4 Altered Metabolome of Amino Acids Species: A Source of Signature Early Biomarkers of T2DM

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Abstract

Diabetes mellitus is a chronic metabolic disease with serious health consequences for a modern civilization that often lead to premature death. With the rapid increase in the number of people diagnosed with type 2 diabetes, early identification of those individuals at higher risk of progression to diabetes is a key criterion enabling the timely intervention or treatment. In recent years, omics-based technologies have given us unprecedented insight into circulating biomarkers in common diseases. Branched-chain amino acids: valine, leucine, isoleucine, and aromatic amino acids, that is, tyrosine and phenylalanine, have been demonstrated as the most consistent metabolite biomarkers for diabetes, in particular type 2.

Therefore, amino acids quantification in biological material, primarily in plasma could be a valuable prognostic tool for determining metabolic abnormalities leading to this disease. Revealing these interactions and possible mechanisms may prove beneficial for the prediction and treatment.

Keywords

Amino acids · Branched-chain amino acids · Insulin resistance · Prediabetic state · Diabetes · Type 2 diabetes mellitus · Metabolic disease · Biomarker · Metabolomics

Abbreviations

2-AAA	2-aminoadipic acid
2-h PG	2-h plasma glucose test
3-HIB	3-hydroxyisobutyrate
AAAs	Aromatic amino acids
AAs	Amino acids
AILS	AminoIndex LifeStyle diseases test
AKT	Protein kinase B, PKB
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
BAIBA	β-aminoisobutyric acid
BCAAs	Branched-chain amino acids
BCKA	Branched-chain α-ketoacid
BCKDC	Branched-chain α-ketoacid dehydrogenase complex

BCKDH	Branched-chain α-ketoacid dehydrogenase
BHBA	3-hydroxybutyrate
BMI	Body Mass Index
Cit	Citrulline
CoA	Coenzyme A
CVD	Cardiovascular disease
Cys	Cysteine
DAG	Diacylglycerol
DM	Diabetes mellitus
FAAs	Free amino acids
FFAs	Free fatty acids
FGF21	Fibroblast growth factor 21
FHS	Framingham Heart Study
FOXO	Forkhead Box O transcription factor
FPG	Fasting plasma glucose
GC	Gas chromatography
GDM	Gestational Diabetes mellitus
GDR	Glucose disposal rate
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
GSK-3	Glycogen synthase kinase-3
HbA1c	Glycated hemoglobin
HECP	Hperinsulinemic-euglycemic clamp procedure
His	Histidine
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IDF	International Diabetes Federation
IGF	Insulin-like growth factor
IGT	Impaired glucose tolerance
Ile	Isoleucine
Ins120 min	2-h post-challenge insulin
IR	Insulin Resistance
IRAS	Insulin Resistance Atherosclerosis Study
IRS-1	Insulin receptor substrate 1
JNK	c-Jun N-terminal kinase
LC	Liquid chromatography
Leu	Leucine
L-GPC	linoleoyl- glycerophosphocholine
LPC	Lysophosphatidylcholine
MDC	Malmö Diet and Cancer Study
MetS	Metabolic syndrome
METSIM	Metabolic Syndrome in Men Study
mmBCFA	Monomethyl branched-chain fatty acids
MS	Mass spectrometry
mTORC1	Mammalian target of rapamycin complex 1

NEFAs	Non-esterified fatty acids
NGT	Normal glucose tolerance
NMR	Nuclear magnetic resonance
OGTT	Oral glucose tolerance test
Orn	Ornithine
PCs	Phosphatidylcholines
PFAAs	Plasma-free amino acids
Phe	Phenylalanine
PPARα	Peroxisome proliferator-activated receptor α
Pro	Proline
QMDiab	Qatar Metabolomics Study on Diabetes
RISC	Relationship of Insulin Sensitivity to Cardiovascular Risk study
ROS	Reactive oxygen species
RQ	Resting respiratory quotient
SABRE	Southall And Brent REvisited Study
Ser	Serine
T1DM	Type 1 Diabetes mellitus
T2DM	Type 2 Diabetes mellitus
TCA	Tricarboxylic acid cycle
TKR	Tyrosine kinase receptor
Trp	Tryptophan
TüF	Tübingen Family study for T2DM
Tyr	Tyrosine
UCD-T2D	University of California-Davis T2DM rat model
Val	Valine
VFA	Visceral fat area
α-HB	α-hydroxybutyrate
α-KB	α-ketobutyrate

Introduction

Amino acids are defined as organic compounds containing amino($–NH_2$) and carboxyl(–COOH) functional groups along with a side chain (R group) specific to each amino acid (AA). They are referred as the building blocks of proteins needed for animal nutrition and basic units for synthesis of hormones and neurotransmitters (Lopez and Mohiuddin 2021). Human proteins are made up of 20 AAs, 9 of which are considered “essential” because they cannot be synthesized from other metabolites in the human body (White and Newgard 2019). AAs play a key role in many metabolic pathways, and quantification of free amino acids (FAAs) in biological fluids and tissues has historically provided nutritional information used in the diagnosis of various diseases, particularly metabolic impairments (Nagao and Kimura 2020).

During the last decade, many studies have consistently reported the positive association of plasma or serum FAAs with insulin resistance (IR) and diabetes in

individuals from different ethnic groups and with varying degrees of obesity in large prospective and cross-sectional human studies (Newgard et al. 2009; (Giesbertz and Daniel 2016; Chen et al. 2019; Bi and Henry 2017). Most of these publications demonstrated that increased levels of branched-chain amino acids in plasma, serum, and also in urine (Branched-chain amino acids (BCAAs); valine, leucine, isoleucine) are associated with obesity, IR, and diabetes, in particular type 2 diabetes mellitus (T2DM) (Newgard et al. 2009; Shah et al. 2012; McCormack et al. 2013; (Würtz et al. 2013; Bi and Henry 2017; Nie et al., 2018; Yousri et al. 2015).

Hyperaminoacidemia observed in obesity may be related to increased IR. Insulin resistance is believed to reduce the BCAA catabolism by suppressing the enzymatic activity of branched-chain alpha-keto acid dehydrogenase complex (BCKDC), which is considered as the plausible mechanism explaining the increased BCAA levels in obese or diabetic individuals (Bi and Henry 2017). Elevated BCAA levels have often been shown to predict the development of T2DM well in advance of its actual occurrence, what is very interesting from a diagnostics standpoint (Wang et al. 2011; Yamakado et al. 2015; McCormack et al. 2013; Ianni et al. 2017).

In addition, there is a body of evidence suggesting the correlation between IR and changes in aromatic amino acids (AAAs), that is tyrosine and phenylalanine, as well several other AAs (Bi and Henry 2017; Chen et al. 2019). Therefore, AAs quantification in biological material, primarily in plasma, may be a useful indicator of the presence of metabolic abnormalities leading to diabetes. This chapter reviews molecular and clinical associations between altered AA levels and diabetes development and interprets underlying biochemical mechanisms.

Diabetes Mellitus

Diabetes mellitus (DM) is a highly prevalent chronic metabolic disease with major health implications for a modern civilization (Freitas et al. 2017; Gan et al. 2020), and the eighth major mainstay of mortalities around the globe (Hameed et al. 2020). It is characterized by an increase in blood glucose level and inability to utilize glucose in adipose and muscle tissues (Zhao et al. 2017; Al-Abbasi 2012). Among the three main types of DM, i.e.,: type 1 (T1DM); type 2 (T2DM); and gestational (GDM), the T2DM is most commonly diagnosed (about 90% of cases) ("Diagnosis and Classification of Diabetes Mellitus," 2004; Gan et al. 2020).

T2DM is characterized by abnormal glucose and lipid metabolism, resulting from resistance to the effects of insulin and insufficient response to the secretion of this hormone. As the disease progresses, there may also occur a partial β-cell insufficiency and deficiency in insulin production. ("Diagnosis and Classification of Diabetes Mellitus," 2004; Gan et al. 2020; Chen et al. 2019).

According to the International Diabetes Federation (IDF) data, the global diabetes incidence has continuously increased each year, with an estimated 578 million cases by 2030 and 700 million people diagnosed with diabetes by the year 2045. Moreover, in 2019 one in two (50.1%) people living with diabetes were undiagnosed (Saeedi et al. 2019).

Chronic hyperglycemia is a common feature related to all DM subtypes that may lead to long-term damages (Association 2016; Freitas et al. 2017) and several vascular, neurological, immunological, and biochemical pathological changes (Al-Abbasi 2012). As the disease progresses, it is accompanied by multiple complications and dysfunction of various organs (Association 2016). Most commonly occurring is microvascular complications, e.g., diabetic retinopathy, nephropathy, and neuropathy; macrovascular complications, including coronary atherosclerotic heart disease with increased risk of cardiovascular events (Rawshani et al., 2017), and vascular disease of the lower extremities (peripheral artery disease), which is a leading cause of nontraumatic limb amputations (Vamos et al. 2010), as well as diabetic nephropathy, hypertension, and cerebrovascular disease, (Zhao et al. 2017). It is also associated with hypercholesterolemia, obesity, and other nutritional disorders (Al-Abbasi 2012).

Diabetes, particularly type 2, imposes a heavy financial strain on health care systems everywhere and shortens the life expectancy of diabetic patients (*International Diabetes Federation (IDF) (*2015*) Diabetes Atlas. 7th Edition, International Diabetes Federation, Brussels, Belgium. – References – Scientific Research Publishing*, no date), (Association 2016; Freitas et al. 2017; Zhao et al. 2017). The global T2DM prevalence is rising rapidly, particularly among those living in low- and middle-income countries (Ahola-Olli et al. 2019; Gan et al. 2020). T2DM is associated with increased mortality risk and reduced health-related quality of life, causing an immense social costs burden (Ahola-Olli et al. 2019; Chen and Gerszten 2020). The still high global incidence of T2DM and the accompanying increase in the number of its complications require earlier diagnosis and more effective treatment of this disease (Zhao et al. 2017).

The Need for New Biomarkers for Diabetes

Metabolic disorders are often present for years before becoming clinically apparent. In the early stages of T2DM, patients may have difficulties to recognize any symptoms of the disease (Zhao et al. 2017). By the time that relative insulin deficiency manifests as hyperglycemia and a T2DM is diagnosed, significant pancreatic β-cell failure already occurs. Early identification of individuals at higher risk of progression to diabetes allows the timely intervention to delay or prevent diabetes onset (Wang et al. 2011; Ahola-Olli et al. 2019).

Currently, most early screening and diagnostic methods for DM are directly related to glucose levels (Gan et al. 2020), which include fasting plasma glucose (FPG) measurement or 2-h plasma glucose (2-h PG) measurement in 75-g oral glucose tolerance (OGT) test (Association 2018; Gan et al. 2020). Moreover, the glycated hemoglobin (HbA1c) test is also often used to diagnose diabetes, providing an overall picture of average blood glucose levels over a period of past 3 months. In addition, several other clinical and laboratory predictors could be used in gauging diabetic status, such as body mass index and c-peptide measurement (Wang et al. 2011; Al-Abbasi 2012).

Meanwhile, many overweight to moderately obese people are found to have completely normal fasting plasma glucose and hemoglobin A1c levels, making them undiagnosed as prediabetic despite underlying metabolic abnormalities (Bi and Henry 2017). Therefore, there is a great need for biomarkers allowing an early diagnosis of prediabetic or diabetic patients (Wang et al. 2011).

In recent years, omics-based technologies have given us unprecedented insight into circulating biomarkers of common diseases. Application of metabolomics allowed identification of biochemical changes occurring prior to the onset of diabetes and provided additional information about pathophysiological mechanisms leading to DM. Amino acids and other metabolites were proposed as predictive markers indicating early metabolic perturbances. Diagnosis of patients at risk of diabetes onset is crucial to introduce changes in life style and prevention of disease development (Würtz et al. 2013; Guasch-Ferré et al. 2016; Ahola-Olli et al. 2019; Wang et al. 2011).

Amino Acids in Metabolic Signaling and Insulin Resistance

Despite the relevant role of the glucose-related pathways, here we are concentrating mostly on the protein metabolism influence on IR development. Many amino acids, especially BCAAs, are important nutritional signals that possess direct and indirect effects (Lynch and Adams 2014). The BCAA metabolic pathway crosses with the mechanism for IR (George 2017).

Evidence that BCAAs may not only have a "reporter quality" but may also contribute to IR and T2DM comes from cell culture and animal studies that propose sustained activation of complex 1 (mTORC1) (Newgard et al. 2009; Giesbertz and Daniel 2016). Proposed mechanisms (explaining how increased levels of BCAAs might be linked to metabolic disease) involve stimulation of the mTOR/p70S6K pathway and phosphorylation of IRS-1 at multiple serine sites (Nie et al. 2018). In this context, leucine is known to activate the nutrient sensing complex, mTORC1, which results in uncoupling of insulin signaling at an early stage of IR and other metabolic disorders. However, numerous observations indicate that BCAA-mediated mTORC1 activation is not necessary or sufficient to induce IR, and subsequent metabolic dysfunction, (Lynch and Adams 2014; Yoon 2016). Recently, in a rat model of diabetes (University of California-Davis T2DM rat model, UCD-T2D) an untargeted metabolomics study was performed showing that elevated plasma BCAA levels were not observed until 6 months after the onset of diabetes. This rules out the causal role of BCAAs in the occurrence of T2DM in the studied model (Yoon 2016; Biswas et al. 2019).

Zhao et al. (2020) worked with high-fat diet-induced obese (DIO) mice. Supplementation of these animals with BCAAs leads to heavy hepatic metabolic disorders, such as suppressed lipogenesis and increased glucose production. They also found that this impairs hepatic AKT2 signaling. BCAA supplementation stops AKT2 activation through mTorc1- and mTorc2-dependent pathways and promotes AKT2 degradation. As a matter of fact, the signaling pathways are other key elements in the development of IR. AKT is responsible for the transcription factors Forkhead box O (FOXO) activation, regulating the energy metabolism. FOXOs modulate the adipogenesis

process in the adipose tissue, they retain the beta cells function during oxidative stress, avoiding their replication, and they are a candidate as regulators of glucose production in the liver (Gross et al. 2009). Tyrosine kinase receptor (TKR) is an insulin receptor (IR), whose first targets are Insulin Receptor Substrate (IRS) 1, 2, 3, and 4. To better understand the most important targets, scientists knocked down these receptors in mice. While the knockdown of IRS3 or 4 showed no or limited effects on mice, knocked down IRS1 or 2 developed, respectively, IR and T2DM (Kubota et al. 2017).

An alternative mechanism referred to the BCAAs dysmetabolism suggests that deficiencies in BCAA metabolism are linked to IR and T2DM by the accumulation of BCAA levels in plasma, as well possibly toxic intermediates (Yoon 2016). BCAAs inefficient metabolism or incomplete oxidation, especially isoleucine and valine can result in anaplerotic stress and an imbalance between anaplerosis and cataplerosis that might cause suboptimal mitochondrial function in states of T2DM. For example, reduced mitochondrial branched-chain α-ketoacid dehydrogenase (BCKDH) activity results in the accumulation of branched-chain α-ketoacid (BCKA) and α-ketobutyrate (α-KB), which result in the restriction of propionyl-Coenzyme A (CoA)-derived metabolites to tricarboxylic acid (TCA) cycles, inducing anaplerotic stress and decreased amino acid fuel delivery to mitochondria (Fiehn et al. 2010; Adams et al. 2009; Yoon 2016).

Incomplete mitochondrial oxidation is thought to be the major cause for increased BCAA concentrations, independently of established IR. In obese subjects, the adipose tissue is characterized by an accumulation of fat, an increased pro-inflammatory state, and alterations in hormone and cytokine secretion that may strongly affect the mitochondrial function in peripheral tissues (Ianni et al. 2017).

In the adipose tissue of obese and T2DM patients with IR, as well in models of obesity in rodents, the expression of genes encoding BCAAs-metabolizing enzymes is significantly downregulated by an undefined mechanism compared to metabolically healthy controls – leading to elevated plasma BCAA levels (Lackey et al. 2013; Lynch and Adams 2014; Yoon 2016). Indeed, defective BCAA oxidation results in consequent accumulation of branched-chain keto acids and branched-chain fatty acids in peripheral tissues (Ianni et al. 2017). A high level of free fatty acids (FFAs) drives tissues, such as liver and skeletal muscles, toward IR, promoting the accumulation of such bioactive lipids as diacylglycerol (DAG) and ceramide (Zabielski et al. 2019). DAG accumulation impairs insulin signaling in liver (Fig. 1). Impaired insulin signaling in muscle results in increased proteolysis, which contributes to the release of BCAAs into the circulation and further supply of substrates for mitochondrial oxidation (Ianni et al. 2017).

However, the metabolism of BCAAs throughout the body is highly dependent on other organs; the expression of these enzymes in other organs such as the liver and muscle must be considered. Growing experimental evidence has posited that these impairments in the ability to metabolize BCAAs in adipose tissue may extend to other tissues (Lynch and Adams 2014). Expression of genes encoding enzymes of BCAA metabolism was downregulated in muscle and liver tissue of T2DM patients. Similar results were obtained in rats (Shin et al. 2014; Yoon 2016).

Zhou et al. (2019), using an integrative pathway analysis in human and mouse populations, showed that IR induced by obesity is connected with the modulations of

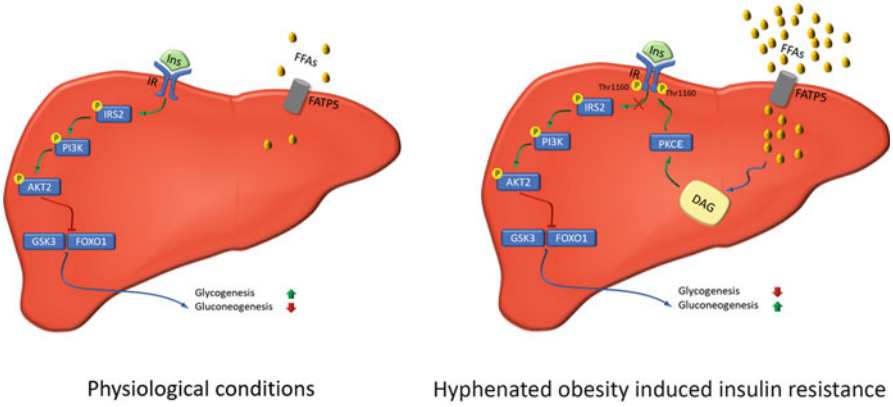

Fig. 1 Diacylglycerol (DAG) accumulation impairs insulin signaling in the liver. In physiological conditions, insulin arrives at the liver, activating the insulin receptor (IR) and starting a signaling cascade. This leads to the sequential activation of insulin receptor substrate 2 (IRS2), phosphatidylinositol-3-kinase (PI3K), and protein kinase B beta (AKT2). AKT2, inhibiting glycogen synthase kinase 3 (GSK-3), promotes the activation of glycogen synthase, increasing glycogenesis. AKT2 inhibition of Forkhead box protein O1 (FOXO1) suppresses the transcription of glucose 6-phosphatase, lowering gluconeogenesis. In the presence of a nonregulated amount of NEFAs in the plasma, they enter the liver through the fatty acids transport protein 5. This brings to an accumulation of DAG in the liver. DAG promotes the membrane translocation of PKCε, an isoform of PKC responsible for the phosphorylation of the Thr1160 on the insulin receptor. The conformational changes in the kinase active site impair its signaling function, leading to a loss of regulation on glycogenesis and gluconeogenesis

genes of the BCAA catabolism. They demonstrated that the BCAA catabolism is impaired in the obese state. In obese mice, restoring the catabolism of BCAAs lowers their levels and improves insulin sensitivity.

On the other hand, this defective enzymatic activity in adipose tissue could be compensated for by increased BCKDC activity in the liver (Lynch and Adams 2014; Yoon 2016). As a result, some individuals may be characterized by a more global reduction in the capacity to metabolize BCAAs, which may contribute to an increase in circulating BCAA concentrations to higher ranges that is related to the development of future T2DM and IR (Shin et al. 2014; Lynch and Adams 2014).

Contribution of BCAAs to IR is a complex process in which multiple, not fully understood, mechanisms are involved. A schematic presentation of possible mechanisms by which BCAAs contribute to development of IR is depicted on Fig. 2.

Altered Amino Acid Profiles in Insulin Resistance and Diabetes

In 1969, Felig et al. (1969) found that of 20 plasma amino acids measured, the concentrations of the three BCAAs: valine, leucine, isoleucine, and aromatic amino acids, phenylalanine and tyrosine, were increased, and glycine was lowered in plasma of obese subjects compared with age- and sex-matched lean individuals.

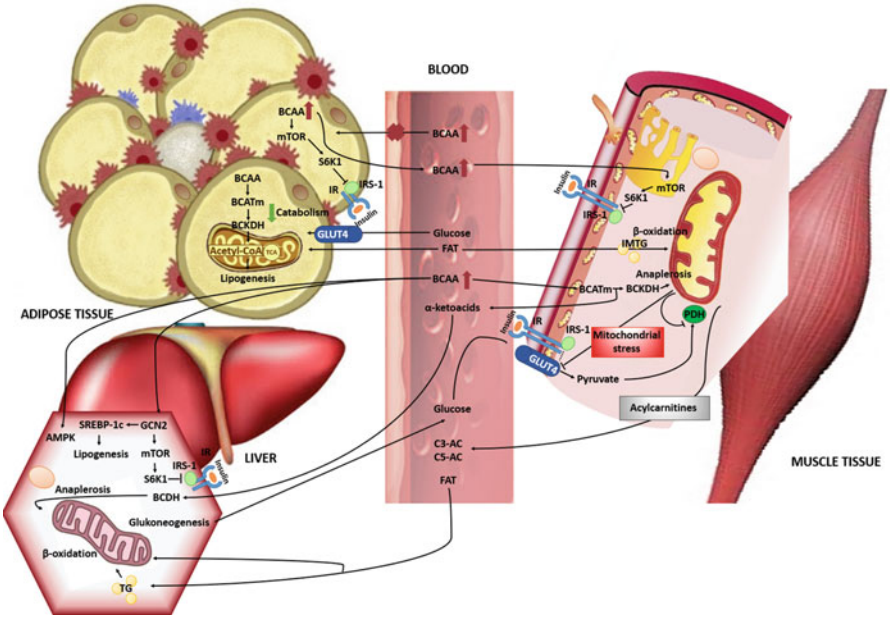

Fig. 2 A schematic view of possible mechanisms by which BCAAs contribute to development of insulin resistance. The first of proposed mechanisms involves stimulation of the mTOR/p70S6K pathway. High BCAA levels therefore inhibit IRS-1 and may impair insulin signaling. This causes cells to become less responsive to the secretion of insulin, resulting in insulin resistance

The concentration of each of the amino acids elevated in obesity correlated directly with serum insulin, suggesting that this increase was a manifestation of IR. In 2009, Newgard et al. (2009) replicated these findings of increased circulating BCAAs, tyrosine, and phenylalanine, and decreased glycine in obese insulin-resistant subjects compared to lean insulin-sensitive individuals. Broader investigations of a role of AA concentrations in the context of various pathophysiological processes have coincided with the advent of metabolomics as a tool for studying human diseases (White and Newgard 2019). Metabolomics provides a snapshot of physiological/patophysiological processes (Zhao et al. 2017).

Since then, an overwhelming number of published data have repeatedly described several changes in metabolites, including increases in BCAA and other AA levels associated with visceral obesity (Yamakado et al. 2012) and IR (Palmer et al. 2015; Seibert et al. 2015; Nakamura et al. 2014; Nagao and Kimura 2020). A summary of current human studies reporting association of amino acids with insulin resistance and T2DM is presented in Table 1. Systemic BCAAs are strongly associated with IR and further T2DM development (Felig et al. 1969; Ferrannini et al. 2013; Wang et al. 2011; Würtz et al. 2013; Biswas et al. 2019). Moreover, numerous human studies have consistently demonstrated that concentrations of BCAAs in plasma as well as in urine have the quality to predict diabetes development (Yousri et al. 2015; Giesbertz and Daniel 2016). The correlation between IR and elevated circulating

Table 1 Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections

Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
(Gall et al. 2010)	2010	The RISC study (Relationship of Insulin Sensitivity to Cardio-vascular Risk), comprising a nondiabetic cohort	Nondiabetic subjects representing a broad spectrum of insulin sensitivity and glucose tolerance	399 subjects	fasting plasma	Nontargeted fashion on three separate mass spectrometry platforms, UHPLC-MS/MS (+/− ESI) and GC-MS (+EI)	α–hydroxybutyrate was shown as an early marker for both insulin resistance and impaired glucose regulation
(Fiehn et al. 2010)	2010		Overweight to obese T2DM and nondiabetic Gullah-speaking African-American women with or without a UCP3 g/a missense polymorphism	44 obese T2DM and 12 obese nondiabetic African-American women	Plasma	Gas chromatography–mass spectrometry	AA levels and their derivatives (i.e., Leu, 2-ketoisocaproate, Val, Cys, His) were increased significantly in T2DM subjects Leu and Val concentrations rose with increasing HbA1c, and significantly correlated with plasma acetylcarnitine concentrations
(Tai et al. 2010)	2010		Nonobese Asian-Indian and Chinese men from a large cross-sectional study carried out in Singapore	263	Plasma	MS-based metabolic profiling	Increased levels of Ala, Pro, Val, Leu/Ile, Phe, Tyr, Glu/Gln, and Orn, and a cluster of branched-chain and related amino acids were associated with IR. Increased abdominal adiposity and leptin, and decreased adiponectin and IGF-binding

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Table 1 (continued)

Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
							protein 1 were also correlated with IR
(Wang et al. 2011)	2011	Nested case-control study in the Framingham Offspring Study Prospective cohort Malmö Diet and Cancer study – replication cohort	Normoglycemic individuals	2422 (201 developed diabetes. during 12 years) metabolite profiling on the samples from 189 cases and 189 controls (mean age 57 years, 42% women) 163 cases and 163 controls (mean age 58 years, 55% women)	Plasma	Targeted approach using liquid chromatography with a triple quadrupole tandem mass spectrometry	Ile, Leu, Val, Tyr, and Phe had highly significant associations with future diabetes. The results were replicated in an independent, prospective cohort. These findings underscore the potential key role of amino acid metabolism early in the pathogenesis of diabetes and suggest that amino acid profiles could aid in diabetes risk assessment
(Cheng et al. 2012)	2012	2 nested case-control studies designed to investigate predictors of diabetes mellitus and cardiovascular disease	Individuals free of diabetes mellitus and cardiovascular disease	1761 individuals free of diabetes mellitus and cardiovascular disease at the original examination from two large, well-characterized	Plasma	LC-MS	Metabolic risk factors, such as obesity, insulin resistance, high blood pressure, dyslipidemia were associated with multiple metabolites including branched-chain amino acids, other hydrophobic amino acids, tryptophan breakdown

				clinical cohorts in the Framingham Heart Study (FHS; $N = 1015$) and Malmö Diet and Cancer Study (MDC; N = 746). In MDC, a diagnosis of new-onset diabetes after the baseline examination (mean follow-up time 12.6 years)			products, and nucleotide metabolites. Moreover, a particularly strong association of insulin resistance traits with decreased Gln and increased Glu was observed. High glutamine-glutamate ratio was associated with lower risk of incident diabetes in FHS, but not in MDC
(Shah et al. 2012)	2012	Interventional	Nondiabetic individuals (37.4% African-Americans and 62.6% White) who had lost ≥4 kg during 6 months (phase I)	500	Plasma	Targeted mass spectrometry-based profiling	A cluster of metabolites comprising BCAA levels and related analytes predicted improvement in HOMA-IR independent of the amount of weight loss
(Stančáková et al. 2012)	2012	Population-based Metabolic Syndrome in Men (METSIM)	Nondiabetic or newly diagnosed type 2 diabetic Finnish men	9369 (4.7-year follow-up)	Plasma	Proton nuclear magnetic resonance spectroscopy	The levels of Leu, Ile, Tyr, and Ala increased and the levels of Gln and His decreased with increasing glycemia, reflecting, at least in part, insulin resistance (except for Gln) Only 1 of 43 risk single nucleotide polymorphisms

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Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
							regulating hyperglycemia, the glucose-increasing major C allele of rs780094 of GCKR, was significantly associated with decreased levels of Ala and Ile and elevated levels of Gln
(Wang-Sattler et al. 2012)	2012	Prospective cooperative Health Research in the Region of Augsburg (KORA) cohort Cross-sectional EPIC-Potsdam cohort – replication cohort	German individuals	4261 1297 cross-sectional KORA S4 study: 91 T2DM patients; 1206 individuals with non-T2DM (including 866 participants with NGT, 102 with i-IFG and 238 with IGT) 1010 prospective KORA S4→F4 study 876 non-T2DM individuals 91 incident cases of T2DM (mean follow-up 7 years) 641 NGT individuals	Serum	Liquid chromatography and flow injection analysis–mass spectrometry	Three metabolites (Gly, lysophosphatidylcholine (LPC) (18:2), and acetylcarnitine) had significantly altered levels in IGT individuals as compared to those with normal glucose tolerance. Lower levels of Gly and LPC were found to be predictors not only for IGT but also for T2DM, and were independently confirmed in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort

				118 incident IGT (mean follow-up 7 years)			
(Yamakado et al. 2012)	2012		Obese Japanese subjects	1449 (985 men and 464 women)	Plasma	LC-MS following derivatization	Accumulated visceral fat altered the peripheral amino acid profile. A multivariate logistic regression model of PFAAs could distinguish visceral obesity. This profile can be used as a predictor of elevated visceral obesity and a risk assessment tool for metabolic complications
(Magnusson et al. 2013)	2013	Matched case-control study derived from the population-based Malmö Diet and Cancer Cardio-vascular Cohort (MDC-CC)	Nested case-control study (MDC-CC) 4577 free of myocardial infarction or stroke (CVD) 253 incident CVD cases (during a mean follow-up time of 12.2 years) were matched with 253 controls. Cross-sectional study CVD case-control material from MDC-CC 506 (253 cases and 253 controls) and 564 free from prevalent CVD for cross-sectional analyses of DM-AA score in relation to intima-media thickness (IMT) of the common carotid artery (CCA), and classified using six-graded		plasma	LC-MS	Fasting plasma levels of Ile, Tyr and Phe were shown to predict diabetes development. The combination of these three AAs also predicted future cardiovascular events during long-term follow-up most likely through increased propensity of atherosclerosis BCAAs and AAAs were identified as novel markers of CVD development and as an early link between diabetes and CVD susceptibility

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Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
			plaque score Distinct cohort of subjects who underwent exercise stress testing with myocardial perfusion imaging: 83 cases with inducible ischemia and 83 control subjects				
(McCormack et al. 2013)	2013	Cross-sectional cohort and prospective longitudinal cohort (for 18 months)	Healthy individuals in Boston (21 African-Americans, 36 White, and 12 others) aged 8 –18 years. A subset of 17 individuals who were pre- or early-pubertal, aged 8–13 years, were enrolled in a prospective longitudinal cohort	69 (40 boys and 29 girls) – cross-sectional cohort 17 participants with a complete data – prospective longitudinal cohort	Plasma	Targeted LC-MS/MS-based profiling	Elevations in the concentrations of BCAAs were significantly associated with BMI in the cross-sectional cohort. In the subset followed in longitudinal study, baseline BCAA levels were positively associated with HOMA-IR measured 18 months later. Elevated BCAA levels were significantly associated with obesity in children and adolescents, and may independently predict future IR
(Würtz et al. 2013)	2013	Prospective cohort	Nondiabetic young Finnish adults from the	1680 (769 men and 911 women)	Fasting serum	High-throughput NMR spectroscopy	Circulating BCAAs (Ile, Leu, Val) and aromatic amino acids (Phe and Tyr) from fasting serum were shown as

			Cardiovascular Risk in Young Finns Study				predictors of the insulin resistance index, but not of glycemia, at 6-year follow-up in young, normoglycemic adults, with most pronounced associations for men
(Floegel et al. 2013)	2013	European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study Cooperative Health Research in the Region of Augsburg study – replication cohort (KORA) Tübingen Family study for T2DM (TüF)	German individuals TüF a family history of T2D, a BMI .27 kg/m^2, and previous impaired glucose tolerance or gestational diabetes mellitus	2282 randomly drawn (EPIC)-Potsdam subcohort (62.0% women) 800 (42.2% women) incident cases of T2DM (mean follow-up 7 years) and 876 KORA subcohort 91 incident cases of T2DM (mean follow-up 7 years) and 76 Caucasians TüF cohort	serum	Flow injection analysis tandem mass spectrometry (targeted metabolomics approach)	Phe was independently associated with increased risk of T2D and Gly was associated with decreased risk of T2DM
(Ferrannini et al. 2013)	2013	Two observational cohorts: the prospective, observational cohort study Relationship between Insulin	1,261 nondiabetic participants from the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study, with 3-year	1,261 and 2,580	Fasting plasma	Targeted LC-MS/MS-based profiling	α-hydroxybutyrate (α-HB) and linoleoyl-glycerophosphocholine (L-GPC) were identified as joint markers of insulin resistance (IR) and glucose intolerance. BCAAs; Leu, Ile, Val, and

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Table 1 (continued)

Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
		Sensitivity and Cardiovascular Disease (RISC) study and 2,580 from the family-based, observational familial Botnia Prospective Study	follow-up Subjects from 13 countries in Europe and 2,580 from the Botnia Prospective Study with 9.5-year follow-up Subjects from the West coast of Finland				three major glucogenic amino acids (Ala, Glu, Arg) were increased, whereas Gly was significantly decreased, in progressors versus nonprogressors. Increased concentrations of BCAAs and fatty acids, such as oleate, were positively related α-HB, whereas L-GPC and insulin sensitivity were reciprocally related to α-HB
(Wang et al. 2013)	2013	Nested case–control study from the Framingham Heart Study	Normoglycemic participants	2422 188 individuals who developed diabetes during 12 years and 188 propensity-matched controls	Plasma	Targeted LC-MS/MS-based profiling	The metabolite 2-aminoadipic acid (2-AAA) was most strongly associated with the risk of developing diabetes
(Mai et al. 2013)	2013	Part of a sample from a population from Eastern Germany, the Sorbs	German individuals with normal glucose tolerance, isolated impaired fasting glycemia, impaired glucose	1019 subjects Subjects with normal glucose tolerance (NGT; $n = 636$), isolated impaired fasting glycemia (IFG;	Serum	LC-MS	Alterations in serum concentrations of several acylcarnitines, in particular tetradecenoylcarnitine (C14:1), tetradecadienylcarnitine (C14:2), octadecenoylcarnitine (C18:

			tolerance or type 2 diabetes	$n = 184$), impaired glucose tolerance (IGT; $n = 87$), T2DM ($n = 112$)			1), and malonylcarnitine/ hydroxybutyrylcarnitine (C3DC+C4OH) are associated not only with T2D but also with prediabetic states
(Xie et al. 2014)	2014		Healthy obese and healthy lean participants (Chinese, $n = 105$ and American, $n = 72$)	106 healthy obese and 105 healthy lean participants	Serum	LC-MS and GC-MS	Metabolite profiles were significantly different between lean and obese participants. A cluster of obesity-associated changes in specific amino acids (BCAAs), fatty acids, acylcarnitine, and organic acid metabolites was identified in the obese participants but not in the lean participants. These metabolites were also associated with IR. Additionally, differences in serum metabolites and metabolic alterations, including BCAAs, fatty acids, UA, and creatinine, in obese humans occurred in a gender-dependent manner. Notably, three BCAAs (Ile, Leu, and Val) were correlated with IR and were differentially expressed

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Table 1 (continued)

Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
							in obese men, but not in obese women
(Nakamura et al. 2014)	2014	Cross-sectional cohort	T2DM Japanese subjects	51 (23 men and 28 women)	Plasma	LC-MS followed by precolumn derivatization	Glu, Tyr, Ala, Pro, and BCAAs were strongly correlated with the insulin-related variables such as C-peptide, insulin and HOMA-IR. Glu, Ala, Trp, and BCAAs were negatively correlated with adiponectin levels. The PFAA profiles in diabetic patients were strongly associated with hyperinsulinemia and hypoadiponectinemia, which might become risk evaluation factors for the development of cardiovascular diseases
(Roberts et al. 2014)	2014	Longitudinal, community-based Framingham Heart Study (FHS) HERITAGE		2067	Plasma	LC-MS	β-aminoisobutyric acid (BAIBA) plasma concentrations were inversely correlated with cardiometabolic risk factors (fasting glucose, insulin,

		Family Study, sedentary subjects were recruited for a 20-week program of supervised exercise training					HOMA-IR, triglycerides, and total cholesterol) in humans and were increased during exercise training
(Tillin et al. 2015)	2015	Cross-sectional and prospective study in South Asian and European cohort from the SABRE (Southall And Brent REvisited) Study	European and South Asian nondiabetic men	1,279 European and 1,007 South Asian nondiabetic men 801 European and 643 South Asian participants Diabetes developed in 227 (35%) South Asian and 113 (14%) European men. after 19 years of the follow-up period	Serum	Nuclear magnetic spectroscopy	Concentrations of Ile, Phe, Tyr, and Ala were significantly higher in South Asian men, while cross-sectional correlations of AAs with glycemia and insulin resistance were similar both in Europeans and South Asians. Stronger adverse associations were observed between branched chain and aromatic AAs, particularly tyrosine, and incident diabetes in South Asian men
(Seibert et al. 2015)	2015	Cross-sectional study	Nondiabetic individuals	182 (118 women and 64 men)	Plasma	LC-MS	14 out of 24 AA levels were significantly higher in males than females; the only Gly was lower in males. Glu, Ile, Leu, and Tyr levels had the strongest correlation with steady-state plasma glucose. This association was similar in women and men,

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Table 1 (continued)

Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
							independent of obesity, and similar to traditional markers of insulin resistance. In comparison to women, men tended to have a more unfavorable AA profile with higher AA levels associated with insulin resistance and less glycine. However, the degree of association between a direct measurement of insulin resistance and AA levels were similar between sexes and equivalent to several traditional markers of insulin resistance
(Allam-Ndoul et al. 2015)	2015		Overweight/obese subjects with or without metabolic syndrome (MetS) and normal weight subjects without MetS	200 (101 men and 99 women)	Fasting plasma	Mass spectrometry-based metabolite profiling	BCAAs were associated with obesity and MetS. Overweight/obese participants irrespective of their MetS status had higher plasma BCAA levels than normal weight participants. Obesity-associated MetS appeared to worsen the difference with normal weight subjects. Leu and Ile levels were correlated with HOMA-IR among obese individuals

							without MetS. Among obese subjects with MetS Leu levels were correlated to HOMA-IR whereas Leu and Ile were correlated with plasma glucose levels
(Yamakado et al. 2015)	2015		Japanese subjects	3701 Visceral fat area (VFA) and 2-h post-challenge insulin (Ins120 min) values were determined in 865 and 1,160 subjects, respectively. The cohort analysis was carried out, in which the individuals without these diseases were selected from a total of 2984 (1877 men) individuals (2,729 subjects without DM -, 91.5%; 2,695 individuals without metabolic syndrome, 90.3%; 2,336 without	Plasma	LC-MS followed by precolumn derivatization	BCAAs (Ile, Leu) and AAAs (Tyr, Phe) were statistically significantly related to the development of DM; Ile, Leu, Tyr, Ala, and Ser were significantly related to the development of metabolic syndrome; Ile, Leu, Tyr, Val, Ala, Pro, Ser, and Gly were significantly related to the development of dyslipidemia over the 4-year time period after adjusting for age, gender, BMI, fasting plasma glucose (FPG), and HOMA-IR. The PFAA models were able to predict the 4-year risk of developing lifestyle-related diseases, including DM, metabolic syndrome, dyslipidemia, and hypertension. The correlation coefficients of the obtained PFAA models

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Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
				dyslipidemia, 78.3%; or 2,637 without hypertension, 88.4%). Capabilities of the obtained models for predicting 4-year risk of developing new-onset lifestyle-related diseases were examined in a cohort study of 2,984 subjects			against VFA or Ins120 min were higher than single PFAA levels, suggesting their usefulness for future risk prediction
(Yousri et al. 2015)	2015	Cross-sectional case-control study	Individuals with diabetes and controls of Arab and Asian descent embedded in the Qatar Metabolomics Study on Diabetes (QMDiab)	188 T2DM individuals and 181 controls	saliva, blood plasma, and urine	LC-MS and GC-MS	Perturbations in the glycolysis pathway are reflected by increased pyruvate and lactate levels, and perturbations in Phe and Tyr metabolism have been also shown. Increased proteolysis with aminoaciduria is reflected by increased urinary BCAA and AAA levels. The presence of subclinical ketoacidosis in

							some patients is indicated by increased concentrations of 3-hyroxybutyrate and 3-hydroxyisobutyrate. Some of the established biomarkers were identified in more than one biofluid, such as 1,5-AG and 2-hydroxybutyrate. Of the 16 newly identified metabolite associations, many are in pathways that play a role in diabetes, including β-hydroxypyruvate (Gly, Ser, and Thr metabolism), 3-methoxytyrosine and 4-hydroxyphenylpyruvate (Phe and Tyr metabolism), 1,3-dihydroxyacetone (glycolysis pathway) as well as arabitol, gluconate, ribose, and xylonate (nucleotide and pentosemetabolism), thus linking these metabolites for the first time to diabetes
(Su et al. 2015)	2015	Cross-sectional study and longitudinal study		27 subjects (33 to 61 years old) Cross-sectional study that involved 9 lean (7 women and 2 men) and	Adipose tissue	GC-MS	Total adipose tissue mmBCFA content was ~30% lower in obese than lean subjects and increased by ~65% after weight loss in the RYGB group. Adipose tissue

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Table 1 (continued)

Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
				9 obese (7 women and 2 men) subjects Longitudinal study that involved 9 obese subjects (8 women and 1 man), who were studied before and 1 year after Roux-en-Y gastric bypass (RYGB) surgery			mmBCFA content correlated positively with skeletal muscle insulin sensitivity
(Chen et al. 2016)	2016	Cross-sectional and Longitudinal cohort study	Chinese participants at different stages of diabetes development	429 213 51 individuals (47% male) developed diabetes and 162 (27% male) remained free of diabetes after a median follow-up time of 10.0 years and 216 in the cross-sectional study	Serum	LC-MS	Early elevation of Val, Leu, Ile, Tyr, and Phe was closely associated with future development of diabetes, suggesting an important role of these metabolites as early markers of diabetes, highlighting the predictive value of these markers for future development of diabetes

(Palmer et al. 2015)	2015	Multiethnic cohort from the Insulin Resistance Atherosclerosis Study (IRAS)	European American, Hispanic, and African American nondiabetic at baseline subjects	196 72 high insulin sensitivity subjects and 75 Low insulin sensitivity subjects 146 76 converted to T2DM during a 5-year follow-up period and 70 nonconverters	plasma	Tandem mass spectrometry (MS/MS)	Gly was decreased and Val, Leu, Phe, and combined Gln and Glu were increased in insulin-resistant subjects. Ethnic-stratified results were strongest in European Americans. Comparing amino acid profiles between subjects that converted to T2DM yielded a similar pattern of associations: decreased Gly and increased Val, Leu, and combined Gln and Glu
(Lee et al. 2016)	2016		Nondiabetic participants of the Insulin Resistance Atherosclerosis Study (IRAS)	685 (290 Caucasians, 165 African Americans, and 230 Hispanics)	Plasma	Mass spectrometry	Plasma BCAAs were associated with incident diabetes and underlying metabolic abnormalities, although the associations were generally stronger in Caucasians and Hispanics
(Tricò et al. 2017)	2017	Cross-sectional and longitudinal study at the Yale Pediatric Obesity Clinic	Nondiabetic adolescents	78 nondiabetic adolescents 16 subjects after a mean follow-up of 2.3 years	Plasma	Nuclear magnetic resonance spectroscopy	BCAAs and α-hydroxybutyrate concentrations during an oral glucose tolerance test (OGTT) characterized insulin-resistant youth and predicted worsening of glycemic control

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Table 1 (continued)

Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
(Haufe et al. 2017)	2017	Prospective study	Overweight to obese individuals before and after 6 months on hypocaloric diets reduced in either carbohydrates or fat	109	Plasma (and urine)	GC-MS	3-HIB relates to insulin sensitivity but is not associated with intramyocellular fat content in overweight to obese individuals. Moreover, changes in 3-HIB rather than changes in BCAAs are associated with metabolic improvements with weight loss
(Harris et al. 2017)	2017		Sedentary, 50- to 65-year-old women with a stable weight basal conditions and during a hyperinsulinemic-euglycemic clamp procedure (HECP) with and without concomitant ingestion of protein ($n = 15$) or an amount of leucine that matched the	30	Plasma	GC-MS	The decrease in plasma 3-HIB concentration and increase in plasma FGF21 concentration induced by insulin and glucose infusion during hyperinsulinemic-euglycemic clamp procedure (HECP) is blocked by protein ingestion and the protein-induced increase in circulating 3-HIB and decrease in circulating FGF21 were associated with a marked impairment in insulin-stimulated glucose disposal. Val and FGF21 metabolism are involved in the pathogenesis of insulin resistance in skeletal muscle

			amount of protein ($n = 15$)				induced by protein ingestion in humans
(Shi et al. 2018)	2018	Nested case–control study within the Swedish cohort of the Västerbotten prospective population-based intervention program	Swedish incident diabetes cases and nondiabetic controls	503 participants at baseline who developed T2DM after a median time of 7 years. Among the 503 pairs of selected participants, 187 case-control pairs at 10-year follow-up	Plasma	LC-MS	Identified metabolites strongly correlated with insulin resistance and/or beta cell dysfunction. Changes in phosphatidylcholines (PCs) with odd-chain fatty acids, branched-chain amino acids, 3-methyl-2-oxovaleric acid, and glutamate over time along with disease progression among diabetes cases. Among other findings, PCs containing odd-chain fatty acids (C19:1 and C17:0) and 2-hydroxyethanesulfonate were associated with the likelihood of developing T2DM
(Flores-Guerrero et al. 2018)	2018	Prevention of renal and vascular end-stage disease (PREVEND) cohort	Residents from Groningen, the Netherlands	Prospective study 6244 subjects 301 cases of T2DM (during median follow-up for 7.5 years)	Plasma	Nuclear magnetic resonance spectroscopy	High BCAA levels were associated with insulin resistance and with increased risk of type 2 diabetes. This association was independent of multiple risk factors, HOMA-IR, and β cell function

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Table 1 (continued)

Associations between amino acids and insulin resistance and T2DM – summary of current studies mentioned in the respective sections							
Reference	Year	Type of study	Subjects	n	Type of material	Method	Main conclusions
(Yamakado 2018)	2018				Obtained model uses plasma AA levels, which correlate to visceral fat area, as an indicator of prediabetic visceral fat accumulation	AminoIndex LifeStyle diseases (AILS) test as a multivariate formula using plasma AA levels for lifestyle-related disease risk screening	Evidence is accumulating that the features of AILS include its ability to assess the risk for diabetes, suggesting it can be used for diabetes prediction
(Chen et al. 2019)	2019	Prospective nested case-control Hitachi Health Study	Nondiabetic Japanese working adults	4754 individuals 284 T2DM cases during a 5-year follow-up and 560 controls	Serum	LC-MS	High concentrations of Val, Leu, Ile, Phe, Tyr, Ala, Glu, Orn, and Lys were associated with an increased risk of incident T2DM. High Gln levels were associated with a decreased risk of incident T2DM. These AAs may be novel useful biomarkers in the identification of people at risk of T2DM before overt symptoms. Insulin resistance may account for or mediate the relationship between these AA and risk of incident T2DM

BCAA levels has been confirmed in several studies involving different ethnic groups and degrees of obesity (Newgard et al. 2009; Shah et al. 2012; McCormack et al. 2013; Würtz et al. 2013; Bi and Henry 2017). FAA profiles, particularly BCAA levels, are altered prior to the development of T2DM and are significantly associated with future diabetes diagnosis (Wang et al. 2011). These changes in plasma-free amino acids (PFAAs) can predominantly result from a metabolic shift caused by early diabetes pathogenesis (Nie et al. 2018) and may serve as a better indicator of impaired IR in prediabetic state than plasma glucose levels (Allam-Ndoul et al. 2015).

In addition, the dietary patterns of protein and BCAAs supplementation also significantly influence the association between BCAAs and IR (Zheng et al. 2016; Nagata et al. 2013; Biswas et al. 2019). Infusion of BCAAs or leucine in humans, as well as dietary intake of BCAAs, reportedly worsened insulin sensitivity (Zheng et al. 2016; Nagata et al. 2013; Harris et al. 2017; Shah et al. 2012; Biswas et al. 2019), while low BCAAs consumption has been correlated with improvement in metabolic health and alleviating IR (amelioration of IR) (Biswas et al. 2019). High dietary consumption of BCAAs is suggested to increase the risk of incident IR and may accelerate the progression of metabolic disorders, such as metabolic syndrome, and diabetes, and is not associated with the pancreatic β-cells dysfunction and hyperinsulinemia in adults. Higher total dietary BCAA intake was associated with an increased risk of T2DM in three prospective cohort studies (Nagata et al. 2013; Zheng et al. 2016; Nie et al. 2018). Moreover, these results were similar in Asian population (Nie et al. 2018).

The circulating BCAAs (valine, leucine, isoleucine) and AAAs (tyrosine and phenylalanine) have been identified as risk factors for the development of T2DM (Guasch-Ferré et al. 2016; Wang et al. 2011; Floegel et al. 2013; Palmer et al. 2015; Tillin et al. 2015; Chen et al. 2016; Stančáková et al. 2012; Wang-Sattler et al. 2012; Chen et al. 2019). Evidence concerning other amino acids is inconsistent (Chen et al. 2019). For example, an inverse correlation of glutamine with T2DM risk has been shown in some (Stančáková et al. 2012; Cheng et al., 2012) but not all of the performed studies (Floegel *et al.*, 2013; Tillin et al. 2015; Wang-Sattler et al. 2012). Glutamate was positively associated with the occurrence of T2DM in cohort studies of Finnish from the Botnia Prospective Study (Ferrannini et al. 2013) and American adults from the Framingham Heart Study (FHS) cohort (Cheng et al. 2012), as well as Swedish cohort of the Västerbotten prospective population-based intervention program (Shi et al. 2018), whereas relations of glutamate were nonsignificant in the Malmö Diet and Cancer Study (MDC) cohort free of diabetes at baseline Swedish individuals (Cheng et al. 2012).

Glycine was reported to be inversely associated with the incidence of T2DM in Germany and West coast of Finland (Floegel et al. 2013; Wang-Sattler et al. 2012; Ferrannini et al. 2013). It was also negatively correlated with incident diabetes in European but not in South Asian men (Tillin et al. 2015). Moreover, limited evidence suggests a positive association with T2DM in the case of alanine (Ala) and histidine as well as ornithine (Guasch-Ferré et al. 2016; Chen et al. 2019). Stronger correlation between BCAA concentrations and insulin sensitivity was observed in men, with

clear differences associated with age and ethnicity (Ianni et al. 2017). Consistent with this view, the population-based Cardiovascular Risk in Young Finns Study demonstrated that BCAAs, along with the aromatic amino acids: phenylalanine and tyrosine, were associated with IR selectively in men (Würtz et al. 2013). Xie et al. (2014) demonstrated that the serum metabolite profiles, verified with two independent groups of participants (Chinese, $n = 105$ and American, $n = 72$) of the obese population are gender-dependent. BCAA levels were correlated with IR and differentially expressed in obese men, but not in obese women (Xie et al. 2014). Another study found that 14 out of 24 measured AA levels were significantly higher in males than females; the only glycine was lower in males. Glutamic acid, isoleucine, leucine, and tyrosine levels had the strongest correlation with steady-state plasma glucose. This association was similar in women and men, independently of obesity, and similar to traditional markers of IR. In comparison to women, men tended to have a more unfavorable AA profile with higher AA levels associated with IR and less glycine. However, the degree of association between a direct measurement of IR and AA levels were similar between sexes and equivalent to several traditional markers of IR (Seibert et al. 2015).

The correlation between BCAA concentrations and T2DM development is also significantly modified by ethnicity, with the association in Caucasians and Hispanics while it does not appear in African Americans (Lee et al. 2016).

The cohort studies on the Asian population found BCAAs as a valid of the future risk of T2DM (Tillin et al. 2015; Tai et al. 2010), while another study of a predictive model in American Indians did not develop a reporter concept of BCAAs (Zhao et al. 2015).

Large, longitudinal studies confirmed that PFAAs analysis can predict the future susceptibility of lifestyle-related diseases (Wang et al. 2011; Yamakado et al. 2015), which is a significant strength of these investigations. Various prospective, case-controlled and nested studies on the subjects of different ethnic origins have shown elevated levels of BCAAs and other amino acids are associated with the prediabetic state, IR, and T2DM. Elevations in PFAA concentrations may independently predict the future development of diabetes, metabolic syndrome, dyslipidemia, or hypertension over a 4-year period, even after adjusting for commonly accepted risk factors such as age, sex, body mass index, fasting plasma glucose, IR, waist circumference, blood pressure, and lipid variables. From 2,984 Japanese subjects in the cohort study, 2,729 individuals without DM were included in the follow-up study to investigate the ability to predict the 4-year risk of developing new-onset DM. For single PFAA levels, BCAAs (isoleucine, leucine) and AAAs (tyrosine and phenylalanine) were significantly related to the development of DM over the 4-year time period after adjusting for age, gender, BMI, fasting plasma glucose (FPG), and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). Multiple linear regression analysis with variable selection models were constructed between PFAA levels and the visceral fat area (VFA) and 2-h post-challenge insulin (Ins120 min) values for predicting 4-year risk of developing new-onset lifestyle-related diseases. The correlation coefficients of the obtained PFAA models against VFA or Ins120 min were higher than single PFAA levels, suggesting their usefulness as versatile markers for health monitoring for future risk prediction (Yamakado et al. 2015).

The positive associations of BCAAs and aromatic amino acids with T2DM risk have subsequently been replicated in multiple other cohorts. Similarly, the Framingham Offspring Study, conducted among 2,422 normoglycemic individuals over a 12-year period, showed that BCAAs (isoleucine, leucine, valine) and AAAs (phenylalanine and tyrosine, and also tryptophan) levels have highly significant associations with future diabetes. The others were aromatic amino acids (tryptophan, phenylalanine, and tyrosine). A combination of three amino acids predicted future diabetes (with a more than fivefold higher risk for individuals in top quartile), highlighting the potentially crucial role of amino acid metabolism in early diabetes pathogenesis (Wang et al. 2011).

Chen and his colleagues verified the close correlation of valine, leucine, isoleucine, tyrosine, and phenylalanine with IR and the future development of diabetes in Chinese populations after 10 years of follow-up, suggesting an important role of these metabolites as early markers of diabetes (highlighting the predictive value of these markers for the future development of diabetes) (Chen et al. 2016).

Cheng et al. (2012) performed one of the most promising investigations to determine the plasma concentrations of 45 distinct metabolites and examine their relation to cardiometabolic risk among 1,761 individuals from two large, well-characterized clinical cohorts in the Framingham Heart Study (FHS; N=1015) and the Malmö Diet and Cancer Study (MDC; N=746). Metabolic risk factors, such as obesity, IR, high blood pressure, dyslipidemia were associated with multiple metabolites including branched-chain amino acids, other hydrophobic amino acids, tryptophan breakdown products, and nucleotide metabolites. Moreover, a particularly strong association of IR traits with decreased glutamine and increased glutamate was observed. High glutamine-glutamate ratio was associated with lower risk of incident diabetes in FHS, but not in MDC (Cheng et al. 2012). The prospective roles of circulating amino acids as reporter molecules of insulin sensitivity and diabetes were further investigated in 1,680 individuals from the population-based Cardiovascular Risk in Young Finns Study. Circulating BCAAs (isoleucine, leucine, valine) and aromatic amino acids (phenylalanine and tyrosine) from fasting serum were shown as predictors of the insulin resistance index, but not of glycemia, at 6-year follow-up in young, normoglycemic adults, with most pronounced associations for men. They were associated with HOMA-IR at baseline and for men at 6-year follow-up, while for women only leucine, valine, and phenylalanine predicted 6-year HOMA-IR ($P < 0.05$). These observations suggest that altered metabolism of BCAAs and AAAs precedes the development of IR in early adulthood, before the onset of impaired fasting glucose levels, which at least partially explains how these amino acids are associated with the risk of future type 2 diabetes (Würtz et al. 2013).

Tillin et al. performed the cross-sectional and prospective analyses of ethnicity, amino acids level, and diabetes in a South Asian and European cohort from the SABRE (Southall And Brent REvisited) study. The study was performed on 801 European and 643 South Asian participants with 19 years follow-up period. The authors concluded that branched-chain and aromatic amino acids may contribute to excess risk of diabetes development (Tillin et al. 2015).

Stančáková et al. investigated amino acid levels with proton nuclear magnetic resonance spectroscopy in the population-based Metabolic Syndrome in Men (METSIM) Study, including 9,369 nondiabetic or newly diagnosed T2DM Finnish men. The levels of leucine, isoleucine, tyrosine, and alanine increased, and the levels of glutamine and histidine decreased with increasing glycemia, reflecting, at least in part, insulin resistance (Stančáková et al. 2012).

In a prospective nested case-control study, conducted among Japanese employees during a 5-year follow-up, fasting serum concentrations of several amino acids, including valine, leucine, isoleucine, phenylalanine, tyrosine, alanine, glutamate, ornithine, and lysine were associated with an increased risk of incident T2DM. High glutamine levels were associated with a decreased risk of incident T2DM (Chen et al. 2019).

In another study performed among Chinese population, GC-MS-based metabolic profiling showed increased levels of several amino acids, involving BCAAs (leucine, isoleucine) as well as alanine, and serine while substantial lower levels of 2-ketoisocaproic acid as early biomarkers of T2DM. Lower concentrations of 2-ketoisocaproic acid, a product of leucine deamination, may indicate a reduced rate of conversion of leucine to 2-ketoisocaproic acid in T2DM (Zeng et al. 2009). Similarly, Fiehn et al. observed an increase in certain AA levels and their derivatives (i.e., leucine, 2-ketoisocaproate, valine, cystine, and histidine). It is noteworthly, that plasma leucine concentration was significantly increased by ∼50%, and its initial catabolic metabolite, 2-ketoisocaproic acid (α-ketoisocaproate), was significantly increased by ∼27%. Mean plasma valine level was ∼20% higher in T2DM subjects vs. nondiabetic weight/age matched African-American women, but this difference was not statistically significant. In addition, leucine and valine concentrations rose with increasing HbA1c, and significantly correlated with plasma acetylcarnitine concentrations (Fiehn et al. 2010).

Several other studies have observed the predictive ability of PFAA analysis in evaluating the risk of developing lifestyle-related diseases and associated cardiovascular diseases. Magnusson et al. (2013) investigated the metabolite profiles among 4577 subjects among whom in case of 253 first-incident of cardiovascular disease (CVD) (myocardial infarction or stroke) occurred during a mean follow-up time of 12.2 years. Fasting plasma levels of isoleucine, tyrosine, and phenylalanine were shown to predict diabetes development with a four- to sixfold increased risk for participants in the top quartile. The combination of these three amino acids may also predict future cardiovascular events during long-term follow-up. McCormack et al. reported that elevations in BCAA concentrations are significantly associated with obesity in children and adolescents, and may independently predict future IR. Elevated BCAA levels significantly correlated with BMI in the cross-sectional cohort. In the subset of participants followed longitudinally, baseline BCAA levels were positively associated with HOMA-IR measured 18 months later (McCormack et al. 2013). Metabolic profiling performed in baseline and after 6 months in plasma samples from 500 participants after at least 4 kg of weight loss in phase I revealed a cluster of metabolites comprising BCAA levels and related analytes that could predict improvement in HOMA-IR independently of the amount of weight lost

(Shah et al. 2012). Nakamura et al. (2014) recruited 51 Japanese subjects diagnosed with T2DM and measured their PFAA profiles. Several amino acids: BCAAs, glutamate, tyrosine, alanine, and proline were strongly correlated with the insulin-related variables such as C-peptide, insulin, and HOMA-IR. They also observed that the levels of BCAAs, glutamate, alanine, and tryptophan were negatively correlated with adiponectin concentrations. Adiponectin plays a pivotal role in the regulation of insulin sensitivity and metabolism. Adiponectin concentrations have shown to be decreased in obese people or diabetic patients and are strongly related to IR and hyperinsulinemia in humans (Ziemke and Mantzoros 2010). These results indicated the significant relationship between PFAA profiles, adiponectin levels, and IR (Nakamura et al. 2014).

A cross-sectional and longitudinal study conducted at the Yale Pediatric Obesity Clinic showed that BCAA and α-hydroxybutyrate concentrations during an oral glucose tolerance test (OGTT) characterize insulin-resistant youth and predict worsening of glycemic control (Tricò et al. 2017). Furthermore, in a nested case-control study within the Swedish cohort of the Västerbotten prospective population-based intervention program, an untargeted metabolomics of plasma samples from 503 case-control pairs at baseline (median time 7 years before diagnosis) and samples from a subset of 187 case-control pairs at 10-year follow-up was performed. As a result 46 metabolites allowing T2DM prediction were reported (Shi *et al.*, 2018). Identified metabolites strongly correlated with IR and/or beta cell dysfunction. Among diabetes cases, changes in phosphatidylcholines (PCs) with odd-chain fatty acids, branched-chain amino acids, 3-methyl-2-oxovaleric acid, and glutamate were observed over time along with disease progression. Prospective associations between plasma BCAA levels and T2DM risk were also established in a population-based Prevention of renal and vascular end-stage disease (PREVEND) cohort. BCAA concentrations were determined by nuclear magnetic resonance spectroscopy in 6244 subjects, among whom 301 cases of T2DM were ascertained during a mean follow-up period of 7.5 years. High levels of BCAAs were confirmed as previously identified predictive biomarkers of IR and T2DM. This association was independent of multiple risk factors, HOMA-IR and β cell function (Flores-Guerrero et al. 2018)

The association between the level of circulating BCAAs, insulin resistant obesity, and T2DM prompted consideration of BCAA levels as a predictor for future IR or T2DM in order to develop screening PFAA-based tests. Analysis of general health check-up data from 8070 subjects revealed that the amino acid balance of precipitants that developed diabetes within 4 years was similar to that of individuals with diabetes, suggesting that changes in amino acid metabolism may occur prior to the onset of diabetes. Based on these findings, an index known as the AminoIndex LifeStyle diseases (AILS) test was developed. The obtained model was created as a multivariate formula using plasma AA levels, which correlate to visceral fat area as an indicator of prediabetic visceral fat accumulation. Plasma AA levels of asparagine, glycine, alanine, valine, tyrosine, and tryptophan levels were included in the AILS (risk of diabetes) formula derived in this study. Alanine, valine, tyrosine, and tryptophan levels were significantly higher in individuals who developed diabetes within 4 years compared with levels in those who were not diagnosed with diabetes during this period,

while levels of glycine were significantly lower. The AILS test was investigated whether its values normalize with such interventions as dietary and exercise counseling for 3 months. AILS values decreased significantly in individuals who managed to reduce body weight and waist circumference (Tochikubo et al. 2016), suggesting that early risk assessment using AILS could support early interventions in at-risk populations (Nagao and Kimura 2020). After commercialization in 2011, tests based on PFAAs were adopted in over 1500 clinics and hospitals in Japan, and numerous clinician-led studies have been performed to validate these tests. Evidence is accumulating that the features of AILS include its ability to assess the risk for diabetes, suggesting it can be used for diabetes prediction (Yamakado 2018).

Amino Acids Metabolic Intermediates As Markers for Insulin Resistance and Diabetes

Many recent studies suggest that not only BCAAs, but also BCAA catabolic enzymes and metabolic intermediates may play a key role in determining the relationship between BCAAs and other amino acids, and IR (Biswas et al. 2019). Obesity-related increase in BCAA levels may be the result of changes in amino acid metabolism, particularly decreased rates of their oxidation in adipose tissue (She et al. 2007; Lackey et al. 2013; Nagao and Kimura 2020; White and Newgard 2019). Recent metabolomics studies on diabetes have identified changes in plasma concentrations of BCAA-derived branched-chain keto acids, short branched-chain fatty acids, and various acylcarnitines as new entities with predictive qualities (Giesbertz and Daniel 2016).

Altered levels of BCAA-catabolic intermediates was shown to influence metabolic maladaptations during obesity and IR (Gall et al. 2010). Circulating BCKA and its association with heightened fasting plasma glucose and IR have been established by the Relationship of Insulin Sensitivity to Cardiovascular Risk (RISC) study group (Gall et al. 2010). Elevated plasma BCKAs were also confirmed by a few studies on mice (Lian et al. 2015).

Other intermediates related to BCAA catabolism are long-chain monomethyl and odd-numbered fatty acids. They can be derived from an odd-chain starter CoA with chain elongation in the fatty acid synthase complex. Monomethyl branched-chain fatty acids (mmBCFA) content in adipose tissue was reduced in obese compared to lean subjects, increased after weight loss in obese individuals, and correlated positively with insulin sensitivity (Su et al. 2015).

Val-derived metabolite, 3-hydroxyisobutyrate (3-HIB), is produced in skeletal muscle in response to forced expression of Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α). 3-HIB, secreted from muscle cells is shown to regulate trans-endothelial fatty acid transport (White and Newgard 2019). Circulating 3-HIB is positively associated with blood glucose levels in diabetic patients, suggesting that this metabolite may promote excessive lipid accumulation and impaired insulin action in skeletal muscle (White and Newgard 2019). In overweight and obese subjects, lower circulating 3-HIB levels, but not BCAA, were associated

with metabolic improvement after weight loss (Haufe et al. 2017; Biswas et al. 2019) and were also correlated with insulin-stimulated glucose utilization in older obese women (Harris et al. 2017; Biswas et al. 2019). These results indicate that 3-HIB may serve as a signaling metabolite in IR and T2DM.

Interestingly, elevated plasma levels of β-aminoisobutyric acid (BAIBA) due to enhanced valine catabolism have also been shown to increase with exercise and are inversely correlated with such metabolic risk factors as fasting glucose, insulin, HOMA-IR, triglycerides, and total cholesterol in a randomized large human cohort enrolled in the longitudinal Framingham Heart Study (Roberts et al. 2014). BAIBA is identified as a novel small molecule myokine that increases brown adipocyte-specific genes expression in white adipose tissue and fatty acid β-oxidation in hepatocytes, both in vitro and in vivo, through a PPARα-mediated mechanism. Moreover it was shown to induce a brown adipose-like phenotype in human pluripotent stem cells, and improve glucose homeostasis in mice (Roberts et al. 2014; Biswas et al. 2019). Thus, BAIBA may contribute to exercise-induced protection against metabolic diseases (Roberts et al. 2014).

Newgard et al. showed that BCAAs-derived odd numbered 3- and 5-carbon acylcarnitines have a predictive value for the development of diabetes (Newgard et al. 2009). Acylcarnitines present in plasma and urine may reflect defective BCAAs mitochondrial oxidation. Various acylcarnitines derived from BCAA catabolism may be associated with IR and T2DM (Giesbertz and Daniel 2016). Mai et al. reported that alterations in serum levels of several acylcarnitines, in particular tetradecenoylcarnitine (C14:1), tetradecadienylcarnitine (C14:2), octadecenoylcarnitine (C18:1), and malonylcarnitine/hydroxybutyrylcarnitine (C3DC+C4OH) are correlated not only with T2DM but also with prediabetic states (Mai et al. 2013).

Lysine degradation product, 2-aminoadipic acid (2-AAA) was most strongly associated with the risk of diabetes development in the Framingham Heart Study (Wang et al. 2013). Participants with 2-AAA levels in the top quartile had greater than a fourfold risk of developing diabetes during the mean 12 years follow-up period. Administration of 2-AAA led to a reduction in fasting plasma glucose levels in mice fed both the standard chow and high fat diets. Moreover, treatment with 2-AAA increased insulin secretion from the pancreatic β cell line as well as murine and human islets. Our results suggest that 2-AAA is a diabetes risk marker and a potential modulator of glucose homeostasis in humans (Wang et al. 2013; Chen and Gerszten 2020).

A disadvantage of these new reporter molecules compared to BCAAs is the lower plasma and tissue levels of several species, resulting in higher analytical variation, with some compounds also less stable (Giesbertz and Daniel 2016).

Conclusions

Beyond their contribution as fundamental building blocks of life, amino acids play a key role in various physiological as well as pathological processes. Recent evidences demonstrate that elevated amino acids, especially BCAA levels, are associated with a number of pathologies, including: obesity, IR, T2DM, and CVD. Therefore, the

measurement and monitoring of circulating AA levels in biological fluids could represent a promising method for early detection of the disease and may provide a means of preventing its development. This chapter reviews the latest findings regarding the use of plasma/serum FAAs profiles as novel useful biomarkers in the identification of people at risk of T2DM before overt symptoms as well as focuses on potential targets relating to their signaling pathways, and metabolism that broadens our understanding of their role in insulin resistance and diabetes.

Applications to Prognosis, Other Diseases, or Conditions

In this chapter changes in circulating AAs levels and their correlations with IR, prediabetic state, and T2DM have been reviewed. Several studies have been explored in order to identify and integrate AAs metabolite biomarkers proposed so far. A large body of evidence shows that AAs, especially BCAAs, are associated with obesity, IR, prediabetic state, and T2DM as well other pathological conditions, including cancers, CVD, liver disease, chronic kidney disease (CVD), and ischemic stroke, highlighting the potential use of AAs profiling as a valuable diagnostic tool to access the risk of disease development. The use of AAs as novel disease biomarkers in clinical practice may improve treatment strategies.

Mini Dictionary of Terms

- **HOMA-IR** – Homeostatic Model Assessment (HOMA) is a method for assessing β-cell function and insulin IR from basal (fasting) glucose and insulin or C-peptide concentrations. It approximates insulin resistance.
- **INSULIN RESISTANCE** – A pathological condition in which cells fail to respond normally to the insulin hormone.
- **BIOMARKER** – A measurable biological feature that can distinguish normal condition from pathological condition or indicate a response to an administered therapeutic drug.
- **METABOLOMICS** – A scientific discipline devoted to study changes in metabolome by measurement of small molecule metabolites. Nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS), coupled with liquid chromatography (LC-MS), gas chromatography (GC-MS), or capillary electrophoresis (CE-MS), is usually used to measure metabolites. Not only primary metabolites, but also the substrates and products of metabolism are measured. Metabolites can be measured in a targeted (metabolic profiling) or an untargeted (metabolic fingerprinting) manner.
- **T2DM** – Most commonly diagnosed type of diabetes, characterized by abnormal glucose and lipid metabolism, resulting from resistance to the effects of insulin and insufficient response to the secretion of this hormone. As this disease progresses, there may also occur a partial β-cell insufficiency and deficiency in insulin production.

Key Facts of Amino acids

A number of studies suggest that BCAAs supplementation or BCAA-rich diets has potential benefits for promoting lean body mass in obesity or catabolic disorders. Elevated BCAAs levels have been reported to improve body composition, glycemia levels, and to increase satiety for weight loss (Lynch and Adams 2014).

- In contrast to the potential health-promoting effects of BCAAs under conditions of negative energy balance, higher BCAAs intake may lead to adverse effects on the development of IR (Nie et al. 2018). Increased levels of branched-chain amino acids in plasma, serum, and also in urine are associated with obesity, IR, and diabetes, in particular T2DM.
- Branched-chain amino acids: valine, leucine, isoleucine, and aromatic amino acids, that is tyrosine and phenylalanine, have been demonstrated as the most consistent metabolite biomarkers for diabetes, in particular T2DM.
- Elevated BCAA levels have often been shown to predict the development of T2DM well in advance of its actual occurrence, what is very interesting from a diagnostics standpoint.
- BCAAs may not only have a "reporter quality" but may also contribute to the development of IR and T2DM.

Summary Points

- In the current medical situation, DM is a worldwide epidemic. Diabetes, particularly type 2 portends a poor prognosis and shortens the life expectancy of diabetic patients. Moreover, it imposes a heavy financial strain on health care systems everywhere.
- Early screening and testing of people at risk is the best approach to control the increasing numbers of diabetes occurrences, and it is most effective to recognize the early stages of DM before major systematic damage occurs.
- Various prospective, case-controlled and nested studies on the subjects of different ethnic origins shown that elevated levels of BCAAs and other AAs are associated with the prediabetic state, IR, and T2DM.
- Amino acids quantification in biological material, primarily in plasma, could be a valuable prognostic tool for determining metabolic abnormalities leading to diabetes.
- The activation of mTORC1 by BCAAs has been suggested to trigger IR, and subsequent metabolic disorders.
- Deficiencies in BCAAs metabolism referred as dysmetabolism lead to increased BCAA levels in obesity and/or diabetes. It can also induce the accumulation of possibly toxic intermediates, such as branched-chain keto acids, branched-chain fatty acids, and various acylcarnitines that impair cellular function(s) and may induce IR.
- Various intermediates of BCAAs are now considered as predictive markers that reflect IR and diabetes development.

The second possible mechanism is referred as BCAAs . Deficiency in BCAAs metabolism may induce the accumulation of toxic intermediates, such as branched-chain keto acids, branched-chain fatty acids, and various acylcarnitines that promotes cellular dysfunction, which leads to poorer insulin sensitivity of skeletal muscles.

Accumulation of BCAA break-down products may also interfere with proper mitochondrial function. Overloading of mitochondria with lipid substrates leads to oxidative stress and impaired insulin action contributing to insulin resistance.

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