normal limits.

A specific deficiency of docosahexaenoic acid (C22:6n-3; DHA) has been noted in some children with LCHAD, TFP, and VLCAD deficiency [14, 35, 38]. DHA is an essential component of cell membranes and is necessary for normal retinal and brain function. Whether the cause of the DHA deficiency is related to the low-fat diet or to poor synthesis of DHA from its precursor α-linolenic acid is not known. Supplementing children with LCHAD, TFP, and VLCAD deficiency with preformed DHA (60 mg/day for children less than 20 kg; 100 mg/day for patients >20 kg; 100–200 mg/day for adults) should normalize plasma DHA levels and may slow progression of pigmentary retinopathy and peripheral neuropathy in LCHAD/TFP deficiency [15, 39, 40]. DHA supplements derived from algae rather than fish oils provide good amounts of DHA without other fatty acids.

23.7 Fat-Soluble Vitamins

Treated children with LCHAD, TFP, and VLCAD deficiency may also be at risk for fat-soluble vitamin deficiency because of the low-fat diet. A review of the dietary intake of ten children with LCHAD or TFP deficiency found adequate vitamin A and D intake related to the regular consumption of two to three cups of skim milk per day [7]. Dietary intake of skim milk provides a low-fat source of protein, B vitamins, and vitamins A and D. The intake of vitamins E and K was approximately 50 % of the RDI/RDA. A daily multivitamin and mineral supplement providing the RDA for vitamin A, D, and E seems prudent for patients with LCHAD, TFP, or VLCAD deficiency consuming a fat-restricted diet. Vitamin K is not routinely found in multivitamins, but no biochemical or clinical deficiencies of this micronutrient have been noted.

Vitamin E & LCHADD

2008 measured plasma γ and α tocopherol in 5 subjects with LCHADD

Homozygous or heterozygous for c. 1528G>C
Areflexia on neurologic exam
Chorioretinopathy of LCHADD

Subjects:	Gender:	Age:	γ-tocopherol μM	α-tocopherol μM
1	M	14	1.2	16.7
2	M	15	2.5	10.8
3	M	16	0.8	11.5
4	M	7	1	10.9
5	F	18	0.5	40.3

4 of 5 subjects α-tocopherol deficient (<20 μM)

Fig. 23.10 Plasma γ- and α-tocopherol concentrations in five children with LCHAD

We measured plasma vitamin E in five subjects with LCHAD deficiency who were prescribed a multivitamin with vitamin E and found four of the five had biochemical α-tocopherol deficiency (Fig. 23.10).

Diet records indicated patients were consuming adequate amounts of vitamin E with their supplements, but it did not appear to be absorbed, possibly because fat is necessary for the absorption of vitamin E. Patients were typically consuming their multivitamin with breakfast, a fat-free meal often consisting of fat-free cereal, skim milk, and orange juice. When the multivitamin supplement was moved to dinner, plasma tocopherol concentrations increased. Dinner was a low-fat meal but contained more total fat than the other meals during the day.

23.8 Summary

There are several unique considerations when planning the nutritional therapy of patients with long-chain fatty acid oxidation disorders. The diet should include adequate energy, but excessive energy intake will promote excessive weight gain that is difficult to reverse later in life. Total energy needed to maintain energy balance may be 10–15 % lower among individuals with LCFAOD because of their inability to oxidize fat. Patients consuming low-fat diets have lower metabolites in plasma that, in the case of LCHAD deficiency, slows the progression of retinopathy. However, restricting long-chain fat can sometimes lower total protein and increases the risk for essential fatty acid and fat-soluble vitamin deficiencies. Recommending lean, high-quality protein can improve lean body mass in subjects. Supplementation with small amounts of DHA and a multivitamin including the fat-soluble vitamins A, D, and E can prevent any potential deficiencies. MCT supplementation throughout the day and in particular immediately before exercise provides the muscle a usable source of energy, improves exercise tolerance, and lowers plasma metabolites.

23.9 Essential Fatty Acid Profile Case Example

KR, a 7-year-old female with LCHAD deficiency, is being evaluated in the clinic. Per her 3-day diet record, she is consuming a low-fat diet with added MCT oil. Upon exam, she is growing along her growth curve but remains at the 10th percentile for height and weight. A nutrition-focused physical exam shows clear healthy skin and nails. She has normal, shiny long hair and reports no recent history of hair loss. She has no physical signs and symptoms of an essential fatty acid deficiency. A plasma essential fatty acid (EFA) profile is ordered to check for biochemical EFA deficiency because she follows a low-fat diet with frequent meals, avoids fasting, and is at increased risk for EFA deficiency.

The following week, the profile given below is returned to the clinic. You are asked to interpret the results.

Fatty acid profile – first visit			
Compound	Reference	Patient	
C 8:0	8–47	43	
C10:1	1.8–5.0	7.8	H
C10:0	2–18	70	H
C12:1	1.4–6.6	6	
C12:0	6–90	22	
C14:2	0.8–5.0	6.8	H
C14:1	3–64	30	
C14:0	30–450	98	
C16:2	10–48	18	
C16:1n-9	25–105	86	
C16:1n-7	110–1,130	269	
C16:0 palmitate	1,480–3,730	1,426	L
C18:3n-6	16–150	18	
C18:3n-3 linolenic acid	50–130	37	L
C18:2n-6 linoleic acid	2,270–3,850	1,207	L
C18:1n-9	650–3,500	872	
C18:1n-7	280–740	207	L
C18:0 stearate	590–1,170	648	
C20:5n-3 eicosapentaenoic acid (EPA)	14–100	31	
C20:4n-6 arachidonic acid	520–1,490	316	L
C20:3n-9	7–30	7	
C20:3n-6	50–250	43	L
C20:0 arachidic acid	50–90	46	L
C22:6n-3 docosahexaenoic acid (DHA)	50–250	29	L
C22:5n-6	10–70	13	
C22:5n-3	20–210	38	
C22:4n-6	10–80	11	
C22:1	4–13	5	
C22:0	0.0–96.3	36.5	
C24:1n-9	60–100	82	
C24:0	0.0–91.4	38.8	
C26:1	0.3–0.7	1	
C26:0	0.00–1.30	0.78	
C19:B	0.00–2.98	0.04	
C20:B	0.00–9.88	0.5	
Holman ratio	0.010–0.038	0.022151899	

1. Examine the omega-6 EFA species. Plasma linoleic acid (C18:2n-6), the parent n-6 species, was 1,207 μmol/L, and the elongated product, arachidonic acid (C20:4n-6), was 316 μmol/L, both below the bottom of the normal range.
2. The Holman ratio is normal at 0.022. Based on the concentrations of linoleic acid and arachidonic acid, this patient has biochemical n-6 deficiency, but the traditional marker of n-6 deficiency, the Holman ratio, is normal. The normal Holman ratio is most likely related to the consumption of a low-fat diet.
3. Examine the saturated fatty acid concentrations to confirm why the Holman ratio is normal. Palmitate (C16:0) is 1,426 μmol/L, below the normal range. Stearate (C18:0) is normal but C20:0 is low. This profile demonstrates biochemical evidence of n-6 deficiency and low saturated fatty acids related to the very low-fat diet consumed by this patient.
4. Evaluate n-3 status. Linolenic acid (C18:3n-3) is low, 37 μmol/L, EPA is within normal limits (WNL) at 31 μmol/L, and DHA is low, 29 μmol/L. This profile demonstrates biochemical evidence of n-3 deficiency.

Overall, you observe biochemical n-6 and n-3 deficiency in this young girl following a very low-fat diet.

23.9.1 Nutrition Management Plan

1. Add an algae-based DHA supplement of 100 mg/day.
2. Replace some of the fat in her diet with a good source of n-6 fatty acids such as two teaspoons to one tablespoon of canola oil per day. This could be incorporated into foods such as sautéed vegetables and salad dressing and used when cooking lean meats like boneless, skinless chicken.
3. To keep the total fat low, decrease saturated fat intake in other foods by 10–15 g.

KR returns to the clinic for follow-up 6 months later. She continues to grow along her growth curve. She has started the DHA supplement but sometimes forgets to take it. She takes 100 mg about four times per week. Canola oil has been added to her diet but only about two teaspoons per day. She has no physical signs or symptoms of EFA deficiency, and an essential fatty acid profile is ordered to reevaluate the biochemical EFA deficiency. The following week, the profile shown below is returned from the lab.

Fatty acid profile – return visit 6 months after diet change			
Compound	Reference	Patient	
C 8:0	8–47	42	
C10:1	1.8–5.0	6.9	H
C10:0	2–18	73	H
C12:1	1.4–6.6	5.7	
C12:0	6–90	21	
C14:2	0.8–5.0	5.8	H
C14:1	3–64	34	
C14:0	30–450	137	
C16:2	10–48	36	
C16:1n-9	25–105	55	
C16:1n-7	110–1,130	269	
C16:0 palmitate	1480–3,730	1,662	
C18:3n-6	16–150	10	
C18:3n-3 linolenic acid	50–130	34	L
C18:2n-6 linoleic acid	2,270–3,850	2,143	L
C18:1n-9	650–3,500	872	
C18:1n-7	280–740	236	L
C18:0 stearate	590–1,170	612	
C20:5n-3 eicosapentaenoic acid (EPA)	14–100	27	
C20:4n-6 arachidonic acid	520–1,490	530	
C20:3n-9	7–30	8	
C20:3n-6	50–250	67	
C20:0 arachidic acid	50–90	46	L
C22:6n-3 docosahexaenoic acid (DHA)	50–250	181	
C22:5n-6	10–70	14	
C22:5n-3	20–210	35	
C22:4n-6	10–80	15	
C22:1	4–13	6	
C22:0	0.0–96.3	39.2	
C24:1n-9	60–100	71	
C24:0	0.0–91.4	39	
C26:1	0.3–0.7	1	
C26:0	0.00–1.30	0.69	
C19:B	0.00–2.98	0.08	
C20:B	0.00–9.88	0.7	
Holman ratio	0.010–0.038	0.01509434	

The linoleic acid has increased, but it is still a bit lower than the normal range. The arachidonic acid is normal. The biochemical n-6 deficiency is resolving. The linolenic acid is still low, but the DHA has increased significantly. Because linolenic acid has no independent function, you are not concerned about increasing linolenic acid concentrations.

Continue with the current diet plan of an algae-based DHA supplement and two to three teaspoons of canola oil per day.

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Nutrition Management of Fatty Acid Oxidation Disorders

24

Fran Rohr

Contents

Core Messages

- In both long-chain fatty acid oxidation disorders (LCFAOD) and medium-chain fatty acid oxidation disorders (MCAD), emergency management of acute illness and the avoidance of prolonged fasting are key treatment strategies.
- Chronic nutrition management of LCFAOD depends on the degree of disease severity, with the most severe forms requiring 8–10 % of total energy from long-chain fat.
- Exercise tolerance may be improved in LCFAOD by MCT and carbohydrate supplementation.
- Diets restricted in fat in LCFAOD require monitoring, especially of essential fatty acids and plasma acylcarnitine profiles.
- MCT should be avoided in MCAD; however, the restriction of other fats is not indicated, and a normal, healthy diet is recommended.

24.1 Background

The pathophysiology of fatty acid oxidation disorders is described in detail in Chap. 22. In summary, when fat is needed as an energy source, lipolysis occurs. Plasma-free fatty acids

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Table 24.1 Fatty acid oxidation disorders identified by plasma acylcarnitine analysis [4]

Disorders of carnitine metabolism
Carnitine uptake defect
Carnitine-acylcarnitine translocase deficiency (CACT)
Carnitine palmitoyltransferase I and II deficiency (CPT I and II)
Long-chain fatty acid (12–20 carbons) oxidation disorders
Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)
Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)
Trifunctional protein deficiency (TFP)
Medium-chain fatty acid (6–12 carbons) oxidation disorders
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)
Medium-chain 3-ketoacyl-CoA thiolase deficiency (MCKAT)
2,4 Dienoyl-CoA reductase deficiency
Short-chain fatty acid (<6 carbons) oxidation disorders
Short-chain acyl-CoA dehydrogenase deficiency (SCAD)
Short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency (SCHAD)
Other
Multiple acyl-CoA dehydrogenase deficiency (MADD) or glutaric aciduria II (GAII)

Adapted from Rinaldo et al. [4]

are released, esterified, conjugated with carnitine, and transported into the mitochondria where a series of carbon chain-length-specific enzymes break down the fatty acids into acetyl-CoA for entry in to the Krebs cycle [3]. A defect in any of the enzymes and/or transporters results in a fatty acid oxidation disorder and may cause symptoms associated with energy deprivation to the heart, liver, and/or muscle [4] (Table 24.1). This chapter focuses on the nutrition management of long-chain and medium-chain fatty acid oxidation defects. Short-chain acyl-CoA dehydrogenase deficiency (SCAD) is not treated with a special diet and presents as a benign condition [5].

Box 24.1: Principles of Dietary Treatment of Long-Chain Fatty Acid Oxidation Defects

Minimize long-chain fat as energy substrate by:

- Treating illnesses aggressively
- Avoiding periods of prolonged fasting
- Modifying the diet to:
  - Limit long-chain fat intake
  - Provide alternative energy sources, including:
    - Medium-chain triglycerides
    - Carbohydrate

24.2 Management of Long-Chain Fatty Acid Oxidation Disorders (LCFAOD)

24.2.1 Chronic Nutrition Management

The chronic nutrition management for all LCFAOD is the same except where noted. In LCFAOD, one or more enzymes in the mitochondrial beta oxidation of long-chain fatty acids are deficient; therefore, the main principle of nutrition management is to avoid reliance upon long-chain fat as an energy substrate. This is accomplished by aggressively treating illnesses, avoiding prolonged fasting, and modifying the composition of the diet (Box 24.1).

Specific recommendations depend on the patient's state of health and the severity of the disease. Historically, infants with LCFAOD presented clinically with severe disease and required strict modification of diet. Presently, newborn screening identifies infants early in life who may

Table 24.2 Maximum fasting interval (hours) in patients with MCAD or VLCAD when NOT ill

Age (mo)	Arnold [6] (US)	Spiekerkoetter [14] (Germany)	Dixon [27] (UK)	Derks [28] (Holland)	GMDI [29, 30] (US)
0–1	3–4	3	6	NA	4
1–3	No consensus	4	6	NA	4
4		4	8	NA	5
5		4	8	NA	6
6		6–8	8	NA	7
7		6–8	8	8	8
8		6–8	10	8	8
9		6–8	10	8	8
10		6–8	10	8	8
11		6–8	10	8	8
12		10–12	12	10	8
24+		10–12	12	12	8

have LCFAOD, many of whom are asymptomatic and therefore require less strict nutrition intervention. Clinical judgment determines how to aggressively treat asymptomatic infants while evaluating the extent of their disease and, moreover, how to properly classify the severity of disease. Current molecular and enzyme assessments are imperfect in predicting who is likely to become symptomatic (Chap. 23). As a result, there is wide variation in practice and management of patients with LCFAOD [6, 7].

24.2.2 Treating Illness

Perhaps the most important aspect of treating patients with FAOD is to counsel families about the urgency of seeking medical attention if the patient becomes ill, especially if he or she is not eating well or vomiting. Patients with FAOD are unable to produce sufficient energy from fat and will need carbohydrate as a quick energy source if they have been fasting due to illness. Carbohydrate can be provided in the form of food, medical foods containing glucose polymers (Appendix J), or IV glucose, depending on the patient's ability to tolerate feedings (for specific guidelines, see section: Acute Illness). Families are provided with an emergency protocol that describes the disease, emergency management, and laboratory testing to be done when the patient is ill [8].

24.2.3 Fasting

Fat is normally oxidized for energy when glucose and glycogen stores are depleted, generally after 12 h of fasting (Chap. 6). Ketones are a sign that fat is being used for energy production. In FAOD, fat is not metabolized properly to produce energy, resulting in hypoketotic hypoglycemia. Although an infant with FAOD should tolerate a 12 h fast without difficulty, clinics vary widely in their recommendations for the amount of time allowed between feedings [9], and most recommend shorter feeding intervals than 12 h to provide a margin of safety. The infant's ability to tolerate a fast will depend on the absence or presence of illness, when the last meal was consumed, and current body weight [9]. Table 24.2 shows typical recommendations for maximum fasting intervals in FAOD. Since newborns generally feed every 2–4 h, these recommendations do not usually interfere with the infant's normal feeding schedule. It may be necessary to wake a child at night if he or she has a severe form of FAOD; however, in milder forms of FAOD, this practice may cause overfeeding and unnecessary stress for the family.

Beginning at 6–12 months of age, uncooked cornstarch (1 g/kg/day) may be introduced at bedtime to provide a source of energy over night, but this approach is usually limited to patients with severe disease who are prone to hypoglycemia [7]. It is important to note that hypoglycemia may

Box 24.2: Initiating Nutrition Management in a Patient with a Long-Chain Fatty Acid Oxidation Disorder

Goal: Reduce or normalize plasma acylcarnitines.

- Restrict long-chain fat to 8–25 % of energy intake.
- Supplement: medium-chain triglycerides (MCT) (10–30 % of energy) and DHA 50–100 mg.
- Provide DRI for protein, energy, vitamins and minerals, and essential fatty acids.

not be the first symptom to present when a patient with FAOD is ill; therefore, monitoring blood glucose with glucometers is not recommended as it may lead to a false sense of security for families.

24.2.4 Diet Modifications

For the asymptomatic infant with LCFAOD who is breast-feeding well and is clinically normal (normal liver function tests, creatine kinase, and electrocardiogram), a change in diet may not be necessary. Rather, the infant is monitored closely and provided an emergency treatment protocol to be used in the case of illness [6]. In the severe forms of LCFAOD, the goal of the diet is to reduce long-chain fat and provide an alternative energy source (Box 24.2).

The total amount of fat in the diet is not restricted, only the source of the fat. Recommended long-chain fat intakes range from 8–10 % of energy in patients with severe forms of LCFAOD to 25 % of energy in mild or moderate forms of the disease (Box 24.3). All other nutrients except fat should be provided in amounts to meet the DRI (Appendix G).

Medium-chain triglycerides (MCT) are frequently used as an alternative energy source. MCT use has been shown to reverse cardiomyopathy [10, 11]. MCT are 6–12 carbons in length, readily absorbed via the portal vein, do not require L-carnitine for transport in the mitochondria, and do not depend on long-chain acyl-CoA dehydrogenases for oxidation [12]. They supply 10–30 % of energy in the diet for LCFAOD, depending on the severity of the disease [6, 13, 14]. Higher amounts of MCT are impractical because high concentrations of MCT can cause gastrointestinal cramping, vomiting, and diarrhea [15]. Some prescribe MCT on a weight basis, using 2–3 g/kg in infancy and 1–1.25 g/kg after the first year [16]. While MCT supplementation in LCFAOD is a well-established practice [17], animal studies suggest that long-term use is not successful in preventing, and may aggravate, the cardiac phenotype in mice [18].

Box 24.3: Determining the Amount of Dietary Fat for Patients with LCFAOD

Seven-year-old child with LCFAOD who requires *1,000* cal per day

1. Amount of fat based on *% of total energy intake*

   For example, in *severe* LCFAOD:
   - 30 % of total energy as fat = 300 kcal[1]
   - 10 % of total energy as long-chain fat = 100 cal (11 g LCF)
   - 20 % of total energy as MCT = 200 kcal (24 g MCT)

   (This is approximately a ratio of 2:1 MCT/LCF).
2. Amount of fat based on the *percent of total fat from long-chain fat and MCT*

For example, in *moderate* LCFAOD:
- 30 % of total energy as fat = 300 kcal[1]
- Half of fat calories (50 %) as long-chain fat = 150 kcal (17 g LCF)
- Half of fat calories (50 %) as MCT = 150 kcal (18 g MCT)

(This is approximately a ratio of 1:1 MCT/LCF).

[1] The total amount of fat is the same in both examples because total fat is not restricted in FAOD, only the source of fat. Recommended fat intake for healthy Americans is 25–35 % of energy intake [2].

Table 24.3 Fat content of medical foods used in treating long-chain FAOD in the USA

Formula	Fat % kcal	LCF % fat	MCT % fat	LA mg/100 g	ALA mg/100 g	Ratio N3: N6	DHA/ARA
Enfaport[a]	48	16	84	350	50	7:1	Yes
Lipistart[b]	40	20	78	1767	246	6:1	Yes
Monogen[c]	25	10	90	473	101	4.7:1	No
Portagen[a]	42	13	87	1620	ND	20:1	No
Pregestimil[a]	50	45	55	4700	480	10:1	Yes

LCF long-chain fat, *MCT* medium-chain fat, *LA* linoleic acid, *ALA* alpha-linolenic acid, *N3* omega-3 fatty acids, *N:6* omega-6 fatty acids, *DHA* docosahexaenoic acid
[a]Mead Johnson Nutrition Evansville, IN
[b]Vitaflo USA, Alexandria, VA
[c]Nutricia North America, Rockville, MD

Table 24.4 MCT Supplements

Product	% of kcal as MCT	MCT content	Kcal[a]
MCT Procal™ [b]	92	10 g per 16 g powder (16 g = 1 packet or 2 scoops)	105
MCT oil® [c]	100	14 g per tablespoon	116
Liquigen® [d]	96	13.5 g per 30 mL (1 oz)	135

[a]Per amount listed in column "MCT Content"
[b]Vitaflo USA, Alexandria, VA (vitaflousa.com)
[c]Nestle Nutrition, Florham Park, NJ (nestle-nutrition.com)
[d]Nutricia North America, Rockville, MD (nutricia-na.com)

Long-chain fat may be provided as either breast milk or standard infant formula. FAOD formulas are limited in long-chain fat and contain MCT (Table 24.3). A supplemental source of MCT, in addition to the medical food, is sometimes needed to meet MCT goals in patients with FAOD. The fat content (MCT and long-chain fat) of products for the treatment of FAOD is presented in Table 24.3, and MCT supplements are listed in Table 24.4.

Research studies using another energy source, triheptanoin or C7, are currently underway. Triheptanoin contains seven carbons in the fatty acid side chain and is oxidized to acetyl-CoA and propionyl-CoA, which is converted to succinyl-CoA, resulting in anaplerosis, or the supplying of Krebs cycle intermediates. It is theorized that triheptanoin results in greater energy production in patients with LCFAOD [19].

24.2.5 Diet After Infancy

The diet follows the same basic principles as for infants, except that the source of long-chain fat in the diet shifts from formula or breast milk to food sources. Patients are prescribed a daily limit in the number of grams of long-chain fat allowed in the diet and are counseled about how to count grams of fat in food. Using the grams of fat found on the nutrition label may be accurate enough in mild disorders, but in more severe disorders where fat intake is more strictly controlled, patients are taught to count to 0.5 g accuracy. In some centers, the use of special formulas for FAOD continues past infancy, and in other centers, the patient is weaned off of the special medical food and transitioned to low-fat or fat-free milk with MCT supplementation (Table 24.4).

24.2.6 Supplements

All patients with LCFAOD should be given supplemental DHA (50 mg for patients weighing <20 kg and 100 mg/day if ≥ 20 kg). DHA supplementation stabilizes the retinopathy that is seen in LCHAD [20]. Supplementing specific oils, such as walnut or flax oil, as a source of essential

Table 24.5 Essential fatty acid content of selected oils per mL

Sources of essential fatty acids[a]						
Source	Amount (mL)	Weight (g)	Long-chain fat (g)	Linoleic acid (mg)	α-Linolenic acid (mg)	Energy (kcal)
Flaxseed oil	1	0.9	0.9	114	480	8
Canola oil	1	0.9	0.9	183	84	8
Walnut oil	1	0.9	0.9	476	94	8
Safflower oil	1	0.9	0.9	672	0	
Corn oil	1	0.9	0.9	482	10	8
Soy oil	1	0.9	0.9	459	61	
Sesame oil	1	0.9	0.9	372	3	8
Peanut oil	1	0.9	0.9	288	0	
MCT oil	1	0.9	0	0	0	7.7

[a]Adapted from GMDI.org; data from http://www.nal.usda.gov/fnic/foodcomp/search/. Accessed 9/11/2014

fatty acids may be necessary. Fat-soluble vitamin intake may be low and may require supplementation (Table 24.5).

24.2.7 Diet for Exercise

Providing additional sources of energy for prolonged exercise may help prevent or reduce episodes of rhabdomyolysis in patients with LCFAOD. Recommendations include providing a source of complex carbohydrate and supplemental MCT (0.15–0.2 g per kg total body weight) for improved exercise tolerance [21, 22] (Chap. 23).

24.3 Monitoring of Patients with LCFAOD

Nutritional monitoring of FAOD is described in detail in Chap. 23 and is summarized below. (Box: Nutrition Monitoring of Patients with LCFAOD).

Individuals on fat-restricted diets are at risk for developing deficiencies of essential fatty acids, DHA, and fat-soluble vitamins. Erythrocyte or plasma fatty acid profiles and fat-soluble vitamins should be monitored yearly or more often if clinically indicated.

Plasma acylcarnitine profiles indicate whether the patient is accumulating acylcarnitines that may be toxic and if the fat intake in the diet should be adjusted. When long-chain fat is sufficiently restricted and enough MCT is provided, plasma acylcarnitine concentrations are lower [20]. However, even with fat restriction, acylcarnitine profiles may not completely normalize. A rule of thumb for determining if the diet is well controlled is if the sum of the acylcarnitine species in VLCADD or the sum of the hydroxy-acylcarnitine species in LCHAD or TFP is less than 2 μmol/L [1] (Box 24.4).

Box 24.4: Nutrition Monitoring of a Patient with FAOD

- Routine assessments including anthropometrics, dietary intake, and physical findings (Appendix F)
- Laboratory monitoring
  - Diagnosis specific
    - Plasma acylcarnitine profile
    - Plasma carnitine profile (total, esterified, free)
    - Creatine kinase
    - Liver function tests
    - Blood glucose
- Nutrition laboratory monitoring of patients on fat-restricted diets may include markers of:
  - Essential fatty acid sufficiency (plasma or RBC fatty acid profile)
  - Fat-soluble vitamin status: 25-hydroxy vitamin D, Vitamins A and E
  - Others as clinically indicated

Box 24.5: Summing It Up: Monitoring Acylcarnitines in FAOD

Aim for the sum to be <2 μmol/L [1].

- For VLCAD: Add up values of C:14, C14:1, C14:2, C16, and C18.
- For LCHAD: Add up values of C14OH, C16OH, C18OH, and C18:1OH.

Plasma carnitine is monitored in LCFAOD, but whether or not it should be supplemented is an area of controversy [6]. Some feel carnitine supplementation is indicated if plasma-free carnitine is low. Others feel that carnitine has not been shown to be beneficial and supplementation is not recommended [23]. Other biochemistries are done to monitor the effect of the disease on the muscle (creatine kinase) and liver (glucose, liver function tests) (Box 24.5).

24.4 Nutrition Management of Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)

24.4.1 Chronic Management

Patients with MCAD should avoid medium-chain triglycerides (MCT) but otherwise do not need dietary intervention when they are well. In the past, it was believed that fat restriction was necessary, but this is no longer the practice [7]. Breast feeding is allowed as long as the supply of breast milk is adequate. However, some infants present with MCAD in the first days after birth, often when breast-feeding is being established and the infant's energy intake is insufficient.

Carnitine supplementation in MCAD is controversial, and there is variation in practice [7]. Unlike in LCFAOD, in MCAD, acylcarnitines do not accumulate, so there is less concern about toxicity. In some centers, carnitine is recommended on a daily basis, but in others it is provided only during illness (Chap. 23). Many clinicians base their decision regarding supplementation by monitoring plasma-free carnitine and supplementing if the free carnitine is low.

24.5 Acute Nutrition Management in FAOD (MCAD and LCFAOD)

Patients with FAOD who become ill may be treated at home as long as they continue to eat well and are not vomiting or having diarrhea [24]. Any illness has the potential to be life-threatening and must be evaluated with the guidance of a metabolic physician. The amount of carbohydrate a sick patient at home should consume is described in Chap. 6 and presented in Appendix J.

Individuals with MCAD or LCFAOD are at risk of metabolic decompensation throughout life whenever there is a circumstance that could lead to energy deprivation, including illness, prolonged fasting (for religious or other reasons), vigorous or prolonged exercise, skipping meals/excessive dieting, or vomiting associated with illness, and eating disorders or binge drinking. Iatrogenic causes of prolonged fasting such as sedation, anesthesia, and surgical or dental procedures may require that IV glucose be given as an energy source when the patient is not allowed to eat by mouth. The metabolic physician should be consulted prior to elective surgical procedures and the emergency protocol used in case of an unplanned surgery. Counseling early in life about cautionary measures under these circumstances and continuing to follow patients with FAOD can help prevent problems.

Note that in MCAD, those most at risk for metabolic decompensation include patients who are homozygous for the A985G mutation [25] and patients with illnesses that include vomiting [26]. Special attention needs to be paid to make certain that families understand

the risks associated with FAOD and have sufficient social support to respond quickly to emergency situations.

24.6 Summary

Fatty acid oxidation defects cover a wide range of enzyme deficiencies in the metabolism of long-, medium-, and short-chain fats. Patients with LCFAOD and MCAD can present with severe illness or be asymptomatic. Chronic management of LCFAOD involves the restriction of long-chain fat and supplementation of MCT, a readily used source of energy that is not dependent on enzymes used in long-chain fatty acid oxidation. The degree of fat restriction depends on the severity of disease; those with severe forms are usually restricted to 10 % of energy as long-chain fat and 10–30 % of energy as MCT. MCT supplementation improves exercise tolerance and is associated with a reduction in the accumulation of plasma acylcarnitines. Patients with LCFAOD are monitored closely for signs of cardiac, liver, or muscle dysfunction. Patients with MCAD do not need day-to-day modifications in their diets but are at risk of acute metabolic crisis if fasted due to illness, stress, or other causes. Acute management of both LCFAOD and MCAD is to provide sufficient energy to prevent catabolism.

24.7 Diet Calculation Example

Example 1:
Infant with severe long chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)

Patient history	Nutrient intake goals (per day)
The patient is a three (3) day old female who was admitted to the intensive care unit with hypoglycemia and cardiomyopathy. Her presumed diagnosis is LCHAD based on elevated long-chain hydroxyl-acylcarnitines. She weighs 3.0 kg and is breast-feeding	**Energy:** 110–120 kcal/kg **LCF:** 10 % of energy **MCT:** 25 % of energy **Protein:** 10–15 % of energy

Steps in diet calculation: This child will be placed on Enfaport formula. An interactive step-by-step guide of this diet calculation is available at www.imd-nutrition-management.com.

Example 2:
Infant with severe LCHAD requiring parenteral nutrition (PN) support.

Patient history	Nutrient intake goals (per day)
This patient is a two (2) month-old male infant weighing 5 kg with severe VLCAD who has been on a low fat, MCT-supplemented diet (see Table below for composition of usual diet). This child is admitted to the hospital and is not able to tolerate enteral feeding	**Parenteral goals are the same as the usual enteral diet:** **Energy:** 100 kcal/kg **LCF:** 10 % of energy **MCT:** 30 % of energy **Protein:** 2.5 g/kg Note: MCT is not available in a PN solution but can be given as a drip or bolus feed

Usual diet for patient with LCHAD

	MCT (g)	LCT (g)	Linoleic (mg)	Linolenic (mg)	Protein (g)	Energy (kcal)
94 g Lipistart®	10	1.1	619	88	11.3	398
13 g MCT Procal®	8				1.6	81
3.5 ml Soybean Oil	0	3.3	1,618	216	0	28
1 ml Flaxseed Oil	0	0.9	149	532	0	8
Total	18	5.4	2,386	836	12.9	515
	29.2 % of kcal	**9.3 % of kcal**	**4.2 % of kcal**	**1.5 % of kcal**	**2.6 g/kg**	**103 kcal/kg**

Energy Composition of Diet Components:

Dextrose: 3.4 kcal/g
Trophamine®: 4.3 kcal/g
Intralipid®: 10 % = 1.1 kcal/mL (10 g fat per 100 ml)
20 % = 2.0 kcal/mL (20 g fat per 100 ml)
MCT Oil: 8.3 kcal/g; 1 TBSP = 15 mL = 14 g MCT = 116 kcal

Step-by-Step PN Diet Calculation:

Step 1. Determine the amount of protein and energy to be provided by Trophamine®.
Protein goal/kg × Infant weight = protein provided by Trophamine®
2.6 g/kg × 5 kg = 13 g protein from Trophamine®
13 g protein × 4.3 kcal/g = 56 kcal from Trophamine®

Step 2. Determine the amount of 20 % Intralipid® needed to provide 5.4 g long chain fat.
LCF goal ÷ 20 % Intralipid® (20 g/100 mL)
5.4 g ÷ 0.2 = 27 mL LCT from Intralipid®
27 mL × 2.0 kcal = 54 kcal from Intralipid®

Step 3. Determine the amount of MCT Oil needed to provide 18 g of MCT.
MCT Oil 19 mL = 18 g MCT
18 g MCT × 8.3 kcal/mL = 150 kcal
Provide 1 mL MCT oil via NG drip for 19 h per day

Step 4. Determine total energy provided by Trophamine® + Intralipid® + MCT Oil =
56 kcal + 54 kcal + 150 kcal = 260 kcal

Step 5. Determine remainder of energy to be provided by dextrose
Total kcal required − kcal provided in Step 4
515 kcal − 260 kcal = 255 kcal needed from dextrose

Step 6. Determine number of grams of dextrose to provide 255 kcal
kcals required from dextrose ÷ kcal/g of dextrose = g dextrose
255 kcal ÷ 3.4 kcal/g = 75 g dextrose

Step 7. Determine number of mL of dextrose (D10)
g of dextrose ÷ 10 g/100 mL
75 g ÷ 10 g/100 mL = 750 mL D10

Step 8. Determine hourly rate to run D10
mL of D10 ÷ number of hours
750 mL D10 ÷ 24 h = 31.5 mL/h D10

Nutrition Provided:
Energy: 515 kcals (103 kcal/kg)
Protein: 13 g (2.6 g/kg)
Long-chain fat: 5.4 g (10 % total energy)
Medium-chain triglycerides: 18 g (29 % total energy)

Example 3:
Young boy with mild VLCAD

Patient information	Nutrient intake goals (per day)
The patient is an eight (8) year-old boy with a mild variant of VLCAD. He weighs 25 kg. Calculate the amount of Liquigen® required to meet his MCT goals and the number of grams of fat from food that should be prescribed	**Energy:** 1,900 kcal (per DRI) **Total fat intake:** 30 % (normal fat intake) **LCF:** 20 % of energy **MCT:** 10 % of energy **Linoleic acid:** 10 g (per DRI) **α-Linolenic acid:** 0.9 g (per DRI)

Step-by-Step Diet Calculation

Step 1. Determine grams of MCT needed to provide 10 % of his energy
10 % of 1,900 cal = 190 cal
190 cal ÷ 8.3 kcal/g = 23 g MCT per day

Step 2. Determine how much Liquigen® will provide 23 g MCT
g MCT needed ÷ g MCT in 100 mL Liquigen®
23 g ÷ 45.4 g × 100 = 51 mL Liquigen®

Step 3. Determine mL of Liquigen® to be given three times per day
51 mL ÷ 3 = 17 mL per dose of Liquigen®

Step 4. Determine grams of LCF allowed in the diet
20 % of 1,900 kcal = 380 kcal from LCF
380 kcal ÷ 9 kcal/g = 42 g fat per day

Step 5. Determine distribution of fat intake (assuming 3 meals, 3 snack pattern)
10 g fat per meal (30 g) plus 4 g fat per snack (12 g) = 42 g

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Part V

Disorders of Carbohydrate Metabolism

The Diet for Galactosemia

25

Laurie E. Bernstein and Sandy van Calcar

Contents

Core Messages

- Classical galactosemia can result in life-threatening complications including failure to thrive, hepatocellular damage, and *E. coli* sepsis in untreated infants.
- Initiation of a soy formula or a lactose-free medical food within the first few days of life can mitigate these complications.
- Patients with classical galactosemia are at an increased risk for developmental delay, speech problems, and neurological complications, even with lifelong dietary treatment.
- Dairy products are the primary source of dietary galactose.
- Galactose is produced endogenously and accounts for a greater contribution to the total galactose pool than the small amounts of galactose found in plant-based foods.

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25.1 Background

Galactosemia is an autosomal recessive disorder of carbohydrate metabolism with an incidence of 1 in 40,000–60,000 live births in the United States [1]. Over 250 different mutations have been identified in

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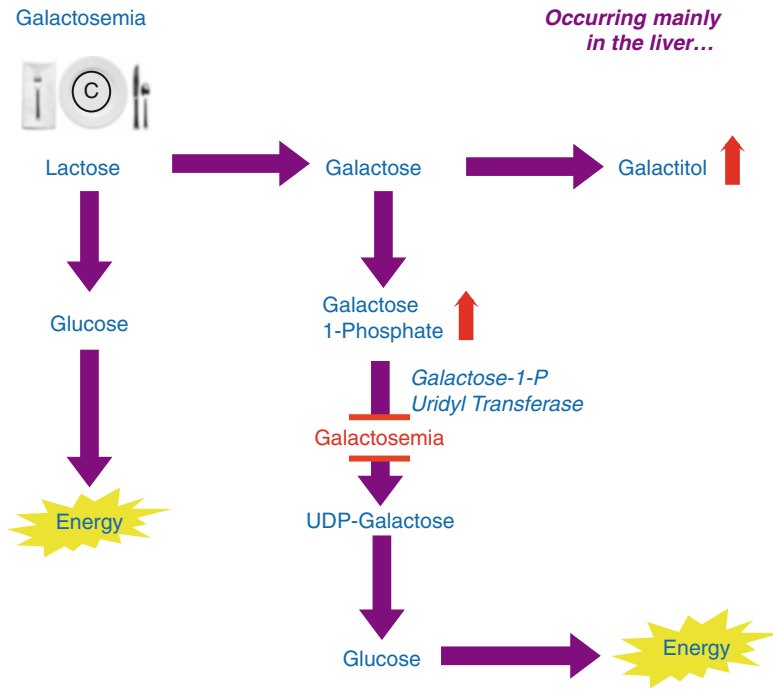


Fig. 25.1 Metabolic pathway of galactosemia

the gene for galactose-1-phosphate uridyltransferase (GALT), located on chromosome 9p13 [2–4].

Lactose is a disaccharide that is hydrolyzed in the small intestine into the monosaccharides glucose and galactose. Galactose must be converted to glucose via the Leloir pathway in order to be used for energy [5]. This occurs primarily in the liver. GALT is the second enzyme in this pathway, and a severe deficiency of GALT leads to classical galactosemia (Fig. 25.1) (Box 25.1).

Box 25.1: Principles of Nutrition Management for Galactosemia

Restrict: Lactose and galactose

Supplement: Calcium and vitamin D[1]

Toxic metabolites: Galactose, galactose-1-phosphate, and galactitol[2]

[1] Intake is often low due to lack of dairy products in diet.

[2] These metabolites accumulate but concentrations are not necessarily related to patient outcome.

Classical galactosemia can result in life-threatening complications including failure to thrive, hepatocellular damage, and *E. coli* sepsis in untreated infants (Box 25.2). Initiation of a soy-based formula or a lactose-free medical food within the first few days of life usually resolves these complications. However, despite identification by newborn screening and early initiation

Box 25.2: Clinical Manifestations of the Newborn with Untreated Classical Galactosemia

- Poor weight gain
- Feeding difficulties
- Jaundice
- Vomiting
- Diarrhea
- Lethargy/coma
- Hypotonia
- Brain edema/bulging fontanel
- Cataracts
- Hepatomegaly
- Coagulopathy
- Sepsis

of a galactose-restricted diet, patients with classical galactosemia often experience long-term complications such as growth delay, speech abnormalities, learning disabilities, motor dysfunction, and, in females, premature ovarian insufficiency, even if diet treatment is continued throughout life [6]. The reasons for poor outcomes in patients with galactosemia remain unclear, and the mechanism for galactose toxicity is not well defined [7, 8].

25.2 Nutrition Management

Newborns with galactosemia require the same nutrients for growth and development as other typically developing infants. Due to the high lactose content, breast milk or milk-based formulas cannot be given to infants with galactosemia, and infant formulas containing minimal or no galactose are required [9–13]. Soy-based formulas containing soy protein isolate or elemental formulas containing L-amino acids are recommended (Box 25.3).

Box 25.3: Infant Formulas for the Treatment of Galactosemia

Soy Formula

Protein source: soy protein isolate (extracted from soybeans)

- *Contains small amounts of galactose*
- Both powdered and liquid soy formulas are acceptable

Elemental Formula

Protein source: L-amino acids

- *Contains no detectable galactose*
- May result in more rapid reduction of galactose-1-phosphate concentrations

Ready-to-feed and liquid concentrate soy formulas contain more galactose than powdered soy formula because liquid formulas have carrageenan added as an emulsifier. However, the galactose in carrageenan is not digested or absorbed by the human gastrointestinal tract [14]. Therefore, the amount of free galactose in liquid soy formulas is equivalent to that found in powdered formula, and any form of soy-based formula is appropriate to recommend for infants. Infants on soy formula do very well, and the galactose-1-phosphate concentration in red blood cells decreases within a couple of months. Occasionally, an infant's galactose-1-phosphate concentration can take up to 6 months to decrease to within treatment range [15], and the metabolic team may suggest a change from soy to an elemental formula to eliminate all galactose [9, 12]. However, it is not known if galactose-1-phosphate concentrations decrease faster when an infant is treated with an elemental formula compared to a soy formula nor if an elemental formula provides any benefits over soy formula in the short- and long-term outcome of infants with galactosemia. It is suggested that soy formulas be used with caution in preterm infants [16]. Thus, an elemental formula is recommended for preterm infants with galactosemia.

Isoflavones may be found in small amounts in soy-based infant formulas [10]. Isoflavones are found in whole soybeans and products including tofu, tempeh, and soy milk. Isoflavones are classified as both phytoestrogens (plant estrogens) and selective estrogen receptor modulators. The phytoestrogenic effects of isoflavones have led to the use of soy foods and isoflavone supplements as alternatives to conventional hormone therapy. However, studies have found no long-term complications associated with isoflavones in infants fed soy-based formulas [10, 17].

The primary source of galactose in the diet is lactose found in dairy products; 100 mL of cow's milk contains approximately 2,400 mg of galactose, and most dairy products must be avoided in the diet for galactosemia. However, during food processing, the lactose content of dairy products often decreases. During cheese production, for example, the whey and casein proteins are separated. Since lactose is water-soluble, it is found primarily in the whey fraction. Various whey proteins are often used in commercial food production and contain a significant amount of lactose. The casein fraction ("curds") is used to produce cheese and contains a significant amount

of residual lactose; however, the lactose content of cheese decreases during the aging process. Several factors determine the final galactose content of cheese, including the type of bacterial cultures used, processing temperature, and length of aging time. Thus, some aged cheeses contain minimal or no galactose in the final product [18, 19]. Sodium and calcium caseinate are added to many processed foods as emulsifiers and stabilizers [20]. Caseinates are produced from casein, but the extensive precipitation and washing results in minimal galactose in the final product [19].

Smaller amounts of galactose are found in many plant products as either free or bound galactose [21, 22] (Box 25.4). Galactose is trapped or "bound" within the plant cell wall of many fruits, vegetables, nuts, seeds, and legumes. Bound galactose cannot be digested in the human gastrointestinal tract because the enzyme alpha-galactosidase is not produced. For this reason, bound galactose does not add to the free galactose pool in the body. Food processing techniques such as heating and fermentation can release free galactose from bound sources in foods, thus increasing the galactose available for absorption [23, 24].

Box 25.4: Bound and Free Galactose

Bound Galactose

- Found in the plant cell wall of many fruits, vegetables, nuts, seeds, and legumes
- Cannot be digested in the human gastrointestinal tract because of the absence of the enzyme alpha-galactosidase
- Does not add to the free galactose pool in the body
- Can be released by ripening, heating and fermentation thereby increasing the galactose available for absorption

Free Galactose

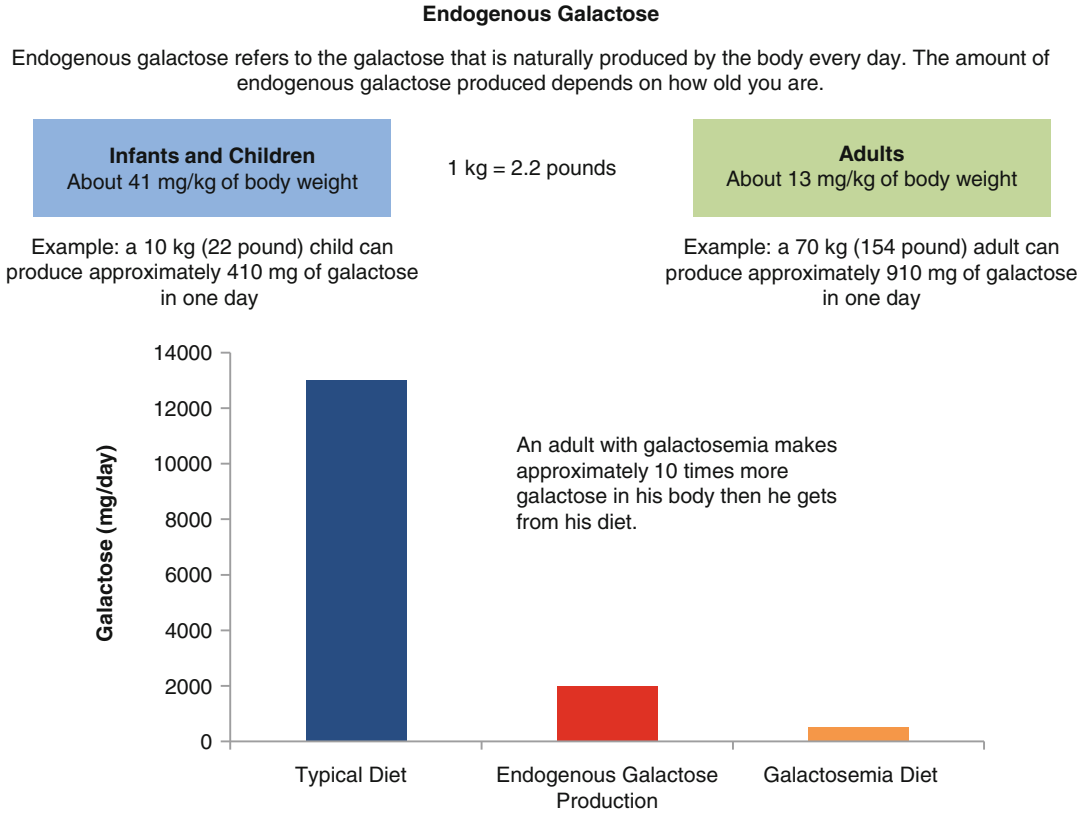
- Most abundant in dairy products as part of lactose
- Found in organ meats and many plants, including fruits, vegetables, nuts, seeds, and legumes
- Readily absorbed in the digestive tract and adds to the free galactose pool in the body

Despite the dietary restriction of galactose, individuals with galactosemia have elevated concentrations of galactose metabolites in blood and urine. This phenomenon is believed to be due, in part, to endogenous production of galactose (i.e., galactose that is produced by the body) (Fig. 25.2).

The endogenous production of galactose is age-dependent with greater amounts produced per kg body weight in infants compared with adults [25]. Using a stable isotope tracer of D-galactose in a continuous intravenous infusion, the endogenous galactose production rate in three healthy, unaffected men and three patients with classical galactosemia was measured [26]. The galactose synthesis rate in both subjects and controls ranged from 0.53 to 1.05 mg/kg per hour resulting in production of gram quantities of galactose per day. Endogenous production of galactose may be an important factor in the development of long-term complications that are seen in older individuals with GALT deficiency [26–28].

Compared to endogenous production, the quantity of galactose in fruits and vegetables is quite small (Fig. 25.3), calling into question the policy of many clinics to recommend their avoidance. A study published in 1991 indicating that tomatoes contained free galactose [8] was the catalyst for many metabolic clinics to recommend restriction of tomatoes and other fruits and vegetables with a higher free galactose content. However, studies never showed improved outcomes in patients who eliminated such foods from the diet, and some clinics continued to freely allow fruits and vegetables without report of complications [29]. A survey of metabolic dietitians found a wide range of clinic policies to restrict various fruits and vegetables in the diet for patients with galactosemia [30] (Fig. 25.4).

Various legumes were recently reanalyzed and found to contain less free galactose than originally reported [19]. The fermentation process used to produce various soy-based



1 Approximate galactose content of a typical diet including 2 cups of milk and 3 servings of fruits and vegetables with a galactose content >20 mg galactose/100 g food.

2 Approximate amount of endogenous galactose produced by an adult with galactosemia.

3 Approximate galactose content of a typical galactose-restricted diet including 3 servings of fruits and vegetables with a galactose content >20 mg galactose/100 g food.

Fig. 25.2 Comparison of the galactose content in a typical diet, the galactose produced endogenously, and the galactose content in the diet for galactosemia

products releases galactose from the bound galactose in this legume, and, thus, relatively higher quantities of free galactose have been found in fermented soy products [30]. Fermented products include soy sauce, miso, natto, tempeh, and sufu (fermented soy cheese). However, the amount of these products used in the diet is typically small. This needs to be considered in the decision to include these products in the diet. In addition, products such as Beano® contain the enzyme α-galactosidase which does break down bound sources of galactose in plant products. Thus, use of these products needs to be avoided.

Based on new analyses and a review of the food science literature, the "allowed" and "restricted" list of various foods and ingredients was modified to include all fruits, vegetables, legumes, non-fermented soy products, caseinates, and a greater number of aged cheeses [30] (Table 25.1).

25.3 Monitoring

Studies have found that children and adolescents with galactosemia have osteopenia of the lumbar spine with decreased markers of bone resorption and formation, despite reporting dietary

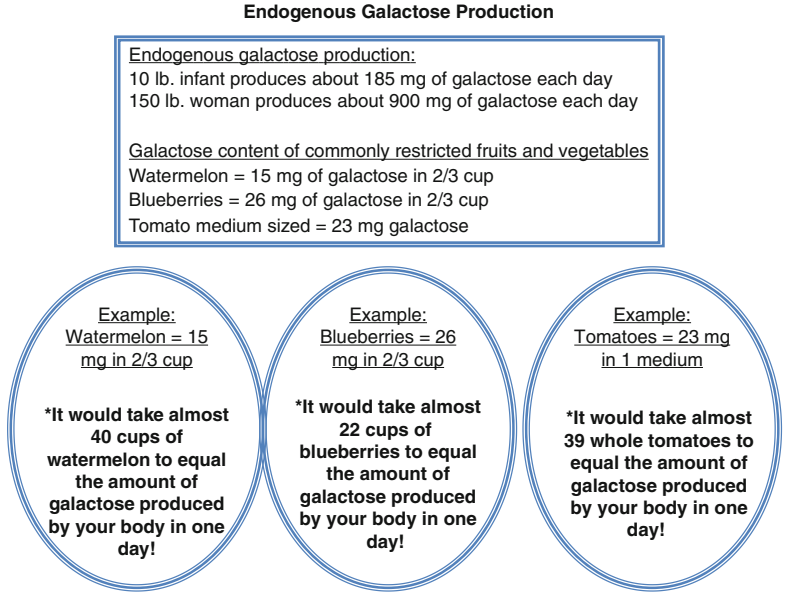


Fig. 25.3 The amount of watermelon, blueberries, or tomatoes that an adult with galactosemia weighing 150 lb would need to consume to equal the approximate amount of galactose produced endogenously in 1 day [41–43]

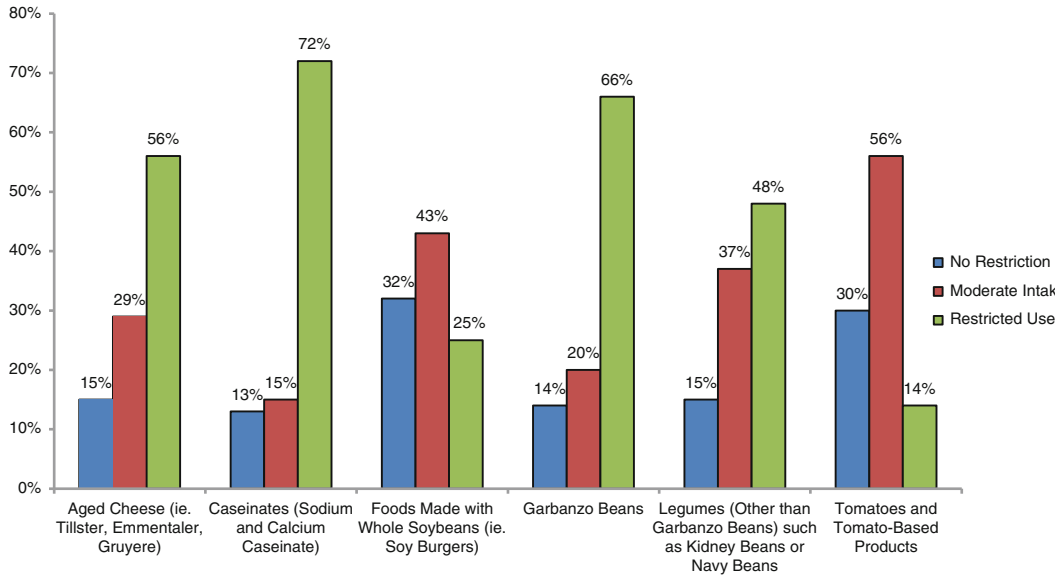


Fig. 25.4 Responses to a survey of metabolic dietitians indicating if a food or ingredient is allowed or restricted or moderation in intake is recommended. Products selected for this figure are now allowed in the diet for galactosemia [30]

intake that meets the established requirements for calcium, magnesium, zinc, vitamin D, and protein [31, 32]. In individuals with galactosemia, serum markers of bone turnover increased from childhood into adolescence, rather than declining with age, as seen in unaffected individuals [33]. Poor nutritional intake, an intrinsic defect in collagen formation, and abnormal

Table 25.1 Allowed and restricted foods and ingredients for individuals with classical galactosemia [30]

Allowed foods and ingredients	Restricted foods and ingredients
Soy-based infant formulas containing soy protein isolate, amino acid-based elemental infant formulas	Breast milk, all milk-based infant formulas
All fruits, vegetables and their juices, pickled fruits and vegetables	All milk-based foods and beverages except for caseinates and aged cheeses, listed above
All legumes (e.g., navy beans, kidney beans, garbanzo beans, soybeans)	Milk-based ingredients including buttermilk solids, casein, dry milk protein, dry milk solids, hydrolyzed whey protein, hydrolyzed casein protein, lactose, lactalbumin, whey
Soy-based products that are not fermented (soy milk, tofu, textured soy protein, hydrolyzed vegetable protein, soy protein concentrate, meat analogs, unfermented soy sauce[a])	All cheese and cheese-based products except those listed above
Aged cheeses: Jarlsberg, Emmentaler, Swiss, Gruyere, Tilsiter, Parmesan aged >10 months, grated 100 % Parmesan cheese, sharp Cheddar cheese	Organ meats, meat-by-products
Sodium and calcium caseinate	Soy products that are fermented (e.g. miso[b], natto[b], tempeh, sufu[b])
All cocoa products except milk chocolate	Fermented Soy sauce[b]
Additional ingredients: natural and artificial flavorings, all gums including carrageenan	

[a]Soy sauce that has not been fermented is made from hydrolyzed soy protein
[b]These fermented soy products are typically used as condiments or ingredients in foods. The amount of these products in the diet needs to be considered when determining acceptability

sex steroids in females have been implicated in the decline in bone mineral density observed in this population [34, 35]. Recommendations suggest initiating bone mineral density (DXA) scans as early as age 4 years with repeat scans every year if the z-score is ≤2 SD below the mean and every 2 years if above this score [36]. While the mechanism of decreased bone density in patients with galactosemia is not well understood, ensuring adequate intake of nutrients associated with proper bone development and maintenance can prevent nutrient deficiencies that could exacerbate the development of osteoporosis.

In 2011, the Institute of Medicine revised the recommended Dietary Reference Intakes (DRI) for calcium and vitamin D [37]. Periodic monitoring of total 25-hydroxyvitamin D to maintain serum concentrations of at least 20–32 ng/mL (50–80 nmol/L) is recommended [38], and intake of vitamin D may need to be greater than the DRI to achieve optimal serum total 25-hydroxyvitamin D concentrations [39]. Panis et al. [40] suggested supplementation of calcium, vitamin D_3, and vitamin K_1 and reported improved bone mineral density after 2 years of supplementation in children and preadolescents with galactosemia [40]. In adults with galactosemia, body mass index correlated with bone density [35].

Box 25.5: Nutrition Monitoring of a Patient with Classical Galactosemia

- Routine assessments including anthropometrics, dietary intake, physical findings (Appendix F)
- Laboratory Monitoring
  - Diagnosis-specific
    - Galactose-1-phosphate (GAL-1-P) in erythrocytes
    - Galactitol in urine
  - Nutrition-related
    - 25-hydroxy vitamin D
- Bone mineral density scans (DXA).

25.4 Summary

Newborn screening and the early introduction of a galactose-restricted diet has reduced mortality in infants with classic galactosemia, but the long-term efficacy of the galactose-restricted diet is limited, and patients with galactosemia remain at risk for developmental and neurological complications. Endogenous production of galactose contributes significantly to the total free galactose pool, and avoidance of small amounts of galactose from plant-based foods is no longer recommended. Recommendations include elimination of dairy products that are abundant in galactose; however, some dairy products, such as certain cheeses and sodium or calcium caseinate, contain negligible amounts of galactose and are allowed in the diet. Monitoring of bone density is recommended for patients with galactosemia because intakes of calcium and vitamin D may be low due to the restriction of most dairy products.

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Glycogen Storage Diseases

26

Johan L.K. Van Hove

Contents

Core Messages

- In glycogen storage diseases, there is insufficient use of glycogen resulting in glycogen buildup, or insufficient synthesis of glycogen.
- There are multiple types of glycogen storage diseases, which can be classified in hepatic and myopathic forms. Types I, III, IV, VI, and IX affect liver primarily.
- Glycogen storage diseases that also affect gluconeogenesis cause severe lactic acidosis on fasting.
- Treatment of glycogen storage disease type I should not only focus on management of hypoglycemia but also on prevention of long term complications.

26.1 Background [1–3]

Glycogen is a polymer of glucose units. Glycogen is the main storage form of glucose. It consists of large strands of glucose bonded together in α-1,4 linkages. Such long uninterrupted strands of glucose are not water soluble. Glycogen has branching points using α-1,6 linkages that result in a globular structure that is water soluble. Glycogen functions as a reserve unit of glucose for energy needs. Large amounts of glycogen are present in the liver where it is used as a reserve of glucose for energy use in the general body. The muscle also contains large

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amounts of glycogen. Muscle glycogen is used for immediate energy needs in the muscle. The brain contains glycogen stores for its own local use.

In the fed state immediately after a meal, glucose is absorbed from such food sources as simple carbohydrates or starches. This will elevate glucose concentrations in the blood. Higher blood glucose concentrations trigger the release of insulin, which then opens the GLUT4 glucose transporters and results in use of glucose by the largest organ of the body, the muscle tissue.

High insulin concentrations suppress lipolysis, and excess glucose is converted to lipid via lipogenesis. The liver uses glucose, as it is available in the blood stream, and at high glucose and insulin concentrations, it makes glycogen (glycogen synthesis) (Fig. 26.1). The brain uses glucose through the insulin-independent GLUT1 transporter.

During the fasting state, glucose concentrations gradually decrease. After a sufficiently long fasting period, glucose concentrations will be low, resulting in very low insulin concentrations. Low insulin concentrations stop the uptake of glucose into the muscle tissue, which spares the use of glucose. Low glucose and insulin concentrations will result in glycogen breakdown in the liver (glycogenolysis) as well as gluconeogenesis, resulting in glucose release and maintaining normal glucose concentrations for organs that are dependent on glucose such as red blood cells and the brain. The low concentrations of insulin are insufficient to suppress lipolysis, and the fat tissue releases free fatty acids and glycerol. These fatty acids are the main source of energy use by the muscle tissue in replacement of glucose. The liver tissue also uses fatty acids for energy use, but when fatty acid concentrations are high, the liver will generate ketones from the metabolism of fatty acids. Ketones can then be used by various tissues, including the brain, replacing glucose as the main fuel source for energy generation. Released amino acids, such as alanine, from the muscle, and glycerol from lipolysis provide substrate to the liver for ongoing gluconeogenesis (Fig. 26.2).

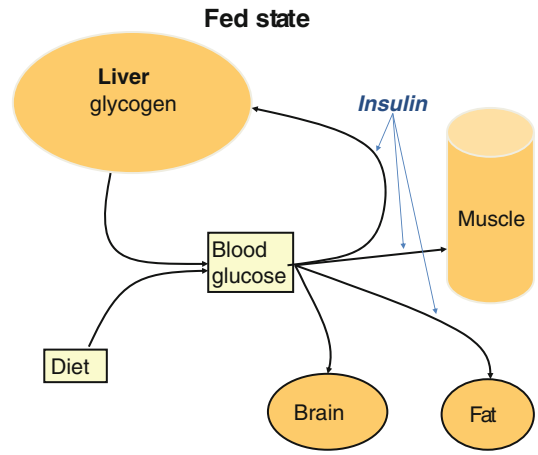

Fig. 26.1 High insulin concentrations suppress lipolysis and glycogenolysis and stimulate lipogenesis

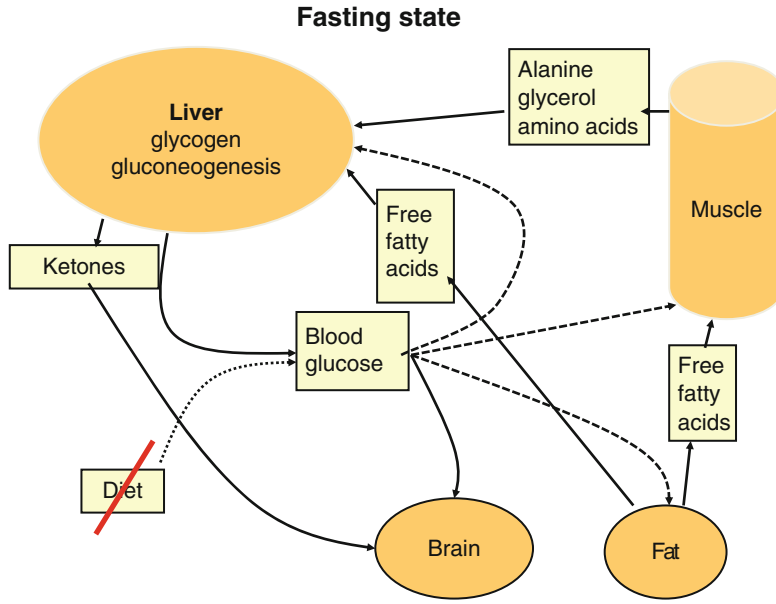

Fig. 26.2 In the fasted state, glucose and insulin concentrations decrease, allowing for glycogenolysis, lipolysis, and ketone production to occur to supply energy to the body

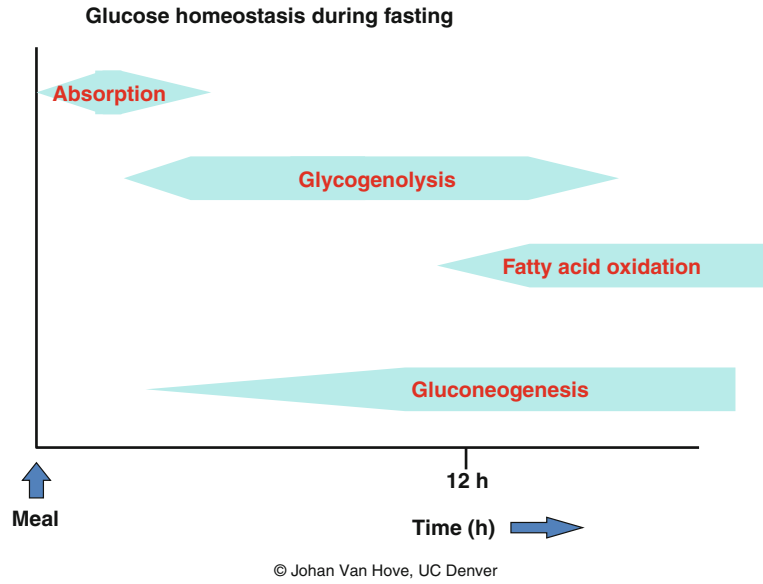

Fig. 26.3 Glucose homeostasis is maintained by the body, first relying on glucose in the post-meal absorption stage to breaking down glycogen and ultimately turning to fatty acid oxidation and ketone production as the energy source for the body

The timing of the transition from the fed to fasted state has important consequences for clinical care. Absorption of nutrients from food such as carbohydrates takes 3–4 h after a regular meal. During this absorptive stage, glycogen synthesis is ongoing. As absorption completes, and glucose concentrations are becoming lower, glycogenolysis starts, aided by gluconeogenesis. This provides for glucose homeostasis from 4 h after a meal to 12–15 h after a meal. When fasting for 12–15 h after a meal, glucose concentrations and hence insulin becomes low enough to allow lipolysis to occur. Lipolysis and fatty acid oxidation become the major fuel sources from 15 to 18 h of fasting and longer, while gluconeogenesis continues (Figs. 26.3 and 26.4).

Standardized fasting tests in normal children show the timing of these metabolic and endocrine switches in the postprandial period (Box 26.1).

Preprandial normal glucose concentrations are between 60 and 120 mg/dl, free fatty acids are <0.5 mM, and ketones 3-hydroxybutyrate and acetoacetate are less than 0.4 mM. After 15 h fasting, free fatty acids are already consistently elevated at >0.6 mM, whereas at 12 h fasting this is still inconsistently elevated. At 18 h fasting, all children have elevated concentrations of ketones with the sum of 3-hydroxybutyrate and acetoacetate >0.9 mM, whereas at 15 h fasting ketogenesis is still inconsistent with some children not yet having ketones, but others already have ketones as high as 2.1 mM. The normal ratio of 3-hydroxybutyrate to acetoacetate is 3:1, which allows one to estimate the amount of total ketones when only 3-hydroxybutyrate concentration is available.

Box 26.1: Normal Biochemical Markers During Fasting

	Glucose	Free fatty acids	Ketones (=3-OH butyrate + acetoacetate)
Preprandial	3.3-6.6 mmol/L	<0.5 mmol/L	<0.4 mmol/L
12 h fasting		Inconsistently elevated	Inconsistently elevated
15 h fasting	3.5–5.3 mmol/L[a]	Consistently elevated >0.6 mmol/L	Inconsistently elevated
18–20 h fasting	2.8–4.9 mmol/L[a]	Consistently elevated >0.6 mmol/L	Consistently elevated >0.9 mmol/L

[a]Age range from 1 month to 15 years of age Bonnefont [1]

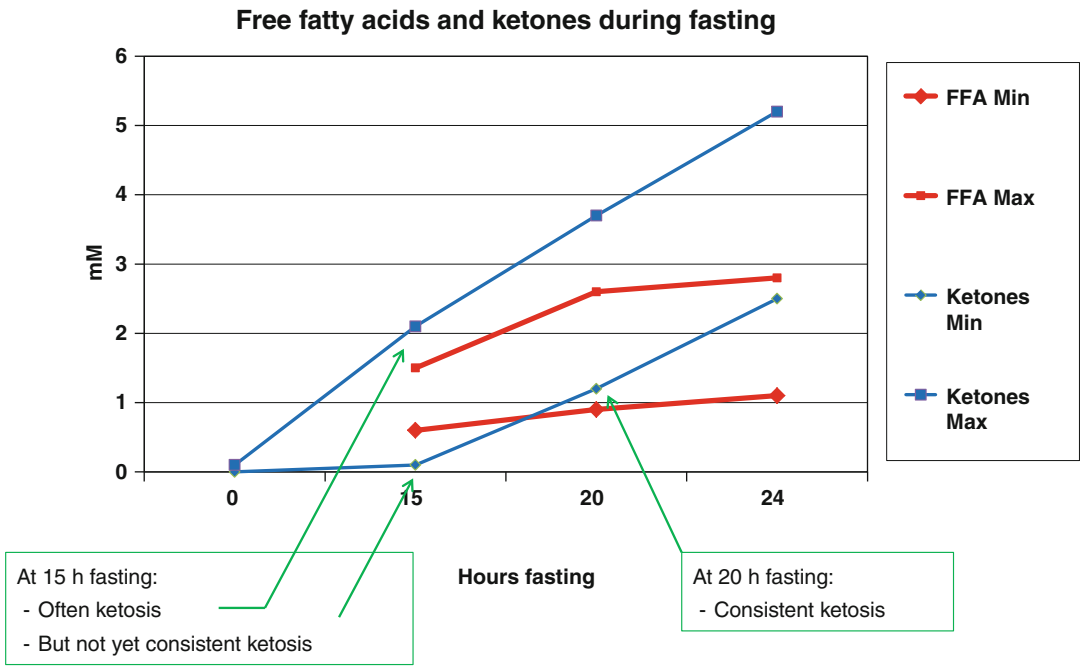

Fig. 26.4 Changes in free fatty acids and ketone production as length of fasting increases

Glycogen is broken down into glucose in two steps. First, upon activation by its kinase, outer glucose moieties are released by the enzyme phosphorylase and transferred onto phosphate to glucose-1-phosphate. Phosphoglucomutase then converts glucose-1-phosphate into glucose-6-phosphate. Phosphorylase can cleave glucose molecules off glycogen but is blocked when it nears an α-1,6 branch point. Glycogen maximally metabolized by phosphorylase is called α-limit dextrin. At that point, debrancher enzyme works to remove the α-1,6 branch points in two sequential reactions. First, the glycosyltransferase part of debrancher enzyme transfers the outer three glucose molecules to a nearby chain in an α-1,4 linkage, leaving only the glucose molecule that is attached by the α-1,6 linkage. This last glucose molecule is then hydrolyzed to glucose by the α-1,6 glycosidase function of debranching enzyme to free glucose, thus completing the removal of the α-1,6 branch. Glucose-6-phosphate in the liver can be released to glucose by glucose-6-phosphatase for exogenous secretion in the blood stream during hepatic glycogenolysis.

26.2 Glycogen Storage Diseases

Glycogen storage diseases affect glycogen synthesis, glycogen breakdown, or glycolysis. Glycogen storage diseases that affect the glycogen pool in the liver cause disturbances of glucose homeostasis in the blood. Glycogen storage diseases that affect the glycogen use in the muscle cause muscle symptoms. As red blood cells are completely dependent on glycolysis, disorders of the glycolytic pathway often involve hemolysis.

Glucose in excess can make glycogen, or glucose can be derived from glycogen. Glucose can also be metabolized to pyruvate in the glycolytic pathway and from there to acetyl-coenzyme A for use in the Krebs cycle. Glucose can be made from precursors in the reverse of the glycolytic pathway during gluconeogenesis. Other carbohydrates such as fructose and galactose convert to glucose for use in multiple organs. Galactose is converted from galactose-1-phosphate into UDP-galactose and from there into UDP-glucose and hence into glucose-1-phosphate entering the pathway of glycogen metabolism. Fructose is

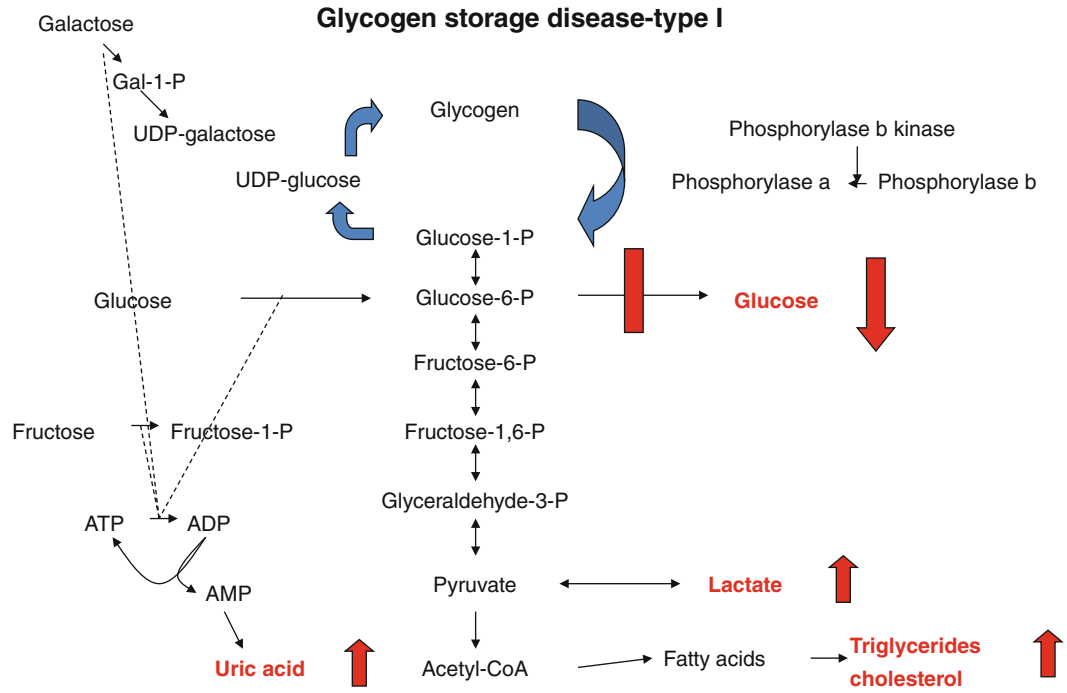

Fig. 26.5 Metabolic pathway of glycogen storage disease type 1

metabolized into fructose-1-phosphate which is broken down by aldolase into dihydroxyacetone phosphate and glyceraldehyde which is then metabolized to glyceraldehyde-3-phosphate, both joining the glycolytic/gluconeogenetic pathway. In classic glycogen storage diseases of glycogen breakdown, such as phosphorylase deficiency, gluconeogenesis is still possible, but the release of glucose from glycogen is blocked resulting in a shortage of glucose. In the hepatic diseases, this will result in hypoglycemia, and in the muscle, this will result in muscle cramping and rhabdomyolysis. Gluconeogenesis occurs in the liver only. Disorders of gluconeogenesis will also result in a shortage of glucose and hence hypoglycemia upon fasting of about 12 h when glycogen stores are depleted but will also result in the accumulation of precursor product pyruvate, which is in equilibrium with lactate. Thus, in isolated gluconeogenetic conditions, such a fructose-1,6-bisphosphatase deficiency, the often mild hypoglycemia will be accompanied by major lactic acidosis. Finally, in glucose-6-phosphatase deficiency, the release of glucose from glucose-6-phosphate is not possible, resulting in an impairment of both glycogenolysis and gluconeogenesis. Due to the combined block of both pathways, hypoglycemia is early and severe and is accompanied by lactic acidosis from the accumulation of gluconeogenetic precursor (Fig. 26.5).

Key clinical features of glycogen storage diseases relate to the organ in which the enzyme is deficient (Box 26.2). Hepatic glycogen is used for the homeostasis of glucose in the blood. Hepatic glycogen storage diseases present with hypoglycemia, usually upon fasting.

Box 26.2: Key Clinical Presentation of GSD

- *Liver*
  - Hypoglycemia
  - Lactic acidosis
  - Hepatomegaly
  - Hyperlipidemia
- *Muscle*
  - Rhabdomyolysis
  - Weakness, wasting, hypotonia
- *Red Blood Cells*
  - Glycolytic defect: hemolytic anemia

Disorders of gluconeogenesis also present with hypoglycemia but have lactic acidosis. In both conditions, there is pronounced hepatomegaly reflecting glycogen storage and steatosis often occurs. The onset of hypoglycemia is dependent on the severity of the condition and the age of the patient. Defects of only glycogenolysis or defects of only gluconeogenesis typically present with hypoglycemia after 8–12 h fasting, whereas glucose-6-phosphatase deficiency that blocks both glycogenolysis and gluconeogenesis can present with hypoglycemia as soon as the absorptive phase is over, usually 4 h after a meal.

Glycogen in the muscle is used when the muscle performs an exercise that requires more energy than can be provided by glucose from the blood stream and aerobic mitochondrial metabolism. This typically occurs with short, intense muscle exercise that exceeds such capacity. At that time, glycogen storage diseases that affect muscle enzymes will result in energy failure of the muscle cells leading to painful cramping and rhabdomyolysis. They can also result in muscle weakness, wasting, and hypotonia. Disorders of glycolysis often result in hemolysis, since the red blood cell is uniquely dependent on glycolysis for its energy needs.

Finally, disorders of glycogen metabolism that result in unusually structured forms of glycogen (branching enzyme and debranching enzyme deficiencies) will result in cytolysis of cells affected with the abnormally formed glycogen resulting in hepatolysis, muscle wasting, cardiac involvement resulting in cardiomyopathy, and neuropathy. Given these differences, the glycogen storage diseases can be categorized as hepatic glycogenoses and myopathic glycogenoses (Table 26.1).

26.2.1 The Hepatic Glycogenoses [4–14]

26.2.1.1 Glycogen Storage Disease Type I

Glycogen storage disease type I is characterized by deficient activity of the glucose-6-phosphatase enzyme system. The glucose-6-phosphatase enzyme is located in the endoplasmic reticulum (ER) (microsomal fraction). Glucose-6-phosphate must first be transported from the cytoplasm into

Table 26.1 Glycogenoses

Glycogenoses	Enzyme deficiency	Gene	Other name
Hepatic glycogenoses			
Glycogen storage disease type I	Glucose-6-phosphatase	*G6PC, SLC37A4*	Von Gierke disease
Glycogen storage disease type III	Debranching enzyme	*AGL*	
Glycogen storage disease type IV	Branching enzyme	*GBE1*	Andersen disease
Glycogen storage disease type VI	Hepatic phosphorylase	*PYGL*	
Glycogen storage disease type IX	Phosphorylase kinase	*PHKA1*, *PHKA2*, *PHKB*, PHKG2	
Glycogen storage disease type 0A	Liver glycogen synthase	*GYS2*	
Glycogen storage disease type XV	Glycogenin	*GYG1*	
	Phosphoglucomutase	*PGM1*	
Myopathic glycogenoses			
Glycogen storage disease type V	Muscle phosphorylase	*PYGM*	McArdle disease
Glycogen storage disease type II	Acid α-glucosidase	*GAA*	Pompe disease
Glycogen storage disease type VII	Muscle phosphofructokinase	*PFKM*	Tarui disease
Glycogen storage disease type 0B	Muscle glycogen synthase	*GYS1*	
	Phosphoglycerate kinase (PGK)	*PGK1*	
Glycogen storage disease type X	Phosphoglycerate mutase PGAM	*PGAM2*	
Glycogen storage disease type XI	Lactate dehydrogenase (LDH)	*LDHA*	
Glycogen storage disease type XIII	β-enolase	*ENO3*	
Glycogen storage disease type XII	Aldolase A	*ALDOA*	
Glycogen storage disease type IIB		LAMP2	Danon disease

the ER by the glucose-6-phosphate carrier (gene *SLC37A4*) before it can be acted on by the catalytic enzyme (gene *G6PC*). Deficiency of the catalytic enzyme glucose-6-phosphatase is called glycogen storage disease type Ia, and deficiency of the glucose-6-phosphate transporter is called glycogen storage disease type Ib. A defect in either of these two functions will result in deficient activity of glucose-6-phosphatase and hence lack of release of glucose derived from either glycogen breakdown or from gluconeogenesis. This results in severe hypoglycemia which occurs early in the postprandial period, about 3–4 h after a meal for young children. During the attempt to generate glucose, the glucose-6-phosphate that cannot be released will be metabolized through the glycolytic pathway resulting in accumulation of pyruvate and hence lactate, to acetyl-CoA resulting in synthesis of triglycerides and cholesterol. Both fructose and galactose cannot be converted into glucose but contribute to the accumulation of glucose-6-phosphate. The accumulation of phosphor-carbohydrates results in a conversion of ATP to ADP and from there to AMP that results in increased production of uric acid. The characteristic biochemical profile thus consists of hypoglycemia, lactic acidosis, hyperuricemia, hypertriglyceridemia, hypercholesterolemia, and hepatic steatosis in addition to hepatic glycogen accumulation. There is pronounced hepatomegaly and nephromegaly, but the spleen is of normal size. Untreated, the children have short stature and a doll-like face. Due to the lower glucose requirements per weight basis, adults often do not exhibit major hypoglycemia, but do exhibit the other metabolic abnormalities (Box 26.3).

Patients with glycogen storage disease type Ib have the same biochemical abnormalities as described above for patients with glycogen storage disease type Ia. The glucose-6-phosphate in the endoplasmic reticulum is also the precursor for reducing equivalents in the neutrophils, which are essential for the generation of the oxidative burst and the killing of bacteria. Patients with glycogen storage disease type Ib have additionally variable neutropenia and decreased neutrophil dysfunction with reduced chemotaxis and killing. This results in increased propensity for infections such as with *Staphylococci* and for chronic inflammatory bowel disease, which can lead to severe diarrhea and malnutrition.

Box 26.3: Signs and Symptoms of GSD-I

GSD-1a: deficiency of glucose-6-phosphatase

GSD-1b: deficiency of glucose-6-phosphate transporter

Impairment of glycogenolysis and gluconeogenesis

Symptoms:

- Hepatomegaly; nephromegaly
- Hypoglycemia: early and severe in infancy
- Lactic acidosis
- Hypertriglyceridemia
- Hypercholesterolemia
- Hyperuricemia
- Short stature, doll-like face
- Neutropenia (1b)

Historically, the diagnosis of glycogen storage disease type 1 was typically made by lack of glucose-6-phosphatase enzyme activity in a liver biopsy in addition to accumulation of normally structured glycogen. In glycogen storage disease type 1b, the deficient activity is only present in fresh tissue and normalizes with freezing and thawing, whereas in glycogen storage disease type 1a, the activity is also deficient in frozen and thawed tissue. This diagnostic method is now commonly replaced by sequencing of the genes *G6PC* and *SLC37A4*. Many mutations have been recorded in both genes.

The nutritional management of glycogen storage disease type 1 is covered in detail in Chap. 26. It consists of maintenance of the glucose concentrations above that which would invoke glycogen breakdown and gluconeogenesis, thus preventing the abnormal biochemical response. This involves providing a consistent glucose input to maintain plasma glucose concentrations above 70–80 mg/dL. Good glycemic control will result in near normalization of lactate concentrations and substantial reduction in triglyceride and uric acid concentrations.

Maintenance of Glucose Concentrations

Maintaining the glucose concentration is achieved by providing a continuous enteral provision of glucose. This can be done by frequent meals throughout the day and, at night, either continuous gastric tube feeding or middle-of-the-night feedings. Acute hypoglycemia should be treated with oral or intravenous glucose, and patients should have warning notes about the risk of life-threatening hypoglycemia such as a MedicAlert bracelet and an emergency room letter. Dislodgement of a gastric feeding tube can result in unforeseen hypoglycemia and should be monitored for. Uncooked cornstarch is a starch that is difficult to digest by human pancreatic amylase and hence results in prolonged absorptive phase, often up to 6–8 h. The child has to be at least 9 months of age to have sufficient pancreatic maturation to be able to tolerate uncooked cornstarch.

Box 26.4: Uncooked Cornstarch
The child must be at least 9 months of age to have sufficient pancreatic maturation in order to tolerate uncooked cornstarch.

Dosing of uncooked cornstarch has to be gradually advanced over several months to allow for the pancreas to adjust to this more-difficult-to-digest starch. Ultimately, a regimen of uncooked cornstarch every 4–6 h, along with meals and snacks (provided an hour before the cornstarch starts to wane), can achieve a good glycemic control. Galactose (lactose)- and fructose (sucrose)-containing products should be avoided since they will result in accumulation of abnormal metabolites. A fat-limited diet can aid in reducing the hypertriglyceridemia. Most patients developed substantial hyperuricemia from adolescence on that is insufficiently controlled by dietary measures, and they should be treated with allopurinol or similar uric acid-reducing agents.

For patients with glycogen storage disease type Ib, neutropenia will respond to treatment with granulocyte-colony stimulating factor (G-CSF). The dose should be carefully controlled as massive splenomegaly with hypersplenism has been reported with high doses. Careful monitoring for infections and use of antibiotics with intracellular killing should be instituted when concern for invasive infections arises. Severe inflammatory bowel disease may require management similar to that of the medical management of Crohn's disease, although fistulas do not tend to occur. Vitamin E use has been reported to improve neutrophil function.

As a result of this treatment, hypoglycemia should be avoided, lactate concentrations should be near normal (i.e., less than 3 mM) and hypertriglyceridemia decrease to at least less than 800 mg/dl. Hepatomegaly persists but is less pronounced. Growth is restored.

Complications of Glycogen Storage Disease Type 1

Several complications are noted in the clinical picture of glycogen storage disease type I over time. The severe hypertriglyceridemia can cause pancreatitis. Excessive hypertriglyceridemia not sufficiently controlled by optimization of the biochemical management partially responds to treatment with fibrates. Rarely, patients develop pulmonary hypertension that can be fatal. Many patients develop significant osteoporosis with pathological fractures, and careful attention to calcium and vitamin D is important. If uricemia is not controlled, then gout can develop. Platelet dysfunction is very common and can result in bleeding, particularly nosebleeds. For planned interventions such as surgery with risk for bleeding, preventive infusion of glucose at high rates can decrease the bleeding tendency and for acute bleeding can be treated with desmopressin acetate (DDAVP). Polycystic ovary syndrome can occur in women; however, many women have had successful pregnancies. Peripheral neuropathy occurs in some patients and can present with painful paresthesias and with loss of sensation.

The main long-term complications of glycogen storage disease type I are renal and hepatic complications. The kidney has elevated renal

blood flow and hyperfiltration, and patients can develop focal segmental glomerulosclerosis resulting in renal failure. Once heavy proteinuria begins (>1 g/day), the disease will progress unrelentingly toward renal failure, and patients will eventually require renal replacement therapy such as dialysis or renal transplantation. There is a high risk for kidney stones. Hypercalciuria and hypocitraturia are contributing risk factors. Rare renal complications include renal tubular dysfunction in patients with very poor metabolic control and renal amyloidosis particularly in GSD type Ib. The liver shows a tendency to formation of hepatic adenoma that can cause intrahepatic acute bleeding. They tend to regress with improved treatment. There is a substantial risk for hepatic carcinoma, most commonly developing in the third or fourth decade, although earlier cases are known. Both the incidence of hepatic adenoma/carcinoma and of the renal focal segmental glomerulosclerosis are reduced with strict lifelong chronic treatment. For patients with tendency toward proteinuria, treatment with angiotensin-converting enzyme (ACE) inhibitors is advised. It is believed that the secondary biochemical alterations of high lactate and triglycerides strongly contribute to these long-term complications. Thus, the management of adults with glycogen storage disease type I should not solely focus on the easily achieved avoidance of hypoglycemia, but rather on the global control of biochemical abnormalities with normalization of lactate and triglyceride concentrations. Finally, liver transplantation is a curative intervention for glycogen storage disease type I. Indications for liver transplantation include hepatic carcinoma and persistent adenoma, medically intractable complications, or poor quality of life with difficult to maintain medical treatment.

Monitoring of treatment of glycogen storage type I includes measurement of a daytime profile of glucose and lactate concentrations on a regular basis and measurement of triglyceride and uric acid concentrations. Annual studies include imaging studies of the liver, preferably by MRI, and evaluation of kidney function by measuring urinary protein, calcium, and citrate. Osteoporosis monitoring is done by periodic DEXA scan for measurement of bone mineral content and with monitoring of calcium and vitamin D status (Box 26.5).

Box 26.5: Monitoring of the Adult Patient with GSD-I

- Laboratory markers
  - Lactate: <4 μmol.
  - Glucose is usually stable.
  - Uric acid <8 mg/dL.
- Liver hepatoma
  - MRI
- Kidney
  - Glomerular filtration rate
  - Proteinuria
  - Stones: calciuria, citraturia
- Bone
  - DEXA scan
  - 25-OH vitamin D

26.2.2 Glycogenolytic Disorders: Glycogen Storage Diseases Types VI and IX

These conditions have isolated defect in glycogen breakdown. Patients present with hypoglycemia and hepatomegaly and have delayed growth that usually improves during puberty. Treatment consists of avoidance of long fasting. Often, a dose of uncooked cornstarch is given at bedtime to decrease the physiologic fasting time associated with sleep.

The phosphorylase kinase enzyme has multiple subunits. The most common defect is in the X-linked PHKA2 gene. Deficient activity can be noted in red blood cells in half the patients, which provides for a more easy diagnosis. The most common diagnostic method is by next-generation sequencing (NGS) of a panel of genes. The PHKG2 subunit is another X-linked subunit, which presents with liver cirrhosis rather than hypoglycemia. There is currently no specific treatment for the cirrhosis.

26.2.3 Glycogen Storage Disease Type III: Debranching Enzyme Deficiency [15–18]

The debranching enzyme has two enzymatic activities: a glycosyltransferase and an α-1,6 glycosidase activity. There are two forms of debrancher enzyme deficiency: in glycogen storage disease type IIIa (85 % of patients), the enzyme is deficient in both liver and muscle, whereas in glycogen storage disease type IIIb (15 % of patients), the enzyme is only deficient in liver but is normal in muscle. The preservation of muscle enzyme activity is caused by mutations in exon 3 where a muscle promoter allows translational start using a secondary start site after the mutation. Isolated deficiency of only one of the two enzyme activities is very rare.

The initial presentation of glycogen storage disease type III is similar to that of glycogen storage disease type I with hypoglycemia, hepatomegaly, growth retardation, and elevated triglycerides and cholesterol, but the lactate and uric acid concentrations tend to be normal. The abnormal form of glycogen results in cytolysis usually with elevated transaminases, and in IIIa elevation of creatine kinase (CK). The hypoglycemia is usually associated with fasting ketosis. The management of hypoglycemia becomes easier with age with few hypoglycemia in adults. Late symptoms develop due to organ damage from the cytolysis associated with the abnormal glycogen. Liver cirrhosis develops in about 5 % of patients. Hepatic carcinoma can occur. Progressive myopathy often develops in adulthood. Cardiomyopathy can develop and can be severe. Polycystic ovary syndrome is common in women. Polyneuropathy has been described.

Initial treatment of glycogen storage disease type III is similar to that of glycogen storage disease type I with frequent meals and supplemental uncooked cornstarch. Since gluconeogenesis is not impaired, galactose and fructose do not have to be so strictly avoided. A high protein diet is indicated to stimulate gluconeogenesis. This has improved myopathy and cardiomyopathy and may reduce the risk of severe liver disease. Cardiac function must be monitored and cardiac conduction defects and arrhythmias specifically evaluated for.

26.2.4 Glycogen Storage Disease Type IV: Branching Enzyme Deficiency [19–22]

In branching enzyme deficiency, abnormal glycogen accumulates which on pathology is evident as diastase-resistant polyglucosan bodies. The primary presentation is with progressive liver disease resulting in cirrhosis and end-stage liver failure which occurs usually around the age of 4–6 years. A few patients have had nonprogressive liver disease, not leading to liver failure. On sequencing, they were found to have mutations that resulted in residual activity. Presentation is with hepatosplenomegaly and failure to thrive. Of note, hypoglycemia is not usually a presenting symptom. A rare severe presentation is with neonatal severe hypotonia and muscle weakness; however, more common is presentation with childhood muscle weakness. Neuropathy is also possible. Patients can develop cardiomyopathy after liver transplantation. Adult polyglucosan body disease is a neurological condition in Ashkenazi Jewish adults that is caused by a specific mutation p.Y329S.

Diagnosis is often made by the recognition of the diastase-resistant polyglucosan bodies on pathology on liver or muscle biopsy. The enzyme assay can be done in the liver or in fibroblasts. The diagnosis can also be made by mutation analysis of the GBE1 gene.

Treatment is usually symptomatic. Most patients with the typical progressive liver disease presentation require liver transplantation for cirrhosis and end-stage liver failure.

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Nutrition Management of Glycogen Storage Disease Type 1

27

Sandy van Calcar

Contents

Core Messages

- Glycogen storage disease types 1a and 1b (GSD-1) are characterized by fasting hypoglycemia and elevated lactic acid, uric acid, cholesterol, and triglycerides. Patients with GSD type 1b are also at risk of neutropenia and inflammatory bowel disease.
- Nutrition management of GSD-1 includes providing supplemental uncooked cornstarch as a source of glucose, avoidance of dietary galactose and fructose, and a moderate restriction of fat.
- Depending on age, overnight continuous feeding or uncooked cornstarch feedings every 4–6 h are necessary to prevent hypoglycemia during the night.
- Frequent home monitoring of blood glucose and adjustments in the diet are needed to prevent hypoglycemia and maintain blood glucose >70 mg/dL or 4 mmol/L.

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27.1 Background

Glycogen storage disease (GSD) type 1, also called Von Gierke disease, results from a deficiency of glucose-6-phosphatase (type 1a) or glucose-6-phosphatase translocase (type 1b),

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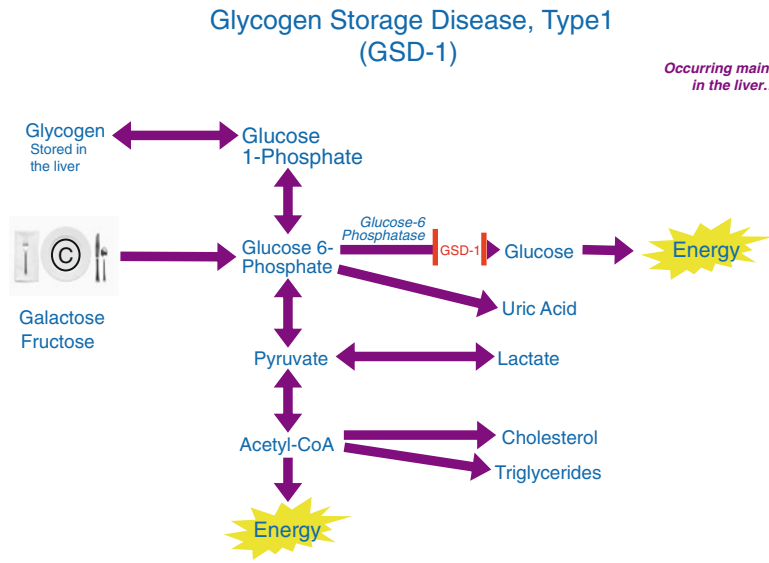

Fig. 27.1 Metabolic pathway of glycogen storage disease type 1

Table 27.1 Glycogen storage diseases

Type	Defective enzyme	Organ affected	Glycogen in the affected organ	Clinical features
I. Von Gierke	Glucose-6-phosphatase or transport system	Liver and kidney	Increased amount; normal structure	Massive enlargement of the liver. Failure to thrive. Severe hypoglycemia, ketosis, hyperuricemia, hyperlipidemia
II. Pompe	α-1,4-glucosidase (lysosomal)	All organs	Massive increase in amount; normal structure	Cardiorespiratory failure causes death, usually before age 2
III. Cori	Amylo-1,6-glucosidase (debranching enzyme)	Muscle and liver	Increased amount; short outer branches	Hepatomegaly, less severe hypoglycemia but may have more severe ketoacidosis without high lactate or uric acid. Increased AST; normal blood lactate and uric acid
IV. Andersen	Branching enzyme (α-1,4 – α-1,6)	Liver and spleen	Normal amount; very long outer branches	Progressive cirrhosis of the liver. Liver failure causes death, usually before age 2
V. McArdle	Phosphorylase	Muscle	Moderately increased amount; normal structure	Limited ability to perform strenuous exercise because of painful muscle cramps. Otherwise patient is clinically normal
VI. Hers	Hepatic phosphorylase deficiency	Liver	Increased amount	Less severe although some patients experience significant hypoglycemia, usually occurring during fasting with hyperketosis; blood lactate is normal but may be elevated postprandially
VII	Phosphofructokinase	Muscle	Increased amount; normal structure	Like type V
VIII	Phosphorylase kinase	Liver	Increased amount; normal structure	Mild liver enlargement. Mild hypoglycemia

Adapted from Biochemistry, 7th Edition., 2012 WH Freeman and Company [1] and Kishnani et al. [2]

Note: Types I through VII are inherited as autosomal recessive. Type VIII is sex-linked

preventing production of glucose from glycogen stores during periods of fasting (Fig. 27.1) and leading to severe hypoglycemia, if untreated. GSD type 1a and type 1b have similar clinical features except GSD type 1b is also characterized by neutropenia and a high incidence of inflammatory bowel disease (Box 27.1). Chapter 26 describes the biochemical and clinical features of GSD, which are also summarized in Table 27.1.

The focus of this chapter is the nutrition management of GSD types 1a and 1b. The goals of nutrition management in GSD type 1 are to maintain adequate blood glucose concentrations (>70 mg/dL or 4 mmol/L), to correct or improve metabolic derangements (elevated lactic acid, uric acid, triglycerides, and cholesterol), and to provide optimal nutrition to support growth and development. A resource for the nutrition management of GSD is available at http://www.gsd.peds.ufl.edu/nutrition.html.

Box 27.1: Principles of Nutrition Management for GSD Type 1 [3]

Restrict: Galactose (and lactose), fructose (and sucrose), excessive fat

Supplement: Carbohydrate (uncooked cornstarch)

Metabolites that accumulate: Lactic acid, uric acid, triglycerides, cholesterol

27.2 Chronic Nutrition Management

27.2.1 Diet in Infancy

The infant with GSD-1 must be fed every 2–3 h during the day to avoid low blood glucose concentrations. Formulas used for GSD-1 do not contain sucrose, lactose, or fructose, such as ProSobee®, Similac Soy Isomil®, Nutramigen®, and Pregestimil®. A soy formula is typically recommended, but if not tolerated, a hydrolyzed or elemental formula is also appropriate. Neither fructose nor galactose can be converted to glucose in patients with GSD-1. Therefore, both must be avoided or severely limited in the diet. As fructose is a component of sucrose, and galactose is a component of lactose, both sucrose and lactose must also be avoided.

Overnight fasting is not allowed, and sufficient glucose must be provided by offering bolus nighttime feeds or by giving a continuous-drip feed, usually via a gastrostomy tube. The calculation for the required amount of glucose is provided below (Box 27.2).

Box 27.2: How Much Glucose Is Enough?

- Use standard glucose infusion rates
  - Infants: 8–10 mg glucose/kg/min
  - Young child: 8 mg glucose/kg/min
  - Older child: 6 mg glucose/kg/min
  - Adult: 4–5 mg glucose/kg/min

Appendix K provides glucose infusion rate information and calculations.

The glucose infusion rate (GIR) is highest for infants (8–10 mg/kg/min) and declines with age. Monitoring of blood glucose concentrations at the end of a continuous nighttime drip feeding helps determine the correct feeding rate to avoid hypoglycemia. Additionally, a formula feeding or cornstarch dose should be given about 30 min before the overnight feeding is turned off to prevent hypoglycemia. If this is not possible, then formula or cornstarch must be given immediately after the drip feeding ends [2].

Although breast milk contains lactose, some clinics allow breastfeeding as long as metabolic control can be maintained [3, 4]. If appropriate metabolic control cannot be maintained, a soy formula may be added (either partially or fully) depending on laboratory and clinical status (Box 27.3).

Box: 27.3: Initiating Nutrition Management in an Infant with GSD-1

Goal: Prevent hypoglycemia.

Step-by-step:

1. Establish glucose needs based on age and fasting tolerance.
2. Determine the amount of formula required to provide a glucose infusion rate of 8–10 mg/kg/min over a 24 h period (see Box 27.2).
3. Avoid fasting. Some infants may require a feeding every 2–3 h to prevent hypoglycemia.
4. If a continuous-drip feeding is used, determine the rate needed to maintain adequate glucose concentrations.
5. Introduce solid foods at 4–6 months of age but avoid fruit, juices, or other food containing fructose/sucrose and/or galactose/lactose.
6. Uncooked cornstarch may be introduced between 6 and 12 months of age. Begin with 0.4 g/kg every 4 h and monitor blood glucose and gastrointestinal tolerance.
7. When introducing the cup, offer soy formula as the first choice.
8. Supplement with sugar-free vitamins and minerals as indicated based on nutrient analysis.

27.2.2 Cornstarch Therapy for GSD-1

Cornstarch is used in the treatment of GSD-1 because it is a slowly digested starch, resulting in a sustained source of glucose that lasts longer than any food source [5]. Uncooked cornstarch is not introduced before 6–9 months of age. Prior to this age, the infant does not have sufficient pancreatic amylase activity to digest the starch, and gastrointestinal distress can be a problem [6]. To improve tolerance, cornstarch can be added to soy formula starting with approximately 25 % of the prescribed dose (unpublished, author's personal clinical experience). Some infants may not be able to tolerate cornstarch during their first year because of continued gastrointestinal distress [3].

The recommended dose of cornstarch depends on the patient's age and weight (Table 27.2) and blood glucose monitoring. The goal is to provide sufficient cornstarch to maintain blood sugar greater than 70 mg/dL (>4 mmol/L) but not to overfeed cornstarch, as it can lead to excessive weight gain, insulin resistance, high lactate levels and increase storage of glycogen (Appendix K) (Box 27.4) [2]. Cornstarch is given uncooked, and it should not be added to acidic beverages, because both heat and acid will break down the starch molecule and reduce its effectiveness. For infants, the cornstarch can be mixed in infant formula. For older individuals, water or diet drinks can be used. Cornstarch should be given after meals to

Table 27.2 Guide for providing uncooked cornstarch in GSD type 1

Age	Cornstarch dose (g/kg)[b]	Frequency	Additional notes
Young children[a]	1.6	3–4 h	Mix in a lactose, sucrose-free formula
			Start gradually with a lower dose to improve GI tolerance
			Pancreatic enzymes (Ku-Zyme)® may improve digestion
Childhood through puberty	1.75–2.5	4–6 h	Provide in water or diet drinks
			Provide at the end of meals as to not affect appetite
Post puberty	1.75–2.5	Bedtime	May need additional doses during the night

Provide uncooked cornstarch divided over 24 h mixed with formula or other appropriate beverages. This guide is not appropriate if the patient is receiving a continuous-drip feeding. Some patients may require more frequent doses of uncooked cornstarch to maintain adequate blood glucose levels.

[a]Some centers start supplemental cornstarch at 6–9 months of age

[b]If patient is overweight, use ideal body weight to calculate cornstarch dose

Box 27.4: Cornstarch Therapy for GSD-1

- Weigh cornstarch with a gram scale, if possible; otherwise, use a household measurement (1 tablespoon of cornstarch weighs 8 g and provides 0.9 g glucose).
- Mix cornstarch into a sucrose, lactose-free formula, water or diet drink.
- Do not mix into acidic beverages.
- Add 1 g of cornstarch to 2–3 mL of fluid.
- Do not cook.
- Consume immediately after mixing into the liquid.
- Store dry cornstarch at room temperature.
- Cornstarch may only be good for 2 weeks after opening a container depending on environmental conditions.
- Argo® is the brand of cornstarch recommended in the United States [2, 7].

Box 27.5: Recommended Macronutrient Composition of the Diet for a Patient with GSD-1

- Carbohydrates (60–70 % of energy)
  - Use complex sources, such as starch
  - Avoid fructose and sucrose
    - No fruit
    - Limited vegetables
  - Limit galactose and lactose
    - 1 serving/day dairy allowed
- Protein (10–15 % of energy)
  - Offer lean sources of protein
  - Include protein of high biological value
- Fat (<30 % of energy)
  - Limit saturated sources
  - Include mono- and polyunsaturated sources
  - Assure adequate essential fatty acid intake

reduce its negative effect on appetite and to extend the time frame in which blood glucose levels remain above 70 mg/dL.

27.2.3 Diet After Infancy

The goal of diet treatment after infancy is to distribute the sources of carbohydrate throughout the day to prevent low glucose concentrations. Fasting time is limited. For children, carbohydrate should be consumed every 4–6 h, and for adults every 8 h or sooner, depending on blood glucose concentrations.

Estimated energy requirements are based on standard calculations considering height, weight, and activity level [8]. Energy is distributed throughout a 24-h period with two-thirds of total energy given throughout the day and one-third given at night. Carbohydrate provides 60–70 % of the total energy in the diet for GSD-1, 10–15 % of energy from protein, with the remainder from fat (Box 27.5).

A list of foods allowed and not allowed in the diet for GSD type 1 is presented in Table 27.3.

There are various methods to determine a meal plan for a patient with GSD-1. One method uses an exchange system based on the carbohydrate content of foods (personal communication, Anne Boney, Duke University). Carbohydrate sources exclude sucrose and fructose and limit lactose and galactose. Both cornstarch and food sources are included in the total carbohydrate prescription. The case study given at the end of the chapter provides an example of diet calculations and developing a meal plan based on the exchange system.

Some centers allow small amounts of galactose and/or fructose in order to improve nutrient intake and variety in the diet of patients with GSD-1, as long as so doing does not compromise metabolic control. For each meal, a maximum intake of 2.5 g of either galactose or fructose (equivalent to 5 g of lactose or sucrose) is allowed [9].

During the night, one-third of total energy is provided either by using continuous tube feedings or by waking the child for intermittent cornstarch feedings. If using tube feedings, any sucrose-, lactose-, and fructose-free formula can be used. Medical foods, such as Enfagrow Soy® (Mead Johnson Nutrition) for toddlers and TOLEREX®

Table 27.3 Foods allowed and foods not allowed in GSD type 1 [2]

Food group	Foods allowed	Foods not allowed
Dairy	Limit to one serving per day 1 cup low-fat milk 1 cup low-fat sugar-free yogurt 1.5 oz hard cheese	Ice cream Sweetened yogurt Sweetened milk
Cereals	Dry and cooked cereal with no added sugar	Cereals with fruit or added sugar
Breads	White, wheat, or rye breads Crackers, matzo English muffins Dinner rolls, biscuits Pita bread	Raisin bread Muffins Sweet rolls Pies Cakes Sweet breads Waffles and pancakes made with sugar
Starches	Brown and white rice Pasta Popcorn Tortillas White potatoes	Any starches with added sugar, milk, cheese Sweet potatoes
Vegetables	All nonstarchy vegetables including asparagus, cabbage, spinach, squash, onion, green beans, turnips, greens	Any with added milk, sugar, cheese Corn, peas, and carrots contain more sugar than other vegetables
Fruit	Lemons and limes Avocados	All other fresh, canned, and dried fruits Tomatoes
Meat	Lean poultry, beef, pork, fish	Organ meats Fatty and processed meats
Legumes/nuts/soy	All beans and nuts Soy and nut based milks without sugar added Tofu and other soy products without sugar added	Any beans, nuts, or soy products with sugar added
Soups	Broth soups made with allowed meats, starches, and vegetables	Creamed soups
Fats	Canola and olive oil Corn, safflower, canola, and soybean oil-based condiments Reduced-fat condiments	*Trans* fatty acids Saturated fats
Sweets	Sugar substitutes, sucralose Dextrose 100 % corn syrup, rice syrup Sugar-free Jell-O and pudding Candies made with dextrose	All other sugars, sweets, syrups, high-fructose corn syrup, honey, molasses, sorbitol, and cane sugar, juice, and syrups

(Nestle Nutrition) for older children and adults, are lower in fat than many pediatric and adult formulas. The amount of formula to prescribe depends on age and weight. The GIR (Appendix K) can be used to determine an initial amount of formula to provide by continuous feeding. Instruct patients to check blood glucose concentrations before the end of the feeding cycle to assure that levels remain above 70 mg/dL (4 mmol/L). If low concentration are measured, the feeding rate can be increased or formula concentrated, as tolerated, to provide additional glucose.

A meal or dose of cornstarch must be given 30 min before discontinuing the feeding or immediately at the end of the pump cycle to prevent rapid-onset hypoglycemia. A recent study suggested that intermittent administration of uncooked cornstarch at night prevented hypoglycemia better than continuous nocturnal feedings of dextrose [10]. While cornstarch feeding during the night is inconvenient, it precludes the problem of pump malfunction in the night causing hypoglycemia. Practice varies from clinic to clinic, and further research is needed before a

definitive recommendation for overnight feeds can be made [11].

27.2.4 Alternative and Adjunct Treatments

Glycosade® (Vitaflo USA) is an alternative to cornstarch. Studies have shown that Glycosade is more easily digested than uncooked cornstarch and may allow for a longer period of fasting before hypoglycemia (<60 mg/dL or 3.5 mmol/L) develops. However, the slower reduction in blood glucose using Glycosade compared to uncooked cornstarch was not significant until glucose concentrations fell below the therapeutic threshold of 70 mg/dL (4 mmol/L) [12].

Another potential option for diet treatment is addition of a source of medium-chain triglycerides (MCT) to the traditional diet. Lower serum concentrations of uric acid and triglycerides have been measured when MCT oil is added to the diet [13]. Addition of MCT oil may also allow for a reduction in the amount of carbohydrate and energy required to maintain adequate glucose control [14].

It is important to consider the micronutrient content of the diet. Unless the diet includes medical food, use of a multivitamin and mineral supplement with additional calcium and vitamin D is often needed. The supplements need to be sucrose- and lactose-free.

Cookbooks emphasizing sugar-free recipes are helpful, although these recipes will likely not limit fructose or lactose. For recipes calling for cow's milk, unsweetened soymilk or rice milk can be used as a substitute. A cookbook written specifically for GSD is available from the Association for Glycogen Storage Disease (www.agsdus.org).

27.3 Nutrition Management in Special Circumstances

27.3.1 Exercise

During periods of physical activity, additional glucose sources are needed to meet the greater energy demand during activity. Hypoglycemia can develop quickly, so extra precautions are needed. To help determine the best management strategy, glucose concentrations should be checked before, during, and after exercise. Additional cornstarch can be incorporated into the diet plan about 30 min before exercise, although gastrointestinal distress may be a problem for some. Another strategy is to give a source of maltodextrin, such as Polycal® (Nutricia North America, Rockville, MD), in water or diet beverages just before, during, and/or after activity. Frequent glucose monitoring is needed to determine the best strategy for each patient.

27.3.2 Illness

An illness can exacerbate the need for glucose and increases the risk of developing hypoglycemia. During minor illnesses, increasing the frequency of carbohydrate feedings may be sufficient, and use of maltodextrin solutions can be effective [15] (Appendix J). If tolerated, reinstitution of continuous-drip tube feedings may help. Patients with GSD should carry an emergency source of glucose, such as Insta-Glucose®, that can be given quickly [2]. However, because of the risk of low glucose concentrations during illness, there should be a low threshold for emergency evaluation and/or admissions. Use of intravenous dextrose (usually 10 % or 12.5 % concentration to meet GIR requirements) provided by peripheral line with appropriate electrolytes is typically recommended. It is important that the patient be able to take carbohydrate and cornstarch by mouth before IV sources are gradually decreased to avoid hypoglycemia [3].

As with illness, a severe injury can increase the need for glucose, and similar procedures as outlined for illness need to be followed. Caregivers and individuals with GSD-1 need to be aware that injuries can cause complications of hypoglycemia so that appropriate measures can be started early.

27.3.3 Surgery

Any surgical procedure causes catabolism and requires additional glucose resources. Thus, patients with GSD-1 undergoing surgery should

have precautions to prevent hypoglycemia. Typically, the fasting time required before the procedure is reduced as much as possible. A peripheral IV source of glucose is typically given during the presurgery fast and surgical procedure and continued postoperatively until adequate oral intake is possible [2]. Frequent monitoring of glucose and electrolytes is necessary.

27.3.4 Pregnancy

Successful pregnancy and infant outcomes have been documented in women with GSD-1 [16]. Carbohydrate requirements are greater during pregnancy, especially during the first trimester. An increase in frequency and severity of hypoglycemia has been noted during pregnancy in some women. Referral to an obstetrics clinic specializing in high-risk pregnancies is suggested, and frequent monitoring is essential throughout pregnancy. Intravenous dextrose has been used during delivery and postpartum period to reduce the risk of hypoglycemia during these times [2].

27.4 Monitoring

A mainstay for management of GSD-1 is home monitoring of glucose concentrations. Glucometers designed for diabetes management can be used. The goal is to maintain blood glucose concentrations ≥70 mg/dL (>4 mmol/L). Blood glucose should be checked in the morning after fasting or at the end of continuous feeding, before meals or cornstarch doses, and after exercise. Caregivers and patients should keep a record of times of meals, cornstarch doses, and blood glucose concentrations to help fine-tune the individual's plan.

Use of continuous glucose monitoring is also an option and provides an average glucose concentration every 5 min. This monitoring is designed for diabetes but can be adapted for GSD. This allows for fine-tuning of maximum fasting times, cornstarch doses, and carbohydrate feedings [2, 17].

Measuring blood lactate concentrations may also be helpful, since lactic acidosis can be present even with normal glucose concentrations and may provide a more sensitive marker of metabolic control in this disorder [2, 18]. A lactate meter can be used to check lactate levels at similar times during the day as recommended for glucose monitoring. The goal is to prevent lactate concentrations >3.5 mmol/L [19]. If elevations are frequently measured, then the diet and cornstarch doses need to be reevaluated.

Other laboratory parameters to measure include growth and plasma or serum concentrations of glucose, uric acid, triglycerides, cholesterol, lactate, and liver function tests. Poor bone mineralization has been found in those with GSD-1; therefore, monitoring of serum or plasma markers of bone metabolism including total 25-hydroxyvitamin D and routine DXA scans is suggested [20] (Box 27.6).

Box 27.6: Nutrition Monitoring of a Patient with GSD-1

- Routine assessments including anthropometrics, dietary intake, and physical findings (Appendix F)
- Laboratory monitoring
  - Diagnosis specific
    - Glucose
    - Lactic acid
    - Uric acid
    - Triglycerides
    - Cholesterol
    - Liver function tests
  - Nutrition monitoring of patients on fructose- and lactose-restricted diets supplemented with cornstarch may include markers of:
    - Nutritional anemia (hemoglobin, hematocrit, MCV, serum vitamin B_{12} and/or methylmalonic acid, total homocysteine, ferritin, iron, folate, total iron binding capacity)
    - Vitamin and mineral status (Total 25-hydroxy vitamin D, zinc, trace minerals)
    - Others as clinically indicated

Box 27.7: Evaluation of Fluctuating Blood Glucose Concentrations in GSD-1

- Is the patient following a consistent feeding and cornstarch schedule?
- Does the dose or frequency of cornstarch need to be increased?
- Does the amount of complex carbohydrate in the diet need to be increased?
- Are blood glucose concentrations related to time of day or periods of activity/exercise?

If glucose and/or lactate control is not adequate and blood sugar concentrations are "bouncing," the following parameters need to be evaluated (Box 27.7).

27.5 Transplantation

Liver and/or kidney transplantation has been performed in patients with GSD1 but is only recommended if the patient is unable to be managed medically or is at risk for hepatic carcinoma [2]. Liver transplantation restores normal fasting tolerance, corrects metabolic derangements, and prevents the development of hepatocellular carcinoma [21, 22]. Catch-up growth has occurred almost universally after transplant, and, in GSD type 1b, improvement in neutropenia has been reported in some, but not all, patients [21]. Patients are at risk for extrahepatic effects of GSD, and renal failure is known to be a complication of liver transplantation in GSD type 1a [2]. Hepatocyte transplantation has been successfully performed in a patient with GSD type 1a [23].

27.6 Summary

The overall goal for nutrition management in GSD-1 is to prevent hypoglycemia by maintaining blood glucose concentrations >70 mg/dL (4 mmol/L). Once glucose has been converted into glycogen and stored in the liver, it cannot be utilized as a source of energy. Avoidance of fasting, distribution of complex carbohydrate sources, and the use of uncooked cornstarch to provide a slow-release source of glucose have allowed for better glucose control and thus improved the long-term outcome of individuals with GSD-1.

27.7 Diet Calculation Example

Example 1:
Child with GSD Type 1A

Patient information	Nutrient intake goals
Six (6) year-old boy weighing 21.5 kg with glycogen storage disease, type 1. The following nutrition calculations provide an example of the steps to determine a nutrition plan	**Energy:** 85 kcal/kg/day **Carbohydrate:** 60–70 % of total kcals **Protein:** 10–15 % of total kcals **Fat:** <30 % of total kcals

Step-by-Step Calculation

Step 1. Calculate the amount of each nutrient required each day.

Energy: 85 kcal/kg × 21.5 kg = 1,828 kcal/day

Total carbohydrate: 0.65 × 1,828 = 1,188 kcal ÷ 4 kcal/g = 297 g CHO

Protein: 0.12 × 1,828 = 219 kcal ÷ 4 kcal/g = 55 g protein

Fat: 0.23 × 1,828 = 420 kcal ÷ 9 kcal/kg = up to 47 g fat

Step 2. Calculate the cornstarch dose required for the day

Cornstarch dose = Recommended range of 1.75–2.5 g/kg every 6 h

For this example, we will use 2.3 g/kg

Cornstarch dose × Child Weight = g cornstarch every 6 h

2.3 g/kg × 21.5 kg = 50 g/dose × 4 doses/d = 200 g total/day

Step 3. Calculate the number of calories provided by 200 g cornstarch

g of cornstarch × g glucose/g of cornstarch = g of glucose

200 g × 0.9 g = 180 g glucose × 4 kcal/g = 720 kcal

Step 4. Calculate the amount of carbohydrate that will be provided by food sources:

Total carbohydrate goal − carbohydrate from cornstarch = kcal from carbohydrate

1,188 kcal − 720 kcal = 468 kcal

468 kcal from carbohydrate ÷ 4 kcal/g = 117 g carbohydrate

Step 5. Determine a feeding schedule that distributes complex carbohydrate and cornstarch doses evenly throughout the day

To use an exchange system, 1 serving = 15 g of complex carbohydrates

117 g total carbohydrate from food ÷ 15 g carbohydrate/exchange = 7.8 or 8 exchanges/day

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Appendix A. Overview of Metabolic Disorders

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Amino acid disorders					
Disorder	Enzyme affected	Biochemical findings	Clinical features	Nutritional modification	Vitamin therapy
Phenylketonuria: severe (classical), moderate (atypical), mild (hyperphenyl-alaninemia)	Phenylalanine hydroxylase	Increased blood phenylalanine Severe: >1,200 umol/L Moderate: 360–1,200 µmol/L Mild: 120–360 umol/L	If untreated, intellectual disability, seizures, hyperactivity, and eczema; normal development with proper treatment	Phenylalanine restriction, ±tyrosine supplementation	None
Maternal phenylketonuria	Phenylalanine hydroxylase	Same as above	Untreated PKU in the mother causes intellectual disability, congenital heart disease, low birth weight, and microcephaly in offspring	Phenylalanine restriction, ±tyrosine supplementation	None
Hyperphenyl-alaninemia (pterin defect)	Dihydropteridine reductase; GTP cyclohydrolase	Mild to moderate hyperphenylalaninemia (see above)	Psychomotor retardation, tonicity disorders, hyperthermia, hypersalivation, difficulty swallowing	±phenylalanine restriction, ±tyrosine supplementation	Tetrahydropterin 2 mg/kg/day orally, ± neurotransmitter supplements
Tyrosinemia type I	Fumarylacetoacetate hydrolase	Increased blood phenylalanine and tyrosine; increased alpha-fetoprotein; urinary succinylacetone	Liver failure; renal tubular acidosis, failure to thrive, vomiting, diarrhea, rickets, porphyria-like crises, hepatic carcinoma	Phenylalanine and tyrosine restriction (diet used in conjunction with NTBC or until liver transplantation is possible)	None
Tyrosinemia type II	Tyrosine aminotransferase	Increased blood phenylalanine and tyrosine	Intellectual disability, photophobia, palmar keratosis	Phenylalanine and tyrosine restriction	None
Homocystinuria (pyridoxine nonresponsive)	Cystathionine ß-synthase	Homocystine in blood and urine, increased methionine and decreased cystine in blood	Dislocated lenses, marfanoid-like skeletal changes, intravascular thromboses, intellectual disability, osteopenia	Methionine restriction; cystine, betaine, and folate supplementation	Betaine 100 mg/kg/day orally
Homocystinuria (pyridoxine responsive)	Cystathionine ß-synthase	Same as above	Same as above	None	Pyridoxine 25–500 mg/day orally
Maple syrup urine disease	Branched-chain keto acid dehydrogenase complex	Elevated blood, urine, and CSF leucine, isoleucine, valine, alloisoleucine	Neonatal form: poor feeding, fluctuating tone, apnea, seizures, death, developmental delay; variant forms: milder ketoacidosis triggered by protein load or illness	Valine, isoleucine, and leucine restriction	Only in variant forms where 100–300 mg/day oral thiamin may enhance residual enzyme activity

Organic acidemias					
Glutaric acidemia type I	Glutaryl-CoA dehydrogenase	Elevated blood, urine, and CSF glutaric acid and 3-OH-glutaric acid, metabolic acidosis	Acute metabolic crisis (vomiting, acidosis) and neurological deterioration triggered by illness; macrocephaly, ataxia, choreoathetosis, developmental delay	Lysine and tryptophan restriction; carnitine supplementation	May have partial response to riboflavin 100–300 mg/day orally
Glutaric acidemia type II	Multiple acyl-CoA dehydrogenase	Elevated blood, urine, and CSF glutaric acid and 2-OH-glutaric acid, metabolic acidosis, hyperammonemia, hypoglycemia (± ketones), impaired fatty acid oxidation	Malformations in most severe form, hypotonia, hepatomegaly, developmental delay	Mild protein and fat restriction; fasting avoidance, ± carnitine supplementation	±Riboflavin 100–300 mg/d orally
Isovaleric acidemia	Isovaleryl-CoA dehydrogenase	Elevated blood, urine, and CSF isovaleric acid; metabolic acidosis, hyperammonemia, hypoglycemia	Poor feeding, vomiting, sweaty-feet body odor, seizures, coma, death if untreated	Leucine restriction; glycine and carnitine supplementation	None
Methylmalonic acidemia	Methylmalonyl-CoA mutase	Metabolic acidosis, ketonuria, hypoglycemia, hyperammonemia, hyperglycinemia	Lethargy, failure to thrive, vomiting, hepatomegaly, hypotonia, coma, death if untreated	Isoleucine, methionine, valine, and threonine restriction; carnitine supplementation	None
Methylmalonic acidemia	Cobalamin processing defect (hydroxocobalamin or adenosylcobalamin)	Metabolic acidosis, ketonuria, ± homocystine in urine and blood, ± folate deficiency	Lethargy, failure to thrive, vomiting, hepatomegaly, hypotonia, coma, death if untreated	Carnitine supplementation, DRI for protein	Hydroxocobalamin 1–2 mg daily to weekly intramuscularly
Propionic acidemia	Propionyl-CoA carboxylase	Metabolic acidosis, ketonuria, hyperglycinemia, hypoglycemia, hyperammonemia	Poor feeding, vomiting, lethargy, hypotonia, seizures, coma, death if untreated, developmental delay	Isoleucine, methionine, valine, and threonine restriction; carnitine supplementation	None
Urea cycle disorders					
N-acetylglutamate synthase (NAGS) deficiency	*N*-acetylglutamate synthase	Hyperammonemia	Lethargy, vomiting, apnea, coma and death if untreated; intellectual disability	Protein restriction; essential amino acid and arginine supplementation (in conjunction with carbamylglutamate)	None

(continued)

Amino acid disorders					
Disorder	Enzyme affected	Biochemical findings	Clinical features	Nutritional modification	Vitamin therapy
Ornithine transcarbamylase (OTC) deficiency; intellectual disability	Ornithine transcarbamylase	Hyperammonemia, respiratory alkalosis	Lethargy, vomiting, apnea, coma and death if untreated; intellectual disability	Protein restriction; essential amino acid and arginine supplementation (in conjunction with nitrogen scavenging medications)	None
Carbamoyl phosphate synthetase (CPS) deficiency	Carbamoyl phosphate synthetase	Hyperammonemia, respiratory alkalosis	Lethargy, vomiting, apnea, coma and death if untreated; intellectual disability	Protein restriction; essential amino acid and arginine supplementation (in conjunction with nitrogen scavenging medications)	None
Citrullinemia	Argininosuccinic synthetase	Hyperammonemia, respiratory alkalosis	Lethargy, vomiting, apnea, coma and death if untreated; intellectual disability	Protein restriction; essential amino acid and arginine supplementation (in conjunction with nitrogen scavenging medications)	None
Argininosuccinic aciduria	Argininosuccinic lyase	Hyperammonemia, respiratory alkalosis	Lethargy, vomiting, apnea, coma and death if untreated; cirrhosis, intellectual disability	Protein restriction; essential amino acid and arginine supplementation (in conjunction with nitrogen scavenging medications)	None
Arginemia	Arginase	±Hyperammonemia	Spastic diplegia and death if untreated, intellectual disability	Restrict protein; essential amino acid supplementation (in conjunction with nitrogen scavenging medications)	None
Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH syndrome)	Defect in mitochondrial transport of ornithine	Hyperornithinemia, hyperammonemia, homocitrullinuria, hyperglutaminemia, hyperalaninemia	Ataxia, lethargy, vomiting, choreoathetosis, seizures, coma, developmental delay	Protein restriction; arginine supplementation	None
Disorders of carbohydrate metabolism					
Galactosemia	Hepatic and erythrocyte epimerase	Galactose in blood and urine	Hepatomegaly, jaundice, vomiting	Restrict galactose; calcium and vitamin D supplementation	None
Galactosemia	Galactokinase	Galactose in blood and urine	Cataracts	Restrict galactose; calcium and vitamin D supplementation	None

Galactosemia	Galactose-1-phosphate uridyltransferase	Galactose in blood and urine; renal Fanconi syndrome	Cataracts, diarrhea, failure to thrive, hepatomegaly, jaundice, vomiting, E. coli sepsis	Restrict galactose; calcium and vitamin D supplementation	None
Pyruvate dehydrogenase complex deficiency	Pyruvate dehydrogenase	Elevated blood pyruvate and lactate, elevated blood alanine	Hypotonia, failure to thrive, seizures, ±dysmorphism, developmental delay	Restrict carbohydrate, provide high-fat diet (50 % of energy) or ketogenic diet	Thiamin 50–100 mg/day orally
Hereditary fructose intolerance	Aldolase B	Decreased blood glucose, phosphate, increased fructose in urine and blood	Nausea, diarrhea, vomiting, hypoglycemia after fructose ingestion; if untreated: failure to thrive, liver and kidney disease, seizures, death	Restrict fructose, sucrose, and sorbitol	None
Fatty acid oxidation disorders					
VLCAD deficiency LCHAD deficiency	Very long-chain acyl-CoA dehydrogenase; long-chain hydroxyacyl-CoA dehydrogenase	Hypoketotic hypoglycemia, ±hyperammonemia	Cardiomyopathy, failure to thrive, hypotonia, hepatomegaly, lethargy, coma	Fasting avoidance; ± long-chain fat restriction (10–25 % of energy); MCT oil, ±carnitine, and ± essential fatty acid supplementation	None
MCAD deficiency	Medium-chain acyl-CoA dehydrogenase	Hypoketotic hypoglycemia, mild hyperammonemia and metabolic acidosis	Metabolic decompensation with fasting (lethargy, vomiting, coma) and hepatomegaly	Fasting and MCT avoidance; fat 30 % of energy; ±carnitine	None
SCAD deficiency SCHAD deficiency	Short-chain acyl-CoA dehydrogenase; short-chain hydroxyacyl-CoA dehydrogenase	Hypoketotic hypoglycemia, ±hyperammonemia, metabolic acidosis	Poor feeding, vomiting, failure to thrive; ±developmental delay	Fasting avoidance; ±carnitine	None

Adapted from Hendricks KM, Duggan C, Walker WA. Manual of Pediatric Nutrition, 3rd edition. 2000, Hamilton, Ontario; B.C. Decker, Inc. Hamilton, Ontario.

Appendix B. Websites Containing Educational Resources for Dietitians Managing Inherited Metabolic Diseases

B.1 Website to Accompany the Book

Nutrition Management of Inherited Metabolic Disorders: Lessons from Metabolic University: http://www.imd-nutrition-management.com (online diet calculations)

B.2 Educational Websites for Dietitians

Educational tools developed by the Inherited Metabolic Disease Clinic at Colorado Children's Hospital, Aurora, CO: http://www.ucdenver.edu/academics/colleges/medicalschool/departments/pediatrics/subs/genetics/clinical/IMDNutrition/Pages/IMDNutritionHome.aspx

New England Consortium of Metabolic Programs, Boston Children's Hospital, Boston, MA: http://newenglandconsortium.org

Genetic Metabolic Dietitians International: www.gmdi.org

Metabolic Genetics and Nutrition Program, Emory University, Atlanta GA: http://genetics.emory.edu/clinical/index.php?assetID=261

Cristine M. Trahms Program for Phenylketonuria, University of Washington, Seattle WA: http://depts.washington.edu/pku/

B.3 Parent/Support Groups Sites with Educational Materials

National PKU Alliance: http://www.npkua.org/Education.aspx

Canadian PKU and Allied Disorders: http://www.canpku.org/canpku-resources

B.4 Industry Websites with Educational Materials

Nutrica Learning Center: http://www.nutricialearningcenter.com

Vitaflo: http://www.vitaflousa.com/resources/

PKU Academy: www.pkuacademy.org

Mead Johnson Nutrition: http://www.meadjohnson.com/pediatrics/us-en/clinical-support/metabolic-toolkit

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Appendix C. Nutrient Composition of Frequently Used Parenteral Fluids

C.1 Carbohydrate

Carbohydrate is provided as IV dextrose.

Dextrose contains 3.4 kcal/g.

Dextrose solutions range from 5 % (D5W) to 25 % (D25W) by weight.

Solutions containing 12.5 % dextrose and higher cannot be given peripherally and require a central line (peripherally inserted central catheter or PICC, port or central access).

Percent solution	CHO content (g/100 mL)	kcal/100 mL
D5W (5 %)	5	17
D10W (10 %)	10	34
D25W (25 %)	25	85

C.2 Protein

Protein is provided as crystalline amino acid solutions.

TrophAmine 6 % and 10 % (B. Braun) and Aminosyn-PF 7 % (Hospira) are frequently used solutions.

Amino acid solutions provide 4 kcal/g.

Percent solution (%)	Amino acid content (g/100 mL)	kcal/100 mL
3.0	3	12
3.5	3.5	14
5.0	5	20
10	10	40

C.3 Fat

Fat is provided as a lipid emulsion.

Intralipid 10 % and Intralipid 20 % are frequently used lipid emulsions.

Percent solution of fat emulsion	kcal/100 mL
10 %	1.1 kcal/mL
20 %	2.0 kcal/mL

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Appendix D. Studies Describing Protein and Energy Intakes

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Author and year	Condition	Reporting country	Protein intake	Energy intake	Other
Acosta (1994) [1]	PKU *N* = 25 from diagnosis Period 1 = 0–3 months of age Period 2 = 3–6 months of age	USA	Fed protein substitute Group A 3.12 g/100 kcal Group B 2.74 g/100 kcal	Group A Period 1,580 ± 28 kcal and period 2,680 ± 30 kcal/kg Group B Period 1,591 ± 30 kcal and period 2,709 ± 34 kcal/kg	Prospective longitudinal study Group A infants tolerated increase Phe Group A improved growth vs. Group B
Schäefer (1994) [2]	PKU *N* = 82 (0–6 years)	Germany	Natural/total protein g/kg/day <2 years ~0.7 ± 0.1/2.2 ± 0.25 3–6 years ~0.5 ± 0.1/2 ± 0.25 g/kg/day	–	Retrospective review. Some growth retardation in first 2 years
Thomas (1994) [3]	PA *N* = 12 Dx (3–790 days)	Saudi Arabia	Initial consult 1.0–3 g/kg Final consult 1.6–3 g/kg	115–145 kcal/kg 99–273 kcal/kg	Retrospective review. Protein recommendations include amino acid supplements. Intakes prescribed based on Ross nutrition support protocol
Schulz (1995) [4]	PKU *N* = 99 (12–29 years) Groups ± protein supplement	Germany	Group with protein supplement: 112–138 % RDA Group without protein supplement: 90–104 % RDA	Protein: 8–20 % energy Fat: 11–40 % CHO: 36–80 % Protein: 5–16 % Fat: 26–47 % CHO: 43–66 %	Retrospective review. Dietary survey. Group without protein supplement had inadequate intake of some nutrients and higher Phe levels
MacDonald (1996) [5]	PKU *N* = 19 (1–16 years)	UK	Allocated protein g/kg/day <1 year: 3 2–5 years: 2.5 6–10 years: 2.0 >11 years: 1.5	Mean (SD, range) 105 % (17 %, 77 %–178 %) EAR	Prospective longitudinal study No correlation with energy intake and plasma Phe level Distribution of protein substitute affects 24 h Phe variability
Krauch (1996) [6]	PKU Low- and high-tolerance groups at various ages	Germany	Recommended protein intake: 3 months: 2.2 g/kg 10 months: 2.0 g/kg 3 years: 1.7 g/kg 8 years: 1.4 g/kg 12 years: 1.1 g/kg 16 years: 0.9 g/kg	"Average energy intake"	Excess of several amino acid for some patients when fed protein at these levels
Acosta (1998) [7]	PKU *N* = 35 (0.5–6 months)	USA	Mean 17.3 ± 0.6 g	660 ± 18 kcal	Prospective longitudinal study Normal growth seen, supporting MRC recommendations for supplementary protein of 3 g/kg/day

Thomas (2000) [8]	Organic acidemia *N* = 6	USA		All children consumed energy <RDA for age	Retrospective review. Adequate growth seen at <RDA energy. May be related to decreased mobility
Arnold (2002) [9]	PKU *N* = 28 (2–18 years)	USA	Total protein g/day > RDA: 2–4 years: 30 4–7 years: 35 g 7–11 year: 40 >12 years: 50–55	–	Retrospective chart review. No general growth impairment. Hypoprealbuminemia predicted linear growth restriction
Yannicelli (2003) [10]	MMA/PA *N* = 16 (0.03–3 years)	USA	Total protein g: mean ± SD: <6 months: 15 ± 0.9 6 to <12 months: 18.3 ± 1.1 1 to <4 years: 25.1 ± 2.46	Energy kcal mean ± SD: <6 months: 645 ± 10 6 to <12 months: 741 ± 92 1 to <4 years: 1,062 ± 100	Multicenter outpatient study. Those increasing in length achieved 98 % and 115 % of WHO/FAO/UNU energy and protein intakes, respectively. Those that did not achieved 87 % and 104 %, respectively
Gillingham (2003) [11]	LCHAD/TFP deficiency *N* = 10 (1–10 years)		Mean 2.5 g/kg/day (1.3–5 g/kg/day)	Protein 12 % energy: MCT: 12 LCT: 11 CHO: 66	Prospective study. No growth deficiencies observed
Dobbelaere (2003) [12]	PKU *N* = 20 (0.7–7 years)	France	Mean ± SD Total protein: 1.67 ± 0.23 g/kg/day Natural protein mean 9.8 g/day	67–12 % RDA (Mean 89 %)	Prospective, cross-sectional study Patients shorted and lighter than reference populations. No relationship between protein and calorie intake and growth retardation
MacDonald (2004) [13]	PKU *N* = 25 (2–10 years)	UK	Protein equivalent at 2 g/kg and 1.2 g/kg	–	Randomized crossover study Poorer Phe control seen with lower amount of protein substitute
Hoeksma (2005) [14]	PKU *N* = 174 (0–36 months)	Netherlands	Mean ± SD Total protein: 2.33 ± 0.42 g/kg/day Natural protein 0.99 ± 0.34 g/kg/day	Mean ± SD For first year of life 27 kJ ± 2.6 kJ/kg/day	Retrospective study. Height growth not clearly related to protein intake. Natural protein rather than total protein correlated with head circumference

(continued)

Author and year	Condition	Reporting country	Protein intake	Energy intake	Other
Acosta (2005) [15]	UCD *N* = 17 Median 4.4 months (0.22–38.84 months) Protocol: protein ~50 % FAO/WHO/UNU or as tolerated. 40–70 % of protein from medical food	USA	Intakes as % FAO/WHO/UNU 0 to <6 months: 70 6 to <12 months: 62 12 to <24 months: 89 24 to <36 months: 59 36 to <48 months: 35, 81	Intakes as % FAO/WHO/UNU 0 to <6 months: 110 6 to <12 months: 110 12 to <24 months: 89 24 to <36 months: 45.132 36 to <48 months: 70.89	Longitudinal study. Adequate intakes resulted in anabolism and linear growth without increasing ammonia. Some still failed to ingest recommended protein and energy intakes
Touati (2006) [16]	MMA and PA *N* = 137 (85 MMA, 52 PA) *n* = 56 Dx 1970–1987 *n* = 81 Dx 1988–2005 *n* = 39 severe disorder Dx >1988	France	Severe patients on amino acid (AA) supplements: 40 % at 3 years, 50 % 6–11 years Intake at 3, 6, 11 years g/kg/day: Group without AA supplements: Natural protein: 0.92, 0.78 0.77 Group with AA supplements: Natural protein: 0.75, 0.74 0.54 Total protein: 1.29, 1.17, 0.89	Energy intake kcal/kg/day No AA supplements vs. AA supplements: 3 years: 93.1 vs. 85.9 6 years: 80.7 vs. 70.2 11 years: 66.4 vs. 52.2	Retrospective review. Since 1988 all patients treated with low-protein diet to tolerance and only occasional use of AA supplements. Metabolic control not different between groups
Nagasaka (2006) [17]	OTC *N* = 7 Follow-up age 3–5 years	Japan	Infancy 1.3–2.0 g/kg Older children 0.7–1.1 g/kg	According to age requirements 1,350–1,660 kcal	Prospective study to determine effect of reintroduction of L-arginine on nutrition, growth, and urea cycle function
Huemer (2007) [18]	PKU *N* = 34 Mean 8.7 years (2–15 years)	Austria	Mean total protein intake g/kg/day: 1.2 ± 0.3 124 % (77–19) DACH 2000 Natural protein 0.3 ± 2 g/kg/day	–	Prospective longitudinal study with cross-sectional component. A significant correlation of fat-free mass with intake of natural protein rather than total protein
Singh (2007) [19]	UCD	USA	EAA supplement to 50 % protein intake:		Intake data from patient charts from author's clinic. Diets must be individualized depending on severity of disorder
			0 to <3 months: 2.1–1.4 g/kg/d	150–101 kcal/kg	
			3–6 months: 1.5–1.2	100–80	
			9 to <12 months: 1.2–1.1	80–75	
			1 to <4 years: 18.6–12.5 g/day	800–1,040 kcal/day	
			4 to <7 years: 21.0–19.0	1,196–1,435	
			7 to <11 years: 22.0–24.0	1,199–1,693	

Ahring (2009) [20]	PKU 10-center survey	Europe	Amino acid supplementation decreased with age (g/kg/day) Infancy: ~2–3 1–10 years: ~1.2–2 1–10 years: ~2–3 (40 % centers) >10 years: ~1.0–1.5		“Normal” energy intake according to national or European recommendations	Structured questionnaire to assess practice differences. Substantial variation in dietary guidelines among countries and within countries. No consensus opinion based on solid scientific rationale
Hauser (2011) [21]	MMA *N* = 29 (2–35 years) Natural protein ± amino acid supplements ± isoleucine or valine	USA	Total protein 0.38–2.94 g/kg/day Natural protein 0.29–2.12 g/kg/day (33–265 % RDA) 18/29 had amino acid supplements 0.21–1.95 g/kg/day		23–86 kcal/kg/day	Prospective study. Wide variation in the dietary treatment of MMA. REE may be overestimated with standard equations due to altered body composition
Rocha (2012) [22]	PKU *N* = 89 (3–30 years)	Portugal	Protein (g/kg/day):		Energy kcal/d:	Prospective study. Prevalence of overweight and obesity, body fat percentage, and central obesity were comparable to controls, however higher than ideal
			<10 years	Natural protein 1.03 ± 0.51	<10 years: 2,171 ± 10–16 years: 2,467 ± 256 >16 years: 2,415 ± 375	
				Protein suppl.: 1.46 ± 0.47		
			10–16 years	Natural protein 0.65 ± 0.36		
				Protein supplement 1.42 ± 0.43		
			>16 years	Natural protein 0.53 ± 0.35		
				Protein suppl. 1.07 ± 0.42		
Gokmen-Ozel (2012) [23]	GA1 *N* = 20 *N* = 9 without encephalopathic crisis (EC) *N* = 11 with EC (2.2–24.1 years)	UK	Patients without EC: Protein intake (median, range) Natural: 4/6 (1.3, 1.3–1.7 g/kg) Protein substitute (1.6,1.3–1.7 g/kg) 2/6 given general protein restriction Patients with EC: Treatment varied. Low-protein diet only. Protein substitute ceased over time		–	Retrospective review. Dietary treatment dependent on age of diagnosis and symptom severity

(continued)

Author and year	Condition	Reporting country	Protein intake	Energy intake	Other
Adam (2012) [24]	UCD 16 IMD centers *N* = 175 *N* = 123 (0–16 years) *N* = 52 (>16 years)	UK	Prescribed protein intake as WHO/FAO/UNU 2007 safe level titrated to metabolic control (g/kg/day): 0–6 months: 2 7–12 months: 1.6 1–10 years: 1.3 11–16 years: 0.9 >16 years: 0.8 Variable use of EAA	–	Cross-sectional data detailing dietary practices from 16 IMD centers in the UK, collected by questionnaire
Adam (2013) [24, 25]	UCD *N* = 464	Europe	Variable for each condition/age/country Use of EAA supplements varied		Survey: dietary treatment varies widely between centers
Boy (2013) [26]	GA1 *N* = 33 (0–6 years) Asymptomatic *n* = 29 Dystonic *n* = 4	Germany	Asymptomatic patients Natural protein Mean 109 % (median 115 %, SD 20 %) DACH recommendations Amino acid supplement Mean 108 % (median 110 %, SD 14 %) of GA1 guideline recommendations Dystonic patients: Mean 121 % (median 122 %, SD 8 %) of DACH recommendations Amino acid supplement Mean 104 % (median 103 %, SD 7 %) of GA1 guideline recommendations	Asymptomatic patients 106 % (median 102 % SD 13 %) of DACH recommendations Dystonic patients (mean 110 %, median 108 %, SD 8 %)	Prospective longitudinal study. Amino acid supplement and energy intake decreased with age in asymptomatic group. Normal weight gain in asymptomatic group but impaired in dystonic group. Reduction in height z-score for both groups
Aldámiz-Echevarría (2013) [27]	PKU BH4 *n* = 38 Diet only *n* = 76 Followed up for 2 and 5 years	Spain	2 years follow-up group g/kg/day: Diet only: Natural protein 0.4 (0.3–0.5) Total protein 1.4 (1.0–2.4) BH4 group: Natural protein 0.8 (0.5–1.0) Total protein 1.5 (0.7–2.2) 5 year follow-up group final intake: Diet only: Natural protein 0.3 (0.2–0.4) Total protein 1.6 (1.2–1.9) BH4 group: Natural protein 0.9 (0.7–1.1) Total protein 1.2 (0.7–1)	–	Retrospective review. Growth impairment also identified in patients on BH4 despite higher intakes of natural protein

Appendix E. Quick Guide to Acylcarnitine Profiles

Carnitine ester	Acylcarnitine name	Clinical correlate
C0	Free carnitine	Primary carnitine deficiency, CPT1
C3	Propionylcarnitine	PA, MMAs, mitochondrial
C3-DC	Malonylcarnitine	Malonic aciduria
C4	Butyrylcarnitine	SCAD, IBD, MADD (GA II)
C4-OH	3-Hydroxybutyrylcarnitine	SCHADD
C5:1	Tiglylcarnitine	BKT, MHBDD
C5	Isovalerylcarnitine 3-methyl-butyrylcarnitine	IVA, SBCAD, MADD
C5-OH	Hydroxyisovalerylcarnitine 2-methyl-3-hydroxybutyrylcarnitine	3MCC, holocarboxylase, biotinidase, BKT, HMG-CoA lyase, 3MGA
C5-DC	Glutarylcarnitine	GA I, MADD
C8	Octanoylcarnitine	MCADD, MADD
C14:1	Tetradecanoylcarnitine	VLCADD
C16	Hexadecanoylcarnitine	CPT II, CACT
C16-OH	Hydroxyhexadecanoylcarnitine	LCHADD/TFP

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Appendix F. Nutrition Care Process

Step 1. Nutrition assessment	
Definition and purpose	Nutrition assessment is a systematic approach to collect, record, and interpret relevant data from patients, clients, family members, caregivers, and other individuals and groups. Nutrition assessment is an ongoing, dynamic process that involves initial data collection as well as continual reassessment and analysis of the patient's/client's status compared to specified criteria
Data sources/tools for assessment	Screening or referral form
	Patient/client interview
	Medical or health records
	Consultation with other caregivers, including family members
	Community-based surveys and focus groups
	Statistical reports, administrative data, and epidemiologic studies
Types of data collected	Food- and nutrition-related history
	Anthropometric measurements
	Biochemical data, medical tests, and procedures
	Nutrition-focused physical examination findings
	Client history
Nutrition assessment components	Review data collected for factors that affect nutrition and health status
	Cluster individual data elements to identify a nutrition diagnosis as described in diagnosis reference sheets
	Identify standards by which data will be compared
Critical thinking	Determining appropriate data to collect
	Determining the need for additional information
	Selecting assessment tools and procedures that match the situation
	Applying assessment tools in valid and reliable ways
	Distinguishing relevant from irrelevant data
	Distinguishing important from unimportant data
	Validating the data
Determination for continuation of care	If upon completion of an initial or reassessment it is determined that the problem cannot be modified by further nutrition care, discharge or discontinuation from this episode of nutrition care may be appropriate
Step 2. Nutrition diagnosis	
Definition and purpose	Nutrition diagnosis is a food and nutrition professional's identification and labeling of an existing nutrition problem that the food and nutrition professional is responsible for treating independently
Data sources/tools for diagnosis	Organized assessment data that is clustered for comparison with defining characteristics of suspected diagnoses as listed in diagnosis reference sheets

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Nutrition diagnosis components	The nutrition diagnosis is expressed using nutrition diagnostic terms and the etiologies, signs, and symptoms that have been identified in the reference sheets describing each diagnosis. There are three distinct parts to a nutrition diagnostic statement
	1. The nutrition diagnosis describes alterations in a patient's/client's status. A diagnostic label may be accompanied by a descriptor such as "altered," "excessive," or "inadequate"
	2. Etiology is a factor gathered during the nutrition assessment that contributes to the existence or the maintenance of pathophysiological, psychosocial, situational, developmental, cultural, and/or environmental problems
	The etiology is preceded by the words "related to"
	Identifying the etiology will lead to the selection of a nutrition intervention aimed at resolving the underlying cause of the nutrition problem whenever possible
	Major and minor etiologies may result from medical, genetic, or environmental factors
	3. Signs/symptoms (defining characteristics)
	The defining characteristics are a typical cluster of signs and symptoms that provide evidence that a nutrition diagnosis exists
	The signs and symptoms are preceded by the words "as evidenced by"
	Signs are the observations of a trained clinician
	Symptoms are changes reported by the patient/client
Nutrition diagnostic statement	A well-written nutrition diagnostic statement should be:
	Clear and concise
	Specific to a patient/client
	Limited to a single client problem
	Accurately related to one etiology
	Based on signs and symptoms from the assessment data
Critical thinking	Finding patterns and relationships among the data and possible causes
	Making inferences
	Stating the problem clearly and singularly
	Suspending judgment
	Making interdisciplinary connections
	Ruling in/ruling out specific diagnoses
Determination for continuation of care	Because the nutrition diagnosis step involves naming and describing the problem, the determination for continuation of care follows the nutrition diagnosis step. If a food and nutrition professional does not find a nutrition diagnosis, a patient/client may be referred back to the primary provider. If the potential exists for a nutrition diagnosis to develop, a food and nutrition professional may establish an appropriate method and interval for follow-up
Step 3. Nutrition intervention	
Definition and purpose	A nutrition intervention is a purposefully planned action(s) designed with the intent of changing a nutrition-related behavior, risk factor, environmental condition, or aspect of health status. Nutrition intervention consists of two interrelated components: planning and intervention. The nutrition intervention is typically directed toward resolving the nutrition diagnosis or the nutrition etiology. Less often, it is directed at relieving signs and symptoms
Data sources/tools for interventions	The American Dietetic Association's Evidence-Based Nutrition Practice Guides or other guidelines from professional organizations
	The American Dietetic Association's Evidence Analysis Library and other secondary evidence such as the Cochrane Library
	Current research literature
	Results of outcome management studies or quality improvement projects

Nutrition intervention components	Planning
	Prioritize diagnoses based on urgency, impact, and available resources
	Write a nutrition prescription based on a patient's/client's individualized recommended dietary intake of energy and/or selected foods or nutrients based on current reference standards and dietary guidelines and a patient's/client's health condition and nutrition diagnosis
	Collaborate with the patient/client to identify goals of the intervention for each diagnosis
	Select specific intervention strategies that are focused on the etiology of the problem and that are known to be effective based on best current knowledge and evidence
	Define time and frequency of care, including intensity, duration, and follow-up
	Implementation
	Collaborate with a patient/client and other caregivers to carry out the plan of care
	Communicate the plan of nutrition care
	Modify the plan of care as needed
	Follow-up and verify that the plan is being implemented
	Revise strategies based on changes in condition or response to intervention
Critical thinking	Setting goals and prioritizing
	Defining the nutrition prescription or basic plan
	Making interdisciplinary connections
	Matching intervention strategies with patient/client needs, nutrition diagnoses, and values
	Choosing from among alternatives to determine a course of action
	Specifying the time and frequency of care
Determination for continuation of care	If a patient/client has met intervention goals or is not at this time able/ready to make needed changes, the food and nutrition professional may discharge the client from this episode of care as part of the planned intervention
Step 4. Nutrition monitoring and evaluation	
Definition and purpose	Nutrition monitoring and evaluation identifies the amount of progress made and whether goals/expected outcomes are being met. Nutrition monitoring and evaluation identifies outcomes relevant to the nutrition diagnosis and intervention plans and goals
Data sources/tools for monitoring and evaluation	Self-monitoring data or data from other records including forms, spreadsheets, and computer programs
	Anthropometric measurements, biochemical data, medical tests, and procedures
	Patient/client surveys, pretests, posttests, and/or questionnaires
	Mail or telephone follow-up
Types of outcomes measured	Nutrition-related history
	Anthropometric measurements
	Biochemical data, medical tests, and procedures
	Nutrition-focused physical findings

Nutrition monitoring and evaluation components	This step includes three distinct and interrelated processes:
	1. Monitor progress:
	Check patient/client understanding and compliance with plan
	Determine whether the intervention is being implemented as prescribed
	Provide evidence that the plan/intervention strategy is or is not changing patient/client behavior or status; identify other positive or negative outcomes
	Gather information indicating reasons for lack of progress
	Support conclusions with evidence
	2. Measure outcomes:
	Select outcome indicators that are relevant to the nutrition diagnosis or signs or symptoms, nutrition goals, medical diagnosis, and outcomes and quality management goals
	3. Evaluate outcomes:
	Compare current findings with previous status, intervention goals, and/or reference standards
Critical thinking	Selecting appropriate indicators/measures
	Using appropriate reference standard for comparison
	Defining where patient/client is in terms of expected outcomes
	Explaining variance from expected outcomes
	Determining factors that help or hinder progress
Determination for continuation of care	Based on the findings, the food and nutrition professional may actively continue care, or if nutrition care is complete or no further change is expected, discharge the patient/client. If nutrition care is to be continued, reassessment may result in refinements to the diagnosis and intervention. If care does not continue, a patient/client may still be monitored for a change in status and reentry to nutrition care at a later date

Appendix G. Dietary Reference Intakes (DRIs)

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G.1 Estimated Average Requirements (Reprinted with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C.)

Life stage group	Calcium (mg/day)	CHO (g/day)	Protein (g/kg/day)	Vit A (µg/day)[a]	Vit C (mg/day)	Vit D (µg/day)	Vit E mg/day)[b]	Thiamin (mg/day)	Ribo-flavin (mg/day)	Niacin (mg/day)[c]	Vit B_6 (mg/day)	Folate (µg/day)[d]	Vit B_{12} (µg/day)	Copper (µg/day)	Iodine (µg/day)	Iron (mg/day)	Magne-sium (mg/day)	Molyb-denum (µg/day)	Phos-phorus (mg/day)	Selenium (µg/day)	Zinc (mg/day)
Infants																					
0–6 months																					
6–12 months			1.0													6.9					2.5
Children																					
1–3 years	500	100	0.87	210	13	10	5	0.4	0.4	5	0.4	120	0.7	260	65	3.0	65	13	380	17	2.5
4–8 years	800	100	0.76	275	22	10	6	0.5	0.5	6	0.5	160	1.0	340	65	4.1	110	17	405	23	4.0
Males																					
9–13 years	1,100	100	0.76	445	39	10	9	0.7	0.8	9	0.8	250	1.5	540	73	5.9	200	26	1,055	35	7.0
14–18 years	1,100	100	0.73	630	63	10	12	1.0		12	1.1	330	2.0	685	95	7.7	340	33	1,055	45	8.5
19–30 years	800	100	0.66	625	75	10	12	1.0		12	1.1	320	2.0	700	95	6	330	34	580	45	9.4
31–50 years	800	100	0.66	625	75	10	12	1.0		12	1.1	320	2.0	700	95	6	350	34	580	45	9.4
51–70 years	800	100	0.66	625	75	10	12	1.0		12	1.4	320	2.0	700	95	6	350	34	580	45	9.4
>70 years	1,000	100	0.66	625	75	10	12	1.0		12	1.4	320	2.0	700	95	6	350	34	580	45	9.4
Females																					
9–13 years	1,100	100	0.76	420	39	10	9	0.7	0.8	9	0.8	250	1.5	540	73	5.7	200	26	1,055	35	7.0
14–18 years	1,100	100	0.71	485	56	10	12	0.9	0.9	11	1.0	330	2.0	685	95	7.9	300	33	1,055	45	7.3
19–30 years	800	100	0.66	500	60	10	12	0.9	0.9	11	1.1	320	2.0	700	95	8.1	255	34	580	45	6.8
31–50 y	800	100	0.66	500	60	10	12	0.9	0.9	11	1.1	320	2.0	700	95	8.1	265	34	580	45	6.8
51–70 years	1,000	100	0.66	500	60	10	12	0.9	0.9	11	1.3	320	2.0	700	95	5	265	34	580	45	6.8
>70 years	1,000	100	0.66	500	60	10	12	0.9	0.9	11	1.3	320	2.0	700	95	5	265	34	580	45	6.8

Pregnancy																					
14–18 years	1,000	135	0.88	530	66	10	12	1.2	1.2	14	1.6	520	2.2	785	160	23	335	40	1,055	49	10.5
19–30 years	800	135	0.88	550	70	10	12	1.2	1.2	14	1.6	520	2.2	800	160	22	290	40	580	49	9.5
31–50 years	800	135	0.88	550	70	10	12	1.2	1.2	14	1.6	520	2.2	800	160	22	300	40	580	49	9.5
Lactation																					
14–18 years	1,000	160	1.05	885	96	10	16	1.2	1.3	13	1.7	450	2.4	985	209	7	300	35	1,055	59	10.9
19–30 years	800	160	1.05	900	100	10	16	1.2	1.3	13	1.7	450	2.4	1,000	209	6.5	255	36	580	59	10.4
31–50 years	800	160	1.05	900	100	10	16	1.2	1.3	13	1.7	450	2.4	1,000	209	6.5	265	36	580	59	10.4

Sources: Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002/2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu

An Estimated Average Requirement (EAR) is the average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group. EARs have not been established for vitamin K, pantothenic acid, biotin, choline, chromium, fluoride, manganese, or other nutrients not yet evaluated via the DRI process

[a]As retinol activity equivalents (RAEs). 1 RAE = 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene, or 24 μg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is twofold greater than retinol equivalents (RE), whereas the RAE for preformed vitamin A is the same as RE

[b]As α-tocopherol. α-Tocopherol includes *RRR-α-tocopherol*, the only form of α-tocopherol that occurs naturally in foods, and the *2R*-stereoisomeric forms of α-tocopherol (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) that occur in fortified foods and supplements. It does not include the *2S*-stereoisomeric forms of α-tocopherol (*SRR*-, *SSR*-, *SRS*-, and *SSS*-α-tocopherol), also found in fortified foods and supplements

[c]As niacin equivalents (NE). 1 mg of niacin = 60 mg of tryptophan

[d]As dietary folate equivalents (DFE). 1 DFE = 1 μg food folate = 0.6 μg of folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach

G.2 Recommended Dietary Allowances and Adequate Intakes, Vitamins (Reprinted with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C.)

Life stage group	Vitamin A (µg/day)[a]	Vitamin C (mg/day)	Vitamin D (µg/day)[b,c]	Vitamin E (mg/day)[d]	Vitamin K (µg/day)	Thiamin (mg/day)	Riboflavin (mg/day)	Niacin (mg/day)[e]	Vitamin B_6 (mg/day)	Folate (µg/day)[f]	Vitamin B_{12} (µg/day)	Pantothenic acid (mg/day)	Biotin (µg/day)	Choline (mg/day)[g]
Infants														
0–6 months	400*	40*	10	4*	2.0*	0.2*	0.3*	2*	0.1*	65*	0.4*	1.7*	5*	125*
6–12 months	500*	50*	10	5*	2.5*	0.3*	0.4*	4*	0.3*	80*	0.5*	1.8*	6*	150*
Children														
1–3 years	**300**	**15**	**15**	**6**	30*	**0.5**	**0.5**	**6**	**0.5**	**150**	**0.9**	2*	8*	200*
4–8 years	**400**	**25**	**15**	**7**	55*	**0.6**	**0.6**	**8**	**0.6**	**200**	**1.2**	3*	12*	250*
Males														
9–13 years	**600**	**45**	**15**	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 years	**900**	**75**	**15**	**15**	75*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	25*	550*
19–30 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
31–50 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
51–70 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4h**	5*	30*	550*
>70 years	**900**	**90**	**20**	**15**	120*	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4h**	5*	30*	550*
Females														
9–13 years	**600**	**45**	**15**	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 years	**700**	**65**	**15**	**15**	75*	**1.0**	**1.0**	**14**	**1.2**	**400i**	**2.4**	5*	25*	400*
19–30 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400i**	**2.4**	5*	30*	425*
31–50 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400i**	**2.4**	5*	30*	425*
51–70 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4h**	5*	30*	425*
>70 years	**700**	**75**	**20**	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4h**	5*	30*	425*
Pregnancy														
14–18 years	**750**	**80**	**15**	**15**	75*	**1.4**	**1.4**	**18**	**1.9**	**600j**	**2.6**	6*	30*	450*
19–30 years	**770**	**85**	**15**	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600j**	**2.6**	6*	30*	450*
31–50 years	**770**	**85**	**15**	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600j**	**2.6**	6*	30*	450*

Lactation														
14–18 years	**1,200**	**115**	**15**	**19**	75*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
19–30 years	**1,300**	**120**	**15**	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
31–50 years	**1,300**	**120**	**15**	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*

Sources: Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu

This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98 %) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevents being able to specify with confidence the percentage of individuals covered by this intake

[a]As retinol activity equivalents (RAEs). 1 RAE = 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene, or 24 μg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is twofold greater than retinol equivalents (RE), whereas the RAE for preformed vitamin A is the same as RE

[b]As cholecalciferol. 1 μg cholecalciferol = 40 IU vitamin D

[c]Under the assumption of minimal sunlight

[d]As α-tocopherol. α-Tocopherol includes *RRR-α-tocopherol*, the only form of α-tocopherol that occurs naturally in foods, and the *2R*-stereoisomeric forms of α-tocopherol (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) that occur in fortified foods and supplements. It does not include the *2S*-stereoisomeric forms of α-tocopherol (*SRR*-, *SSR*-, *SRS*-, and *SSS*-α-tocopherol), also found in fortified foods and supplements

[e]As niacin equivalents (NE). 1 mg of niacin = 60 mg of tryptophan; 0–6 months = preformed niacin (not NE)

[f]As dietary folate equivalents (DFE). 1 DFE = 1 μg food folate = 0.6 μg of folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach

[g]Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages

[h]Because 10–30 % of older people may malabsorb food-bound B_{12}, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with B_{12} or a supplement containing B_{12}

[i]In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 μg from supplements or fortified foods in addition to intake of food folate from a varied diet

[J]It is assumed that women will continue consuming 400 μg from supplements or fortified food until their pregnancy is confirmed and they enter prenatal care, which ordinarily occurs after the end of the periconceptional period – the critical time for formation of the neural tube

G.3 Recommended Dietary Allowances and Adequate Intakes, Elements (Reprinted with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C.)

Life stage group	Calcium (mg/day)	Chromium (µg/day)	Copper (µg/day)	Fluoride (mg/day)	Iodine (µg/day)	Iron (mg/day)	Magnesium (mg/day)	Manganese (mg/day)	Molybdenum (µg/day)	Phosphorus (mg/day)	Selenium (µg/day)	Zinc (mg/day)	Potassium (g/day)	Sodium (g/day)	Chloride (g/day)
Infants															
0–6 months	200*	0.2*	200*	0.01*	110*	0.27*	30*	0.003*	2*	100*	15*	2*	0.4*	0.12*	0.18*
6–12 months	260*	5.5*	220*	0.5*	130*	11	75*	0.6*	3*	275*	20*	3	0.7*	0.37*	0.57*
Children															
1–3 years	**700**	11*	**340**	0.7*	**90**	**7**	**80**	1.2*	**17**	**460**	**20**	**3**	3.0*	1.0*	1.5*
4–8 years	**1,000**	15*	**440**	1*	**90**	**10**	**130**	1.5*	**22**	**500**	**30**	**5**	3.8*	1.2*	1.9*
Males															
9–13 years	**1,300**	25*	**700**	2*	**120**	**8**	**240**	1.9*	**34**	**1,250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 years	**1,300**	35*	**890**	3*	**150**	**11**	**410**	2.2*	**43**	**1,250**	**55**	**11**	4.7*	1.5*	2.3*
19–30 years	**1,000**	35*	**900**	4*	**150**	**8**	**400**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
31–50 years	**1,000**	35*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
51–70 years	**1,000**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.3*	2.0*
>70 years	**1,200**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.2*	1.8*
Females															
9–13 years	**1,300**	21*	**700**	2*	**120**	**8**	**240**	1.6*	**34**	**1,250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 years	**1,300**	24*	**890**	3*	**150**	**15**	**360**	1.6*	**43**	**1,250**	**55**	**9**	4.7*	1.5*	2.3*
19–30 years	**1,000**	25*	**900**	3*	**150**	**18**	**310**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
31–50 years	**1,000**	25*	**900**	3*	**150**	**18**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
51–70 years	**1,200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.3*	2.0*
>70 years	**1,200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.2*	1.8*

Pregnancy															
14–1 years	**1,300**	29*	**1,000**	3*	**220**	**27**	**400**	2.0*	**50**	**1,250**	**60**	**12**	4.7*	1.5*	2.3*
19–30 years	**1,000**	30*	**1,000**	3*	**220**	**27**	**350**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
31–50 years	**1,000**	30*	**1,000**	3*	**220**	**27**	**360**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
Lactation															
14–18 years	**1,300**	44*	**1,300**	3*	**290**	**10**	**360**	2.6*	**50**	**1,250**	**70**	**13**	5.1*	1.5*	2.3*
19–30 years	**1,000**	45*	**1,300**	3*	**290**	**9**	**310**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*
31–50 years	**1,000**	45*	**1,300**	3*	**290**	**9**	**320**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*

Sources: Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); and Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu

This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98 %) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevents being able to specify with confidence the percentage of individuals covered by this intake

G.4 Recommended Dietary Allowances and Adequate Intakes, Total Water and Macronutrients (Reprinted with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C.)

Life stage group	Total water[a] (L/day)	Carbohydrate (g/day)	Total fiber (g/day)	Fat (g/day)	Linoleic acid (g/day)	α-Linolenic acid (g/day)	Protein[b] (g/day)
Infants							
0–6 months	0.7*	60*	ND	31*	4.4*	0.5*	9.1*
6–12 months	0.8*	95*	ND	30*	4.6*	0.5*	**11.0**
Children							
1–3 years	1.3*	**130**	19*	ND[c]	7*	0.7*	**13**
4–8 years	1.7*	**130**	25*	ND	10*	0.9*	**19**
Males							
9–13 years	2.4*	**130**	31*	ND	12*	1.2*	**34**
14–18 years	3.3*	**130**	38*	ND	16*	1.6*	**52**
19–30 years	3.7*	**130**	38*	ND	17*	1.6*	**56**
31–50 years	3.7*	**130**	38*	ND	17*	1.6*	**56**
51–70 years	3.7*	**130**	30*	ND	14*	1.6*	**56**
>70 years	3.7*	**130**	30*	ND	14*	1.6*	**56**
Females							
9–13 years	2.1*	**130**	26*	ND	10*	1.0*	**34**
14–18 years	2.3*	**130**	26*	ND	11*	1.1*	**46**
19–30 years	2.7*	**130**	25*	ND	12*	1.1*	**46**
31–50 years	2.7*	**130**	25*	ND	12*	1.1*	**46**
51–70 years	2.7*	**130**	21*	ND	11*	1.1*	**46**
>70 years	2.7*	**130**	21*	ND	11*	1.1*	**46**
Pregnancy							
14–18 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
19–30 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
31–50 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
Lactation							
14–18	3.8*	**210**	29*	ND	13*	1.3*	**71**
19–30 years	3.8*	**210**	29*	ND	13*	1.3*	**71**
31–50 years	3.8*	**210**	29*	ND	13*	1.3*	**71**

Source: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002/2005) and Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005). The report may be accessed via www.nap.edu

This table (take from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDA) in **bold type** and Adequate Intakes (AI) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98 %) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevents being able to specify with confidence the percentage of individuals covered by this intake

[a]*Total* water includes all water contained in food, beverages, and drinking water

[b]Based on g protein per kg of body weight for the reference body weight, e.g., for adults 0.8 g/kg body weight for the reference body weight

[c]Not determined

G.5 Acceptable Macronutrient Distribution Ranges (Reprinted with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C.)

Macronutrient	Range (percent of energy)		
	Children, 1–3 years	Children, 4–18 years	Adults
Fat	30–40	25–35	20–35
n-6 Polyunsaturated fatty acids[a] (linoleic acid)	5–10	5–10	5–10
n-3 Polyunsaturated fatty acids[a] (α–linolenic acid)	0.6–1.2	0.6–1.2	0.6–1.2
Carbohydrate	45–65	45–65	45–65
Protein	5–20	10–30	10–35

Source: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002/2005). The report may be accessed via www.nap.edu

[a]Approximately 10 % of the total can come from longer-chain *n-3* or *n-6* fatty acids

G.6 Acceptable Macronutrient Distribution Ranges (Reprinted with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C.)

Macronutrient	Recommendation
Dietary cholesterol	As low as possible while consuming a nutritionally adequate diet
Trans fatty acids	As low as possible while consuming a nutritionally adequate diet
Saturated fatty acids	As low as possible while consuming a nutritionally adequate diet
Added sugars[a]	Limit to no more than 25 % of total energy

Source: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002/2005). The report may be accessed via www.nap.edu

[a]Not a recommended intake. A daily intake of added sugars that individuals should aim for to achieve a healthful diet was not set

G.7 Tolerable Upper Intake Levels, Vitamins (Reprinted with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C.)

Life stage group	Vitamin A (μg/day)[a]	Vitamin C (mg/day)	Vitamin D (μg/day)	Vitamin E (mg/day)[b,c]	Vitamin K	Thiamin	Riboflavin	Niacin (mg/day)[c]	Vitamin B_6 (mg/day)	Folate (μg/day)[c]	Vitamin B_{12}	Pantothenic acid	Biotin	Choline (g/day)	Carotenoids[d]
Infants															
0–6 months	600	ND[e]	25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6–12 months	600	ND	38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Children															
1–3 years	600	400	63	200	ND	ND	ND	10	30	300	ND	ND	ND	1.0	ND
4–8 years	900	650	75	300	ND	ND	ND	15	40	400	ND	ND	ND	1.0	ND
Males															
9–13 years	1,700	1,200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14–18 years	2,800	1,800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
31–50 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
51–70 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
>70 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
Females															
9–13 years	1,700	1,200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14–18 years	2,800	1,800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
31–50 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
51–70 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
>70 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND

Pregnancy															
14–18 years	2,800	1,800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
31–50 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
Lactation															
14–18 years	2,800	1,800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
31–50 years	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND

Sources: Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu

A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient

[a]As preformed vitamin A only

[b]As α-tocopherol; applies to any form of supplemental α-tocopherol

[c]The ULs for vitamin E, niacin, and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two

[d]β-Carotene supplements are advised only to serve as a provitamin A source for individuals at risk of vitamin A deficiency

[e]*ND* not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only, to prevent high levels of intake

G.8 Tolerable Upper Intake Levels, Elements (Reprinted with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C.)

Life stage group	Arsenic[a]	Boron (mg/day)	Calcium (mg/day)	Chromium	Copper (µg/day)	Fluoride (mg/day)	Iodine (µg/day)	Iron (mg/day)	Magnesium (mg/day)[b]	Manganese (mg/day)	Molybdenum (µg/day)	Nickel (mg/day)	Phosphorus (g/day)	Selenium (µg/day)	Silicon[c]	Vanadium (mg/day)[d]	Zinc (mg/day)	Sodium (g/day)	Chloride (g/day)
Infants																			
0–6 months	ND[e]	ND	1,000	ND	ND	0.7	ND	40	ND	ND	ND	ND	ND	45	ND	ND	4	ND	ND
6–12 months	ND	ND	1,500	ND	ND	0.9	ND	40	ND	ND	ND	ND	ND	60	ND	ND	5	ND	ND
Children																			
1–3 years	ND	3	2,500	ND	1,000	1.3	200	40	65	2	300	0.2	3	90	ND	ND	7	1.5	2.3
4–8 years	ND	6	2,500	ND	3,000	2.2	300	40	110	3	600	0.3	3	150	ND	ND	12	1.9	2.9
Males																			
9–13 years	ND	11	3,000	ND	5,000	10	600	40	350	6	1,100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 years	ND	17	3,000	ND	8,000	10	900	45	350	9	1,700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 years	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 years	ND	20	2,000	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
>70 years	ND	20	2,000	ND	10,000	10	1,100	45	350	11	2,000	1.0	3	400	ND	1.8	40	2.3	3.6
Females																			
9–13 years	ND	11	3,000	ND	5,000	10	600	40	350	6	1,100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 years	ND	17	3,000	ND	8,000	10	900	45	350	9	1,700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 years	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 years	ND	20	2,000	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
>70 years	ND	20	2,000	ND	10,000	10	1,100	45	350	11	2,000	1.0	3	400	ND	1.8	40	2.3	3.6
Pregnancy																			
14–18 years	ND	17	3,000	ND	8,000	10	900	45	350	9	1,700	1.0	3.5	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	3.5	400	ND	ND	40	2.3	3.6
61–50 years	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	3.5	400	ND	ND	40	2.3	3.6

Life stage group	Arsenic[a]	Boron (mg/day)	Calcium (mg/day)	Chromium	Copper (µg/day)	Fluoride (mg/day)	Iodine (µg/day)	Iron (mg/day)	Magnesium (mg/day)[b]	Manganese (mg/day)	Molybdenum (µg/day)	Nickel (mg/day)	Phosphorus (g/day)	Selenium (µg/day)	Silicon[c]	Vanadium (mg/day)[d]	Zinc (mg/day)	Sodium (g/day)	Chloride (g/day)
Lactation																			
14–18 years	ND	17	3,000	ND	8,000	10	900	45	350	9	1,700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	ND	40	2.3	3.6
31–50 years	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	ND	40	2.3	3.6

Sources: Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu

A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient

[a]Although the UL was not determined for arsenic, there is no justification for adding arsenic to food or supplements

[b]The ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water

[c]Although silicon has not been shown to cause adverse effects in humans, there is no justification for adding silicon to supplements

[d]Although vanadium in food has not been shown to cause adverse effects in humans, there is no justification for adding vanadium to food, and vanadium supplements should be used with caution. The UL is based on adverse effects in laboratory animals, and this data could be used to set a UL for adults but not children and adolescents

[e]*ND* not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake

Appendix H. Maintenance Fluid Requirements

The Holliday-Segar nomogram is a common method used in approximating water loss and calculating the fluid requirement [28].

Box 1: Fluid Requirement Calculation

1. 100 mL / kg for the first 10 kg of weight +
2. 50 mL / kg for the second 10 kg of weight +
3. 20 mL / kg for remaining kg of weight =
4. Total daily fluid requirement

Example: A child weighing 25 kg would require:

1. 100 mL × first 10 kg = 1000 mL +
2. 50 mL × second 10 kg = 500 mL +
3. 20 mL × remaining 5 kg = 100 mL =
4. Total fluid requirement of 1,600 mL/day

Fluid requirements are typically thought of on a 24-h basis, while administration is based on an hourly infusion rate via the delivery pump. To approximate the hourly rate, the "4-2-1" formula can be used [29].

Box 2: Hourly Maintenance Fluid Infusion Calculation (Using 4-2-1 Formula)

1. 4 mL / kg / h for the first 10 kg +
2. 2 mL / kg / h for the second 10 kg +
3. 1 mL / kg / h for the remaining kg =
4. Total hourly infusion rate

Example: For a 25-kg child, the maintenance fluid rate would be:

1. 4 mL / kg / h × first 10 kg = 40 mL / h +
2. 2 mL / kg / h × second 10 kg = 20 mL / h +
3. 1 mL / kg / h × remaining 5 kg = 5 mL / h =
4. Total hourly infusion = 65 mL / h = 1560 mL

L.E. Bernstein et al. (eds.), *Nutrition Management of Inherited Metabolic Diseases: Lessons from Metabolic University*, DOI 10.1007/978-3-319-14621-8,

Appendix I. Interpreting Quantitative Fatty Acid Profiles

Example fatty acid profile			
Compound	**Reference**	**Patient**	
C 8:0	8–47	43	
C10:1	1.8–5.0	7.8	H
C10:0	2–18	70	H
C12:1	1.4–6.6	6	
C12:0	6–90	22	
C14:2	0.8–5.0	6.8	H
C14:1	3–64	30	
C14:0 Myristic Acid	**30–450**	**98**	
C16:2	10–48	18	
C16:1n-9	25–105	86	
C16:1n-7	110–1130	269	
C16:0 Palmitic Acid	**1480–3730**	**1426**	**L**
C18:3n-6	16–150	18	
C18:3n-3 Linolenic Acid	**50–130**	**37**	**L**
C18:2n-6 Linoleic Acid	**2270–3850**	**1207**	**L**
C18:1n-9	650–3500	872	
C18:1n-7	280–740	207	L
C18:0 Stearic Acid	**590–1170**	**648**	
C20:5n-3 Eicosapentaenoic acid (EPA)	**14–100**	**31**	
C20:4n-6 Arachidonic Acid	**520–1490**	**316**	**L**
C20:3n-9	7–30	7	
C20:3n-6	50–250	43	L
C20:0 Arachidic acid	**50–90**	**46**	**L**
C22:6n-3 Docosahexaenoic acid (DHA)	**50–250**	**29**	**L**
C22:5n-6	10–70	13	
C22:5n-3	20–210	38	
C22:4n-6	10–80	11	
C22:1	4–13	5	
C22:0	0.0–96.3	36.5	
C24:1n-9	60–100	82	
C24:0	0.0–91.4	38.8	
C26:1	0.3–0.7	1	
C26:0	0.00–1.30	0.78	
C19:B	0.00–2.98	0.04	
C20:B	0.00–9.88	0.5	
HOLMAN RATIO	0.010–0.038	0.022151899	

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1. Evaluate n-6 status:
   (a) Low linoleic acid
   (b) Low arachidonic acid
   (c) Normal Holman ratio
   - The patient is consuming a low fat diet so the Holman ratio is normal because both n-6 and n-9 are low. The Holman ratio may or may not indicate an essential fatty acid deficiency in metabolic patients.

Compound	Reference	Patient	Low
C18:2n-6 Linoleic acid	2270–3850	1207	Yes
C20:4n-6 Arachidonic Acid	520–1490	316	Yes
C22:5n-6 Docosapentaenoic acid n-6	10–70	13	No
Holman Ratio	0.010–0.038	0.022	No

2. Evaluate saturated fatty acids:
   (a) Low saturated fat acids
   -The patient is consuming a low fat diet.

Compound	Reference	Patient	Low
C14:0 Myristic acid	30–450	98	No
C16:0 Palmitic acid	1480–3730	1426	Yes
C18:0 Stearic acid	590–1170	648	No
C20:0 Arachidic acid	50–90	46	Yes

3. Evaluate n-3 fatty acids:
   (a) Low linolenic acid
   (b) Low in DHA
   (c) ARA:DHA ratio is too high (goal is less than 4)
   (d) Low C22:5n-6 is another indicator of n-3 deficiency.

Compound	Reference	Patient	Low
C18:3n-3 Linolenic acid	50–130	37	Yes
C20:5n-3 Eicopentaenoic acid (EPA)	14–100	31	No
C22:6n-3 Docosahexaenoic acid (DHA)	30–250	29	Yes
ARA:DHA	2.1–4.6	10.8	-

Box 3: Nutrition Management Plan

- Increase n-6 fatty acid intake to correct low linoleic and arachidonic acid concentrations.
- Decrease saturated fatty acid intake in order to maintain low dietary fat intake.
- Begin DHA supplements to correct low DHA concentrations.
- Alpha-linoleic acid is low but as it is primarily needed as a precursor to DHA, additional alpha-linoleic acid is not needed as long as DHA is supplemented in the diet

Courtesy of Dr. Melanie Gillingham, PhD, RD
Oregon Health & Science University, Portland, Oregon

I.1 Plasma Fatty Acids

- Free fatty acids are found in plasma in the ionized form. FFA are elevated with fasting and low with feeding.
- Most plasma fatty acids exist as esters in lipoproteins:
  - Triglycerides
  - Phospholipids
- *Fatty acid profiles include both FFA and plasma fatty acids in lipoprotein.*
- Plasma fatty acids are a short-term marker of dietary intake.
- Red Blood Cell (RBC) fatty acids are a long-term marker of dietary intake.
- Adipose fatty acids reflect dietary intake over years.
- *Plasma and RBC fatty acids are similar in people with repetitive diets such as patients with inherited metabolic diseases*.
- Primary circulating fatty acids include:
  - Stearate
  - Palmitate
  - Oleic
  - Palmitoleic acid
  - Linoleic acid
- Arachidonic acid (C20:4n-6) can fall with DHA supplements.
- With regard to the DRI for fatty acids, the Institute of Medicine states, "The linoleic acid: α-linolenic acid ratio is likely most important in diets that are very low or devoid of arachidonic acid" [30].

Shorthand	Abbreviation	Name
C10:0	–	Capric acid
C12:0	–	Lauric acid
C14:0	MA	Myristic acid
C16:0	PA	Palmitic acid
C16:1n7	PO	Palmitoleic acid
C16:1n7t	t-PO	*Trans* palmitoleic acid
C18:0	SA	Stearic acid
C18:1n9	OA	Oleic acid
C18:1n9t	t-OA	*Trans* oleic acid
C18:2n6	LA	Linoleic acid
C18:2n6t	t-LA	*Trans* linoleic acid
C20:0	–	Arachidic acid
C18:3n6	GLA	Gamma linolenic acid
C20:1n9	-	Eicosenoic acid
C18:3n3	ALA	Alpha linolenic acid
C20:2n6	–	Eicosadienoic acid
C20:3n6	–	Eicosatrienoic acid
C22:0	–	Docosanoic acid
C20:4n6	AA	Arachidonic acid
C24:0	–	Lignoceric acid
C20:5n3	EPA	Eicosapentaenoic acid
C24:1n9	–	Nervonic acid
C22:4n6	DTA	Docosatetraenoic acid
C22:5n6	DPAn6	Docosapentaenoic acid n6
C22:5n3	DPAn3	Docosapentaenoic acid n3
C22:6n3	DHA	Docosahexaenoic acid

Appendix J. Glucose Polymer Protocol

J.1 Nutritional Approach During Illness

What is this about?

As part of the management of your metabolic condition, you have been advised to avoid prolonged fasting. This handout provides a practical, *temporary* approach to managing illness to avoid prolonged fasting during illness. Your specific situation should always be discussed with your metabolic dietitian.

What happens during an illness?

Illness is often accompanied by decreased appetite, nausea, and vomiting which can result in decreased intake of food, hence energy. To compensate for this decreased intake and to provide energy to fight off the infection, the body mobilizes its reserves and breaks down some of its own substances such as proteins, fat, and glycogen to provide energy. This process is called *catabolism*. In patients with inborn errors of metabolism, the breakdown of some of these body substances is impaired, resulting in low blood sugar (hypoglycemia) or in the accumulation of intermediary products in blood, which can then result in complications.

How can you counteract catabolism with nutrition?

During times of illness, catabolism can be counteracted by providing sufficient energy from nutrition. This is best done by consuming sugars (carbohydrates) regularly. Sugars are easily digested and a readily available source of energy from nutrition. They are also well tolerated during illness.

Temporary management strategy requires intake of carbohydrate-rich drinks, preferably *a glucose polymer* at regular intervals during times of illness. The concentration, the volume, and the frequency of intake necessary to prevent catabolism are dependent on the weight and the age of the individual. With this handout we will provide you with information regarding ***your/your child's specific needs. The glucose polymer solution does not provide complete nutrition and should only be used for a very short time (max. 1–2 days).

What to do when there is diarrhea?

With diarrhea, a glucose polymer should be added to an appropriate rehydration solution. This will help prevent catabolism as well as dehydration due to loss of electrolytes.

In conclusion:

With insufficient intake it is important to temporarily incorporate carbohydrate-rich drinks in the nutrition plan. Such intervention will help reduce or prevent the need for hospitalization. It is always necessary to *inform your PCP, your metabolic dietitian, and your metabolic physician* during times of illness. They can provide further instructions.

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J.2 Nutrition Protocol with Illness (e.g., Infection): Oral Treatment [31]

Age	Glucose polymer[a] (% solution)	Dose (ml/kg/h)	Fluid requirement (ml/kg/day)	Energy requirement (Cal/kg/day)
0–6 months	15	7.7	183	110
6–12 months	15	7.0	168	100
1–3 years	20	4.5	110	90
3–6 years	25	3.3	80	80
6–12 years	25	2.6	65	65
or 6–12 years	30	2.25	54	65
12–15 years	30	1.8	42	50
>15 years	30	1.6	38	45

[a]Polycal (Nutricia North America, Rockville, MD)

Original source: Dorothy Francis – Metabolic Dietitian – Royal Children's Hospital – Australia

One teaspoon = 5 ml

One tablespoon = 15 ml

One ounce = approximately 30 ml

Four ounces =120 ml or approximately one-half cup

Polycal: A maltodextrin that contains the same number of calories per grams and with similar structure as a glucose polymer

One level teaspoon of Polycal = 2 g of Polycal = 8 cal

One level tablespoon of Polycal = 6 g of Polycal = 23 cal

15% = 15 g CHO** / 100 mL = app.8 tsp / 4 oz

20% = 20 g CHO / 100 mL = app.11 tsp / 4 oz

25% = 25 g CHO / 100 mL = app.4½ tbsp. / 4 oz

30% = 30 g CHO / 100 mL = app.5½ tbsp. / 4 oz

Appendix K. Calculation of Glucose Infusion Rate and Cornstarch Dosing for Patients with Glycogen Storage Disease

The main priority for GSD type 1 is to prevent hypoglycemia and suppress lactic acidosis. This is achieved by calculating glucose requirements using the glucose infusion rate. Glucose infusion rate (GIR) is expressed as mg/kg/min. Glucose can be provided through formula feeding, nocturnal nasogastric drip feeding, or the use of uncooked cornstarch. Nasogastric drip feedings provide a continuous source of glucose, and cornstarch provides a source of glucose that is slowly released and slowly absorbed.

Recommended GIR [32]	
0–12 months	7–9 mg/kg/min
1–3 years	7 mg/kg/min
3–6 years	6–7 mg/kg/min
6–14 years	5–6 mg/kg/min
Adolescents	4–5 mg/kg/min
Adults	3–4 mg/kg/min

Uncooked cornstarch 1 tbsp = 8 g = 7.2 g carbohydrate (CHO)

K.1 Example GIR Calculations

Example 1

Patient weight: 22 kg

Patient age: 5 years

Current cornstarch dose: 36 g at 6:00, 10:00, 14:00 and 18:00

Step 1. $\frac{7.2\ \text{g CHO}}{8\ \text{g cornstarch}} = 0.9\ \text{g CHO per 1 g cornstarch}$

Step 2. $36\ \text{g cornstarch} \times 0.9\ \text{g CHO} = 32.4\ \text{g CHO}$

Step 3. $32.4\ \text{g CHO} \times 1{,}000\ \text{mg} = 32{,}400\ \text{mg CHO}$

Step 4. $\frac{32{,}400\ \text{mg CHO}}{22\ \text{kg}} = 1{,}472.7\ \text{mg CHO per kg}$

Step 5. $4\ \text{h} \times 60\ \text{min per hour} = 240\ \text{min}$

Step 6. $\frac{1{,}472.7\ \text{mg CHO per kg}}{240\ \text{min}} = \mathbf{6.13\ mg/kg/min}$

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Example 2

You have a 12-year-old patient weighing 43 kg and is experiencing low blood sugar with his current cornstarch dosing regimen. He currently takes 48 g of cornstarch every 4 h starting at 10 am. Calculate the current GIR and the cornstarch dose that is appropriate for a correct GIR.

Current Dose

Step 1. $\frac{7.2\ \text{g CHO}}{8\ \text{g cornstarch}} = 0.9\ \text{g CHO per g cornstarch}$

Step 2. $48\ \text{g cornstarch} \times 0.9\ \text{g CHO per g cornstarch} = 43.2\ \text{g CHO}$

Step 3. $43.2\ \text{g CHO} \times 1{,}000\ \text{mg / g} = 43{,}200\ \text{mg CHO}$

Step 4. $\frac{43{,}200\ \text{mg CHO}}{43\ \text{kg}} = 1{,}004.6\ \text{mg CHO / kg}$

Step 5. $4\ \text{h} \times 60\ \text{min/ h} = 240\ \text{min}$

Step 6. $\frac{1{,}004.6\ \text{mg CHO / kg}}{240\ \text{min}} = \mathbf{4.18\ mg / kg / min}$

New Dose

Step 1. *5.5 mg/kg/min*

(this GIR is provided to you by the metabolic physician, the recommended GIR for 6–14-year-olds is 5–6 mg/kg/min)

Step 2. $5.5\ \text{mg CHO / kg / min} \times 240\ \text{min} = 1{,}320\ \text{mg CHO / kg}$

Step 3. $1{,}320\ \text{mg CHO / kg} \times 43\ \text{kg} = 56{,}760\ \text{mg CHO}$

Step 4. $56{,}760\ \text{mg CHO} \div 1{,}000\ \text{mg} = 56.76\ \text{g CHO}$

Step 5. $56.76\ \text{g CHO} \div 0.9\ \text{g CHO per 1 g cornstarch} = \mathbf{63\ g\ cornstarch}$

Appendix L. Guide to Counting Carbohydrate for Patients with Glycogen Storage Disease

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Food	Serving	Wt in grams	CHO in grams	Grams CHO/g food	Grams food for 1 g CHO	Grams food for 2 g CHO	Grams food for 5 g CHO	Grams food for 7 g CHO	Grams food for 10 g CHO	Grams food for 15 g CHO	Grams food for 20 g CHO
Almonds-dry roasted		28	6.9	0.2	4	8	20	28	41	61	81
Bacon Bits Imitation		25	7.2	0.3	3	7	17	24	35	52	70
Baked Beans		253	50.6	0.2	5	10	25	35	50	75	100
Banana		114	26.7	0.2	4	9	21	30	43	64	85
Cashews-dry roasted		28	9.3	0.3	3	6	15	21	30	45	60
Chicken Mc Nuggets		113	16.5	0.1	7	14	34	48	68	103	137
Chicken Popcorn Chicken (KFC)	1 small order	99	21.0	0.2	5	9	24	33	47	71	94
Corn canned	1/2 cup	82	15.2	0.2	5	11	27	38	54	81	108
Corn Chips	1 oz	28	16.6	0.6	2	3	8	12	17	25	34
Corn Syrup – dark		20	15.3	0.8	1	3	7	9	13	20	26
Corn Syrup – light		20	15.3	0.8	1	3	7	9	13	20	26
Crackers Chicken in a Biskit	14 each	30	17.0	0.6	2	4	9	12	18	27	35
Crackers Parmesan Cheeze-its	25 each	30	19.0	0.6	2	3	8	11	16	24	32
Crackers Parmesan Goldfish	55 each	30	19.0	0.6	2	3	8	11	16	24	32
Crackers Triscuit	7 each	31	21.0	0.7	1	3	7	10	15	22	30
Crackers Wheat Thin	16 each	29	19.1	0.7	2	3	8	11	15	23	30
Dill Pickle Spears		65	2.7	0.0	24	48	120	169	241	361	481
Dressing Kraft Buttermilk Ranch	1 Tbsp	15	0.5	0.0	30	60	150	210	300	450	600
Flour All Purpose White		125	95.4	0.8	1	3	7	9	13	20	26
French Fries – Restaurant		50	20.0	0.4	3	5	13	18	25	38	50
French Fries -Frozen		85	22.5	0.3	4	8	19	26	38	57	76
FunYuns		25	15.9	0.6	2	3	8	11	16	24	32
Green Beans -boiled		62	4.9	0.1	13	25	63	89	127	190	253
Green Beans -canned		68	3.1	0.0	22	44	110	154	219	329	439
Macaroni – Cooked		140	39.7	0.3	4	7	18	25	35	53	71
Olives Black		25	1.6	0.1	16	32	80	112	160	240	320
Olives Green		46	0.5	0.0	92	184	460	644	920	1380	1840
Peanuts-dry roasted		28	6.0	0.2	5	9	23	33	47	70	93
Potato Boiled		135	27.0	0.2	5	10	25	35	50	75	100
Pretzels – Rold Gold		28	15.1	0.5	2	4	9	13	19	28	37
Rice Dream Frozen Dessert		92	23.0	0.3	4	8	20	28	40	60	80

Food	Serving	Wt in grams	CHO in grams	Grams CHO/g food	Grams food for 1 g CHO	Grams food for 2 g CHO	Grams food for 5 g CHO	Grams food for 7 g CHO	Grams food for 10 g CHO	Grams food for 15 g CHO	Grams food for 20 g CHO
Rice-a-Roni Fried Rice	1/2 cup	35	25.0	0.7	1	3	7	10	14	21	28
Rice-a-Roni Rice Pilaf	1/2 cup	35	26.0	0.7	1	3	7	9	13	20	27
Smarties		28	25.0	0.9	1	2	6	8	11	17	22
Strawberries		149	10.5	0.1	14	28	71	99	142	213	284
Tortilla Chips Santitas	10 chips	28	20.0	0.7	1	3	7	10	14	21	28
Waffles Eggos Buttermilk	1 each	39	15.5	0.4	3	5	13	18	25	38	50

Food	Grams food for 1 g CHO	Grams food for 2 g CHO	Grams food for 5 g CHO	Grams food for 7 g CHO	Grams food for 10 g CHO	Grams food for 15 g CHO	Grams food for 20 g CHO
Almonds – dry roasted	4	8	20	28	41	61	81
Bacon bits imitation	3	7	17	24	35	52	70
Baked beans	5	10	25	35	50	75	100
Banana	4	9	21	30	43	64	85
Cashews – dry roasted	3	6	15	21	30	45	60
Chicken McNuggets	7	14	34	48	68	103	137
Chicken popcorn chicken (KFC)	5	9	24	33	47	71	94
Corn canned	5	11	27	38	54	81	108
Corn chips	2	3	8	12	17	25	34
Corn syrup – dark	1	3	7	9	13	20	26
Corn syrup – light	1	3	7	9	13	20	26
Crackers – Chicken in a Biskit	2	4	9	12	18	27	35
Crackers – parmesan Cheez-Its	2	3	8	11	16	24	32
Crackers – parmesan Goldfish	2	3	8	11	16	24	32
Crackers – Triscuit	1	3	7	10	15	22	30
Crackers – Wheat Thins	2	3	8	11	15	23	30
Dill pickle spears	24	48	120	169	241	361	481
Dressing – Kraft Buttermilk Ranch	30	60	150	210	300	450	600
Flour all purpose white	1	3	7	9	13	20	26
French fries – restaurant	3	5	13	18	25	38	50

(continued)

Food	Grams food for 1 g CHO	Grams food for 2 g CHO	Grams food for 5 g CHO	Grams food for 7 g CHO	Grams food for 10 g CHO	Grams food for 15 g CHO	Grams food for 20 g CHO
French fries – frozen	4	8	19	26	38	57	76
Funyuns	2	3	8	11	16	24	32
Green beans – boiled	13	25	63	89	127	190	253
Green beans – canned	22	44	110	154	219	329	439
Macaroni – cooked	4	7	18	25	35	53	71
Olives – black	16	32	80	112	160	240	320
Olives – green	92	184	460	644	920	1,380	1,840
Peanuts – dry roasted	5	9	23	33	47	70	93
Potato boiled	5	10	25	35	50	75	100
Pretzels – Rold Gold	2	4	9	13	19	28	37
Rice Dream frozen dessert	4	8	20	28	40	60	80
Rice-a-Roni fried rice	1	3	7	10	14	21	28
Rice-a-Roni rice pilaf	1	3	7	9	13	20	27
Smarties	1	2	6	8	11	17	22
Strawberries	14	28	71	99	142	213	284
Tortilla chips – Santitas	1	3	7	10	14	21	28
Waffles Eggos buttermilk	3	5	13	18	25	38	50

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