# Chapter 10
# Chronic Diseases and Lifestyle Biomarkers Identification by Metabolomics

**Annalaura Mastrangelo and Coral Barbas**

**Abstract** Chronic diseases, also known as noncommunicable diseases (NCDs), are complex disorders that last for long periods of time and progress slowly. They currently account for the major cause of death worldwide with an alarming increase in rate both in developed and developing countries. In this chapter, the principal metabolomic-based investigations on chronic diseases (cardiovascular diseases, diabetes, and respiratory chronic diseases) and their major risk factors (particularly overweight/obesity) are described by focusing both on metabolites and metabolic pathways. Additional information on the contribution of metabolomics strategies in the ambit of the biomarker discovery for NCDs is also provided by exploring the major prospective studies of the last years (i.e., Framingham Heart Study, EPIC, MONICA, KORA, FINRIK, ECLIPSE). The metabolic signature of diseases, which arises from the metabolomic-based investigation, is therefore depicted in the chapter by pointing out the potential of metabolomics to explain the pathophysiological mechanisms underlying a disease, as well as to propose new therapeutic targets for alternative treatments.

**Keywords** Diabetes • Cardiovascular diseases • Chronic respiratory diseases • Biomarker • Obesity • Risk factors • Metabolomics • Metabolic signature • Longitudinal studies • Metabotypes

A. Mastrangelo • C. Barbas (✉)
Center for Metabolomics and Bioanalysis (CEMBIO), Faculty of Pharmacy, Universidad San Pablo CEU, Campus Monteprincipe, Boadilla del Monte 28668, Madrid, Spain
e-mail: cbarbas@ceu.es



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## Abbreviations

| | |
|---|---|
| 2AA | 2-Aminoadipic acid |
| ArAA | Aromatic amino acids |
| AUC | Area under the curve |
| BA | Bile acid |
| BAIBA | Beta-aminoisobutyric |
| BCAA | Branched-chain amino acids |
| BCKDH | Branched-chain alpha-keto acid dehydrogenase |
| BMI | Body mass index |
| BWHHS | British Women's Heart and Health Study cohort |
| CE | Capillary electrophoresis |
| COPD | Chronic obstructive pulmonary disease |
| CVD | Cardiovascular disease |
| DM-AA | Diabetes-predictive amino acid |
| ECLIPSE | Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points |
| EPIC | European Prospective Investigation into Cancer and Nutrition |
| FA | Fatty acids |
| FAHFA | Fatty acid esters of hydroxy fatty acid |
| FAO | Fatty acids oxidation |
| FIA | Flow injection analysis |
| FINRISK | National FINRISK study |
| FSH | Framingham Heart Study |
| GC | Gas chromatography |
| GD | Gestational diabetes |
| HFA | Hydroxy fatty acids |
| IFG | Impaired fasting glycemia |
| IGT | Impaired glucose tolerance |
| IR | Insulin resistance |
| KORA | Cooperative Health Research in the Region Augsburg |
| LC | Liquid chromatography |
| LDLs | Low-density lipoproteins |
| LysoPC | Lysophosphocholine |
| LysoPEs | Lysophosphoethanolamines |
| MDC-CC | Malmö Diet and Cancer Study-Cardiovascular Cohort |
| MONICA | Multinational monitoring of trends and determinants in cardiovascular disease |
| MS | Mass spectrometry |
| MS/MS | Tandem mass spectrometry |
| mTOR | Mammalian target of rapamycin |
| NCDs | Noncommunicable diseases |
| NGT | Normal glucose tolerance |
| NMR | Nuclear magnetic resonance |
| PC | Phosphocholine |

| | |
|---|---|
| PCa | Alkyl-phosphatidylcholines |
| PL | Phospholipids |
| ROC | Receiver-operating characteristic |
| SABRE | Southall And Brent REvisited cohort |
| S-AMP | Adenylosuccinate |
| T1D | Type 1 diabetes |
| T2D | Type 2 diabetes |
| TMAO | Trimethylamine N-oxide |

## 10.1 Chronic Diseases

Chronic diseases, also known as noncommunicable diseases (NCDs), are medical conditions that last for long periods of time and progress slowly. Often less visible than communicable diseases, they have noninfectious and non-transmissible cause. NCDs are currently the major cause of death worldwide (32 %, in 2012), more than all other causes combined (68 %, in 2012) [1, 2]. Contrary to common perception, the majority of all NCD deaths occur before the age of 70 and mainly in low- and middle-income countries where the access to affordable treatment and effective health-care services is limited [2]. The rapidly increasing burden of chronic diseases is a global threat for the population, not only for the high percentage of deaths but also for its economic, psychological, and social impact. Notably, the indirect costs of chronic diseases (e.g., inability to work, loss of productivity, cost of caregivers, among others) in the USA amounted to five times their direct costs (i.e., treatments, hospitalization) [3, 4].

The WHO has classified the major chronic diseases in four types as:

- Cardiovascular diseases (CVDs)
- Cancers
- Chronic respiratory diseases
- Diabetes

Altogether they accounted for the leading causes of NCD deaths in 2012 (see Fig. 10.1) [2]. In the following sections, the principal chronic diseases will be described, except for cancers, which are fully discussed in Chap. 9. Additionally, the major risk factors leading to chronic diseases will be explored, focusing on overweight/obesity as one of the biggest contributors.

### *10.1.1 Cardiovascular Diseases*

Cardiovascular diseases (CVDs) comprise several disorders of the heart and blood vessels including coronary heart disease (which leads to heart attack), cerebrovascular disease (which leads to stroke), rheumatic heart diseases, and other conditions. CVDs, particularly heart attack and stroke, are the first cause of death globally, with

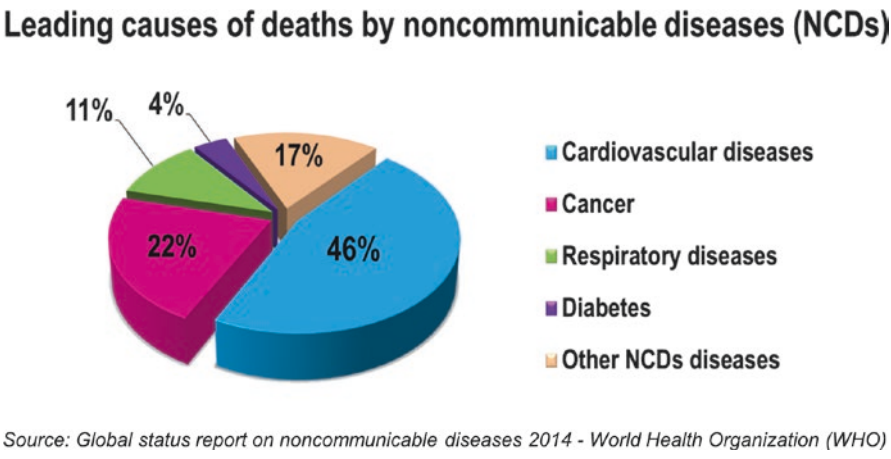


**Fig. 10.1** Pie chart displaying the leading causes of noncommunicable diseases (NCDs) deaths in 2012 [2] [Source: Global status report on noncommunicable diseases 2014 – World Health Organization (WHO)]

the low- and middle-income countries showing a substantial increased mortality over the years. Currently, over 80% of cardiovascular deaths occur in developing countries with a projection to increase [5].

Heart attack and stroke are usually acute events resulting from inadequate blood supply to a portion of myocardium (myocardial ischemia) or of the brain (cerebral ischemia); they are strongly associated with atherosclerosis, which consists of lipid accumulation in large arteries, that narrows the inner surface of the vessels by blocking or severely reducing the normal blood flow. The resulting lack of oxygen and glucose induces the death of the cells, thereby damaging the tissue.

*Atherosclerosis* has a complex etiology; it is initiated by inflammation in the endothelial layer of the artery that allows the low-density lipoproteins (LDLs) to accumulate in the inner layer of the artery, the intima. LDLs, and their oxidized form, then trigger the transmigration of immune cells, particularly monocytes, into the intima by creating plaques that become progressively larger with time. The plaque formation is a slow and silent process that develops over the years and eventually results in the plaque break or in the complete coronary/cerebral artery blockage (heart attack/stroke) causing premature death if untreated.

Although the recovery from the damage is possible, ischemic events often evolve into chronic disabilities that markedly affect the individual long life both emotionally and physically. Most importantly, patients who have suffered a heart attack and stroke have increased likelihood for second coronary and cerebral events [6].

Hence, prevention of atherosclerosis and CVDs is the most effective measure to prevent from premature morbidity, mortality, and disability. Indeed, although the drug therapy (i.e., combining aspirin, statins, beta-blockers, and diuretics) is effective in reducing the number of ischemic events, the identification of high-risk subjects and the preventions from complications remain the best option, both for people with established disease and for those at high risk of developing disease.

### *10.1.2 Chronic Respiratory Diseases*

Chronic respiratory diseases are a group of diseases affecting the airways and the other structures of the lungs. They include asthma and respiratory allergies, chronic obstructive pulmonary disease (COPD), occupational lung diseases, sleep apnea syndrome, and pulmonary hypertension [7]. According to the WHO, hundreds of millions of people are affected by chronic respiratory diseases, with asthma and COPD as the most prevalent lung diseases and major causes of morbidity and mortality worldwide. Indeed, it was estimated that currently 235 million people have asthma, whereas 64 million people suffer from COPD. Besides, in 2002, COPD has been the fifth leading cause of death globally, and it is expected to become the third in 2030 [8].

Asthma and COPD are multifactorial and complex diseases. They are characterized by a remarkable heterogeneity both in the clinical course and in their pathophysiological phenotypes that makes them frequently under-recognized, underdiagnosed, undertreated, and insufficiently prevented.

*Asthma* is a chronic inflammatory disorder mostly common among children where it appears with the same incidence as cancer and diabetes [9]. The typical symptoms include episodes of wheezing, coughing, chest tightness, and shortness of breath, generally in response to environmental exposure to various stimuli (allergens, viral respiratory infections, irritant fumes or gases). Along with a genetic predisposition, they trigger an inflammatory and immune response in the lungs' airways that causes an abnormal narrowing of the airways leading to the typical asthma symptoms [7].

In contrast, *COPD* is a multicomponent and systemic syndrome that affects both lungs and organs outside the lungs. It includes conditions such as emphysema and chronic bronchitis and is characterized by shortness of breath, cough, and sputum production. The principal underlying cause is cigarette smoking both from primary and secondhand exposure that together with occupational dust and chemicals (in high-income country) and indoor and outdoor pollution (mainly in low- and middle-income countries) damages the lungs progressively and irreversibly. COPD progresses slowly and is mostly asymptomatic until the frequent exacerbations and further reductions in airflow make it clinically apparent, generally by the age of 40 [10].

Up to now, no precise diagnosis or definitive therapy is available both for asthma and COPD. The diagnosis is typically based on the pattern of symptoms and the response to therapy over time and is eventually confirmed by the spirometry test. Concerning the therapy, the medicaments commonly employed are bronchodilators (long- and short-acting beta-agonist) and corticosteroids that reduce the inflammation and relieve the symptoms and oxygen administration for patients with chronic respiratory failure. Furthermore, avoiding asthma triggers reduces the severity of the asthmatic attack [11].

Although the management of these diseases is possible, they remain a health threat that need to be monitored over the life span. Indeed, the failure to use appropriate medications or to adhere to treatment can lead to death. Hence, the development

of reliable tests for an early and accurate diagnosis, the reduction of the exposure to the major risk factors, and prevention strategies to control the progression, exacerbation, and complications of the disease are the essential measure to efficiently manage these serious long-term diseases.

### *10.1.3 Diabetes*

Diabetes is a collection of metabolic diseases characterized by chronic high blood glucose levels (hyperglycemia) that, if not well controlled, causes serious damages to the whole body (i.e., the heart, blood vessels, eyes, kidneys, and nerves) and other long-term consequences that impair the quality of life significantly [12].

The WHO estimates that in 2014 diabetes has affected 422 million people in the world, mainly Southeast Asia and Western Pacific Regions, with prevalence among adult population. Over the past few decades, diabetes showed a steady rise (the incidence of diabetes has quadrupled since 1980), particularly in low- and middle-income countries, with an increased frequency in children and young people [13]. Moreover, in 2012, diabetes was the eighth leading cause of death globally with 3.7 million deaths, 1.5 million of which directly caused by diabetes and additional 2.2 million deaths from diseases (i.e., cardiovascular diseases, chronic kidney disease, and tuberculosis) related to higher-than-optimal blood glucose [13].

According to the different etiology underlying the insulin deficiency that causes hyperglycemia, diabetes has been classified in type 1 diabetes (T1D) and type 2 diabetes (T2D) [14]. Other conditions characterized by higher-than-optimal blood glucose have been described including impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG); they are intermediate conditions of hyperglycemia that may result in diabetes (mainly T2D). Although these conditions are not established diseases, they increase the risk for complications (e.g., CVDs) and have to be adequately monitored for life [12]. Additionally, gestational diabetes (GD), which is characterized by hyperglycemia and hyperinsulinemia that occur during pregnancy and usually remits after pregnancy, has been described as a temporary form of diabetes that can be responsible for adverse outcomes during pregnancy, childbirth, and future susceptibility to T2D [14, 15].

Concerning T1D, it is characterized by a selective autoimmune destruction of the pancreatic β-cells that reduces and eventually eliminates insulin production. Commonly with a juvenile onset and with a lesser incidence, it occurs in genetically susceptible individuals that are exposed to environmental factors still not well-defined (hypothetically viral infections, gut microbiota, and specific diet) [16]. In contrast, T2D results from gradual depletion in pancreatic β-cells mass and functions in response to peripheral insulin resistance that makes the body unresponsive to insulin and stimulates its secretion, thereby leading to β-cell exhaustion from failing to compensate the increased insulin demand. Genetic predisposition, ethnicity, older age, and environmental risk factors (i.e., overweight/obesity, inadequate physical activity, smoking, and poor diet) are the major underlying causes of T2D [13].

While no prevention strategies have yet been successful for T1D, since its etiology is still unknown, T2D is potentially preventable through diet and physical activity (remarkably more effective than medication) [13]. Besides, even though they are chronic progressive diseases, several measures can be carried out to assure long and healthy lives for diabetic subjects, including the access to insulin and a strict control of glycemia for T1D and lifestyle interventions and early diagnosis for T2D [13]. Early diagnosis is particularly important for T2D since its symptomatology is less marked than in T1D, and the diagnostic assays generally employed (i.e., fasting blood glucose test, oral glucose tolerance tests, measuring glycated hemoglobin) do not provide prediabetic and diabetic threshold values [14, 17]. It is noteworthy that despite the availability of several diagnostic tests, up to 62 % of T2D cases are undiagnosed and untreated [18]. This underscores the need for enhanced diagnostic tools to allow the delay or even the prevention of the disease onset and its complications.

### *10.1.4 Risk Factors*

Risk factors for chronic diseases can be gathered into three strongly interrelated groups: underlying factors (e.g., globalization, urbanization, socioeconomic determinants, aging), behavioral risk factors (e.g., physical inactivity, alcohol abuse, unhealthy diet, tobacco use), and metabolic/physiological risk factors (e.g., hypertension, hyperglycemia, hyperlipidemia, and overweight/obesity) [19].

Urbanization and globalization have greatly influenced the habits of the developing countries by promoting the rise of untraditional diets; the use of processed foods high in saturated fats, salt, and sugar; an increased tobacco and alcohol use; urban air pollution; and a more sedentary lifestyle, among others. The chronical exposure to these behavioral risk factors then represents the main underlying cause of NCDs and premature death. In 2012, alcohol abuse was responsible for 3.3 million deaths, with NCDs being responsible for more than half. In addition, tobacco causes six million preventable deaths every year, whereas 3.2 million annual deaths have been attributed to insufficient physical activity, and 1.7 million annual deaths from CVDs were attributed to excess of salt intake [2].

Behavioral risk factors are also responsible for metabolic/physiological alterations including hyperglycemia, hypertension, hyperlipidemia, and overweight/obesity that, in turn, contribute to the progression of the disease toward life-impairing complications and premature death.

However, despite the alarming incidence of chronic diseases worldwide, their slow evolution and the dependence on modifiable risk factors have influenced preventing measures which are expected to reduce the prevalence of NCDs by 25 % by 2015 [2].

Among the risk factors for chronic diseases, the condition of being overweight and obese is one of the biggest contributors. It can be considered as a model for the simultaneous investigation of several risk factors underlying the chronic diseases by

providing insights on the interaction patterns that may be responsible for the onset of such complex diseases. Indeed, the excess of body weight, which characterizes the medical condition of being overweight and obese, results from the interaction of genetic and environmental factors and includes at the same time the underlying and behavioral risk factors typical of NCDs. Besides, it is usually associated to the metabolic and physiological changes, such as hypertension, hyperlipidemia, and hyperglycemia, generally present in the NCDs, thereby posing a greater risk for their development. Importantly, the increase of NCDs over the years has mirrored the prevalence of obesity and overweight (e.g., in 2014, overweight and obesity accounted for about 65–80 % of the new cases of T2D in Europe).

#### 10.1.4.1 Overweight and Obesity

Obesity is a complex condition that affects virtually all age and socioeconomic groups, thereby being a global health threat, the "globesity." In 2014, more than 1.9 billion adults worldwide were overweight, and over 600 million of which were obese, with a predominance of women. Furthermore, according to WHO estimates, obesity causes 3.4 million deaths every year, that along with deaths caused by diseases of which obesity is a leading factor, and its strong social and psychological impact has placed obesity at the forefront of public health concern [20].

In clinical and epidemiological practice, the body mass index (BMI) is the parameter internationally recommended to categorize adult underweight, normal weight, overweight, and obesity (see Fig. 10.2). However, since it is an ethnic-independent measurement, the possibility to employ alternative BMI cutoffs in Asia and the Pacific Regions, where the risk of developing chronic diseases is at a lower BMI level than populations of European origin, is under evaluation [21].

Moreover, the waist circumference has been employed as additional measurement of obesity for its relationship with the visceral fat which is independent of the

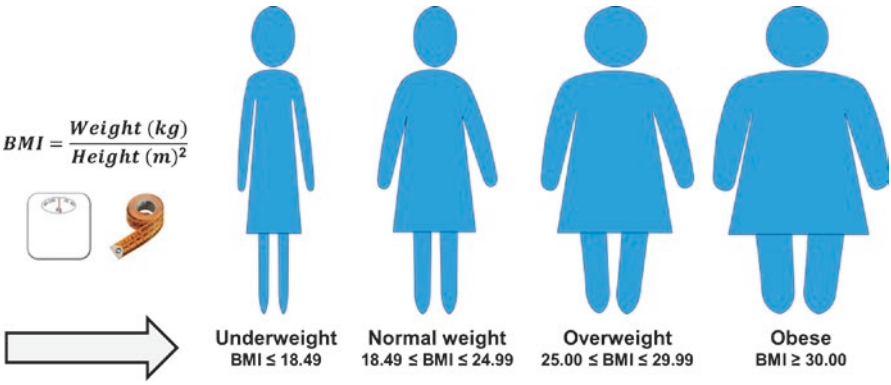


**Fig. 10.2** International classification of adult underweight, overweight, and obesity according to BMI [21]

changes in BMI. It provides sex-specific cutoffs that combined with BMI have demonstrated to have a potential ability to predict the risk of chronic diseases. Indeed, higher waist circumference and BMI have been associated with increased risk of CVDs and T2D by allowing timely interventions [22].

Currently, behavioral strategies (diet and physical activity) and taxing policies (e.g., increased price for sugary beverages and unhealthy food) are the most efficient measures to treat obesity [2]. However, the rapid increase of obesity, particularly in children and developing countries, has boosted the need of strategies aimed to preventing and controlling obesity especially in these vulnerable populations. It is noteworthy that the incidence of childhood overweight is increasing worldwide with 42 million of children (<5 years old) overweight in 2013 [20]. Moreover, childhood obesity is strongly associated with higher cardiometabolic risks in adolescence and higher morbidity and mortality from NCDs, mainly T2D and CVDs, in adulthood [23–25]. Hence, reducing the prevalence of obesity in children would have a long-term effect on reducing the prevalence in adults as well as on the susceptibility to chronic diseases later in life. Importantly, considerable evidences have highlighted that chronic disease risk is present from fetal life and continues cumulatively during the life span [26–29]; life-course investigation and interventions are thus essential in order to face and control the incidence and the premature mortality from NCDs.

## 10.2 Metabolomics and Chronic Diseases

The development of chronic diseases is a complex process. NCDs are characterized by a progressive dysfunction of metabolic and physiological functions in response to chronical exposure to lifestyle factors. From an evolutionary perspective, the rapid cultural change has far outpaced the genetic adaptation by generating a mismatch between the human evolution and the daily life, thereby increasing the susceptibility to chronic diseases [30]. Moreover, because of the polygenic nature of human traits and their adaptive nature, the phenotypic expression of such diseases turned out to be heavily affected by the environment; NCDs are indeed considered as a physiological adaptation of the body homeostasis to harmful lifestyle behaviors.

Thus, metabolomics, which measures the entire set of metabolites of a wide range of biological specimen in a certain time and under particular conditions [31, 32], has emerged as a versatile and valuable tool to investigate the etiology and the pathophysiology of such complex diseases [33]. Indeed, since it is the most proximal to the phenotype among the omics, it offers the possibility to investigate metabolic pathways that play a role in the overall metabolic dysfunction underlying NCDs (either before their onset or during their progression).

Over the years, metabolomics has addressed the investigation of chronic diseases by providing an integrated perspective on how metabolites interact in response to specific exposures by characterizing metabolic signatures of the diseases [34–37].

Besides, metabolomics has demonstrated predictive, diagnostic, and prognostic capabilities that have enabled the study of factors influencing the onset and progression of chronic diseases [38–40]. Metabolomics studies can be indeed classified according to these qualities depending on whether the focus of the study was on the identification of the subjects more susceptible to develop a certain disease in the future, on the early detection of a currently occurring disease, and on the determination of features able to predict the disease outcome or the efficacy of a treatment, respectively [40].

Thus, while predictive studies have the power to tackle the growth of the chronic diseases by anticipating their onset, diagnostic and prognostic studies are able to improve the management of an already overt disease, by preventing its adverse outcome. To achieve this goal, predictive studies require large cohorts (i.e., several thousands of participants), where initially healthy subjects are monitored through a large period of time (i.e., over 10 years) in prospective study designs. During the follow-up period, then, a limited number of healthy subjects will develop the disease by allowing the identification of risk factors, which are strongly related to the disease onset [41] (see Sect. 10.5.3 for more details on the major prospective studies of chronic diseases investigated by metabolomics approaches). Concerning diagnostic and prognostic studies, they employ instead cross-sectional studies aimed to identify biomarkers that allow tracking of the disease state in order to achieve a more effective patient stratification and a more accurate characterization of the disease outcome and the monitoring of the treatment's effectiveness [42].

Novel biomarkers, thus, hold the promise to be relevant tools in the clinical setting (in combination, or not, with traditional biomarkers) by driving a more effective decision-making process that helps the physician in the daily clinical practice. Additional information on the contribution of metabolomics strategies in the ambit of biomarker discovery for chronical diseases will be discussed in detail in the following section.

## 10.3 Metabolomics and Biomarker Discovery

Biomarkers are classified as screening, diagnostic, and prognostic according to their capability on detecting a future disease, a suspected disease, and the progression or remission of overt disease, respectively [43]. Since many diseases result in characteristic changes in the metabolite profiles, several metabolites have been employed as reliable biomarkers for decades [44–47]. Over the last few years, high-throughput technologies such as metabolomics, which broaden the coverage of the metabolome, have been applied with more frequency in the field of the biomarker discovery [33, 41, 48].

An ideal biomarker should be safe and easy to measure, cost-effective during both the discovery and the follow-up processes, and consistent across genders and different ethnicities [43]. Regarding the use of metabolites as diagnostic markers, one of the major challenges in metabolomics is the validation of the compounds statistically significant in small sets of well-selected samples, in a big cohort. While there are numerous screening studies in metabolomics research producing potential

biomarkers, most of the identified biomarkers have failed to replace existing clinical tests. To become a clinically approved test, a potential biomarker should be confirmed and validated using hundreds of individuals and should be reproducible, specific, and sensitive. The reproducibility is assured by validating the biomarkers in other study samples, preferably from an independent cohort. In contrast, concerning sensitivity and specificity, they are essential features of a biomarker as they measure the biomarker's ability to correctly detect subjects with the target condition (true positive rate) and without the target condition (true negative rate), respectively. They are generally computed through the receiver operating characteristic (ROC) curve analysis which provides the C-statistics or area under the curve (AUC) as a measure of the predictive ability of the biomarker model with values that range from 0.5 (random classification) to 1.0 (perfect classification) [49, 50].

However, in multifactorial disorders such as NCDs, single biomarkers rarely own high values of specificity and sensitivity; therefore, a multiple biomarker approach has been increasingly employed over the years to select the simplest combination of biomarkers that produces an effective predictive outcome [43].

Biomarkers (alone and more frequently in combination) can be further employed to generate risk scores as an estimate of the individual's risk of developing a certain disease in the future. The risk scores are usually generated within prospective studies that allow exploring the contribution of a new biomarker in an already existing predictive model [51]. This assessment is carried out by evaluating the discrimination power of the new model (model discrimination), the agreement between the observed outcome and the expected risk (model calibration), and the possibility to refine the stratification of the population into more pragmatic risk categories (i.e., reclassification of the subjects from an intermediate risk level to either an upper or lower risk level, risk reclassification) [52, 53].

Indeed, it is important to point out that for a metabolite to be employable as a biomarker other than in the clinical research, it has to prove to strengthen the predictive model beyond that achieved by conventional biomarkers that are employed in the clinical practice [43].

The discovery of new biomarkers is therefore a challenging task that metabolomics has addressed only recently by providing promising findings mainly in the field of hypothesis-generating biomarkers. This typology of biomarkers, which is focused on explaining the pathophysiological mechanisms underlying a disease, aims to understand the metabolic alteration associated with a disease with the ultimate goal of driving the discovery of a more efficient and personalized treatment or the design of new drugs from an informed perspective.

## 10.4 Study Design and Analytical Considerations

Regarding the workflow in metabolomics, researchers in the field do not agree upon the terms, not only for metabolomics and metabonomics, which were originally considered as different definitions that nowadays are used indistinctively [31, 32,

54], but also for the approach employed (i.e., fingerprinting, global profiling, profiling, among others). Hence, in order to simplify, in the present chapter, they will be referred to as targeted or untargeted metabolomics.

In the targeted approach, specific metabolites of known identity are analyzed. In mass spectrometry (MS), this often involves the addition of multiple stable isotope-labeled standards to the biological sample prior to the extraction and derivatization steps to control for differences in analyte loss during sample processing and to compensate for ionization-suppression effects. Advantages of targeted methods are that (i) identification of compounds is straightforward and (ii) metabolites can be quantified. A disadvantage is their limited metabolite coverage that can include from a small set to several hundred metabolites.

In contrast, untargeted metabolomics involves the simultaneous measurement of as many metabolites as possible in a biological specimen. This approach is generally used in differential analysis of two or more biological or clinical states/treatments; the report consists of differences between the states and is based on signal abundances of raw spectral data. The chemical identity of the signals is not known a priori, and significant chemical/spectral analysis must be performed to define the molecular species. It is noteworthy to point out that while semiquantitative data can be employed in the discovery phase, quantitative data is paramount for implementation in the clinical practice.

In a standard metabolomics workflow, metabolites can come from any biofluid or tissue after convenient extraction and can be detected using various chemical detection platforms including MS and nuclear magnetic resonance (NMR) as the most important. Notably, due to the chemical diversity of the metabolites, no single analytical technique is able to cover the entire metabolome; therefore, whenever possible, a combination of platforms has been increasingly applied over the last few years. The multiplatform approach, indeed, broadens the metabolite coverage and at the same time allows a mutual cross validation of the metabolites that are detected in more than one analytical technique. Concerning NMR, it has the potential for high-throughput fingerprinting, minimal requirements for sample preparation, robustness of the response, and nondestructive nature of the technique. However, only medium to high abundance metabolites will be detected with this approach, and the identification of individual metabolites based on chemical shift signals, which cause sample clustering in multivariate analysis, is challenging in complex mixtures. MS-based metabolite detection is instead a powerful tool for investigations of metabolism due to its sensitivity for low-abundant molecules and flexibility for the detection of multiple chemical molecular classes. MS detection platforms are biased in their compatibility of a particular molecule with a mode of ionization or detection. The ability to globally profile highly complex mixtures of plant extracts is enhanced by coupling chromatography with MS detection. Thus, a “metabolomics platform” refers to the combination of a separation technique and MS. The most commonly utilized metabolomics platforms include liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS), and capillary electrophoresis–mass spectrometry (CE-MS). Following data acquisition and processing, MS-metabolomics data is often expressed as a matrix of molecular features defined by (i) elution time, (ii) mass

(mass/charge ratio), and (iii) abundance of the mass signal. Annotating the detected molecular feature as a metabolite is the major bottleneck in MS-metabolomics workflows [55].

## 10.5 Metabolic Signatures of Chronic Diseases

In the past decade, metabolomics has made remarkable progress in providing new insights into the systemic alteration underlying NCDs: (1) disease-related metabotypes have been described that reflect changes in metabolites (i.e., amino acids, lipids, and organic acids) in body fluids, organs, and/or tissues as consequence of disease or disease-related conditions; (2) the role of new contributors (i.e., gut microbiome) in the development and progression of NCDs has been unveiled; (3) markers of the disorder's onset, progression, and prognosis have been identified in prospective metabolomic-based studies. The metabolic signature of chronic diseases that arises from these discoveries (Fig. 10.3) will be described in the following sections by focusing both on metabolites and metabolic pathways.

### *10.5.1 Metabotypes of NCDs*

In 2009, Newgard et al. described for the first time a metabotype of obesity and insulin resistance (IR) characterized by the increase in branched-chain amino acids (BCAA, i.e., leucine, isoleucine, and valine) and related metabolites (i.e., propionylcarnitine (C3), isovalerylcarnitine (C5), glutamate) in mice ingesting a high-fat diet [36]. This finding was then corroborated by subsequent studies in obese and/or diabetic humans and rodents, by identifying BCAA and their by-products, mainly short-chain acylcarnitines, as sensitive metabolic marker of obesity, IR, and future T2D [56–58]. Interestingly, consistent with these studies, an improvement in insulin sensitivity associated with lower BCAA levels was described for subjects undergoing weight-loss interventions (i.e., dietary, behavioral, bariatric surgery) [59, 60].

Various hypotheses have been proposed for the increase of BCAA and related catabolites in obese and or/diabetic subjects including an increased protein intake, increased proteolysis, reduced protein anabolism, or impaired mitochondrial catabolism. While several studies have ascribed only a marginal role to the first four processes [61–63], the altered BCAA catabolism has been suggested as the principal mechanism underpinning such changes [58]. Recent findings have highlighted a decrease in BCAA-catabolizing enzymes (e.g., branched-chain alpha-keto acid dehydrogenase, BCKDH) in the fat and liver of obese genetically modified mice and rats; insulin was also linked to BCAA catabolism through its action on the hypothalamus [64]. Shin et al. indeed pointed out an inducing effect on the hepatic BCKDH, mediated by the insulin signaling in the brain that was

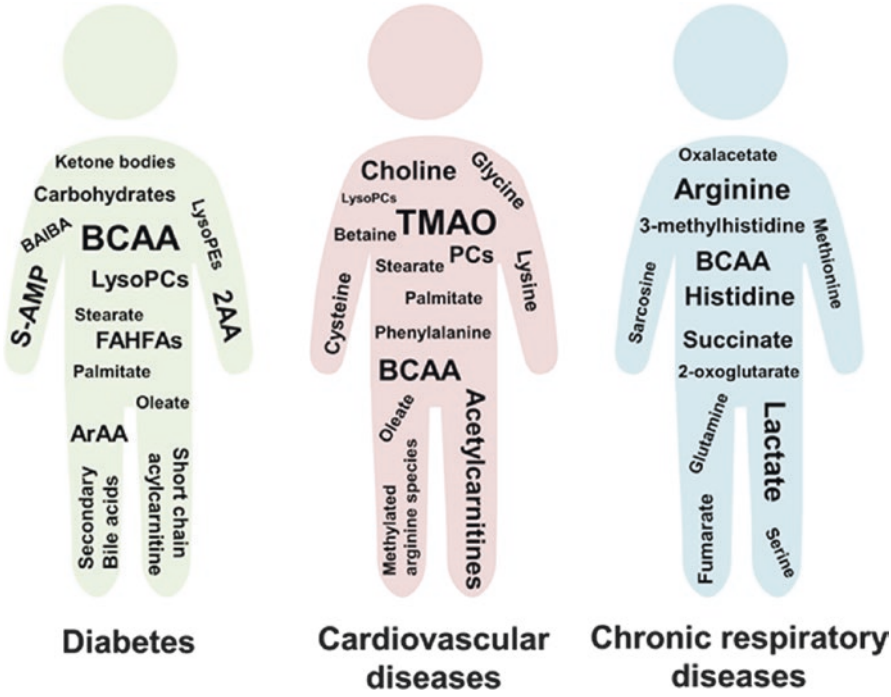


**Fig. 10.3** Metabolic signatures of chronic diseases unveiled by metabolomics. Abbreviations: *BCAA* branched-chain amino acid, *S-AMP* adenylosuccinate, *BAIBA* beta-aminoisobutyric, *ArAA* aromatic amino acids, *PC* phosphocholine, *LysoPCs* lysophosphocholines, *LysoPEs* lysophosphoethanolamines, *FAHFAs* fatty acid esters of hydroxy fatty acids, *2AA* 2-aminoadipic acid, *TMAO* trimethylamine N-oxide

found to be responsible for lowering the plasma levels of BCAA, thereby suggesting for the BCAA a role as marker of hypothalamic IR.

The detrimental effects mediated by BCAA have been attributed to their role on the overactivation of the mTOR (mammalian target of rapamycin) pathway which induces hepatic IR, thus worsening the systemic insulin signaling [65]. High levels of BCAA were also found to affect the fatty acids oxidation (FAO); the hepatic BCKDH is indeed involved in the catabolism of both BCAA and acylcarnitines, and in the case of high BCAA levels, it resulted to be overloaded, thereby producing incomplete FAO by-products (i.e., short-chain acylcarnitines) [66]. These metabolites then have been related to the mitochondrial stress and impaired insulin signaling that characterize T2D [67].

Further alterations in the amino acid metabolism were found to be associated to CVDs and/or related conditions. Wang et al. postulated that an increase in methylated arginine species (i.e., N-mono-methylarginine, asymmetrical dimethylarginine, and symmetrical dimethylarginine), which are related to the inhibition of the nitric oxide production, may serve as a marker of increased risk of coronary artery disease, myocardial infarction, and stroke [68], whereas Wang et al. revealed

changes in the levels of amino acids (glycine, lysine, and cysteine, particularly) in young hypertensive men by shedding light on metabolic variations taking place at an early stage of hypertension [69].

Changes in amino acid metabolism have been reported also for chronic respiratory diseases: Wedes et al. identified the urinary metabolite bromotyrosine, which is generated by the enzyme eosinophil peroxidase, as a noninvasive marker of future asthma exacerbation in children [70]; Jung et al. described alteration in metabolites (i.e., increase in methionine, glutamine, and histidine and decrease in acetate, choline, and arginine) in serum of asthma patients [71]; finally, several studies reported a decrease in plasma BCAA in COPD patients concomitant to cachexia [72–74].

A further example of metabotype of respiratory chronic diseases comprises of metabolites of the TCA cycle (i.e., succinate, fumarate, oxalacetate, cis-aconitate, and 2-oxoglutarate) that were found to be increased in urine and/or serum of asthmatic patients [71, 75]. High levels of lactate were also found in this patients by supporting the hypothesis of an upregulation of the TCA cycle due to a greater effort to breathe for the patients with a reduced oxygenation concomitant to the disease exacerbation [75].

An additional metabotype made up of lipids (i.e., mainly phospholipids and fatty acids) and illustrative of T2D, CVDs, and related conditions has emerged over the years by metabolomics and lipidomics approaches.

Phospholipids (PL) are an important class of lipids involved in NCDs. Generally described as the main components of the cellular membranes and lipoproteins (HDL and LDL mainly), they are involved in various metabolic pathways including signaling events and inflammation that are usually underlying NCDs and their related conditions. For instance, Ha and colleagues reported an altered lipid profile comprising of several PL metabolites, namely, six lysophosphocholines (LysoPCs C14:0, C16:1, C18:1, C18:3, C20:5, and C22:6) and three lysophosphoethanolamines (LysoPEs C18:1, C18:2, and C22:6), in case of diabetes; the lipid profile was also found to correlate to inflammation, oxidative stress, and future diabetes-related complications (i.e., arterial stiffness) [76]. Interestingly, also other LysoPCs (predominantly with long-chain acyl groups, $C \geq 16$) were found to be elevated in prehypertensive young men; these lipids then were described to be highly associated with oxidized LDLs, thereby featuring an increased oxidative stress and inflammation process as potential predictors of future hypertension, atherosclerosis, and CVDs [77].

High levels of fatty acids (i.e., palmitic acid, stearic acid, and oleic acid, among others) have been also associated to increased risk for T2D and CVDs. Yang et al. proposed a link between serum docosahexaenoic, palmitic, and palmitoleic acids and prevalence of hypertension [78]. Increased levels of free fatty acids and their oxidized by-product (beta-hydroxybutyrate, acetoacetate, and acetone) have been also associated to T2D and heart failure [79, 80]. Of note, a new class of fatty acids has been recently discovered by untargeted lipidomics, namely, the fatty acid esters of hydroxy fatty acids (FAHFAs) that consist of a combination of four fatty acids (FA) and four hydroxy fatty acids (HFA) [81]. FAHFAs were described to be present in food, synthesizable by mammalian, and at low levels in obese/insulin-resistant

humans and mice. Besides, conversely to other fatty acids, FAHFAs were described to exert a plethora of beneficial effects on diabetic-related conditions including the enhancement of glucose uptake from the bloodstream, improvement of insulin secretion and sensitivity, and reduction of inflammation. The FAHFAs' discovery represents therefore an important breakthrough in the field of NCDs and a great example of the potential of metabolomics for opening new avenues for the investigation of uncharacterized biochemical pathways in human physiology and diseases as well as proposing therapeutic targets for an alternative treatment of metabolic diseases.

### *10.5.2 New Contributors in Chronic Diseases*

The gastrointestinal tract comprises around 1013 cells (1,183–3,180 bacterial phylotypes) in adult's intestinal microbiome [82], which means $3 \times 10^6$ genes (130-fold higher than the number in human body) for a metabolically active organ that has been proposed as one of the major contributors to human health and disease. Indeed, accumulating evidences highlighted the crucial role of the gut microbiota on the development of chronic diseases (mainly T2D and CVDs) and related conditions (obesity, IR, and atherosclerosis, among others) by its action in several metabolic pathways including lipid metabolism, inflammation, energy metabolism, and insulin signaling [83, 84]. In seminal work, Turnbaugh and colleagues demonstrated that the transplant of microbiota from obese mice to germfree recipients was able to transfer the obese phenotype to the recipients that indeed experienced an increased weight gain in comparison to the mice that received a "lean microbiota" [85]. This study represented an important new insights into the role of the microbiome in the development of a disease or diseases-related condition. Since then, several studies have been carried out to investigate the gut microbiota and its relationship with health and diseases. Concerning the metabolomic-based investigations, various metabolites mirroring the action of the microbiome have been uncovered by providing new insights into how the microbiota interacts with the host and which metabolic pathways are involved in the gut–host cross talk [86].

Wang et al. identified a novel metabolite, namely, the trimethylamine N-oxide (TMAO), with a pro-atherogenic action that was found to be generated by the action of the gut microbiota on the dietary phosphatidylcholine (PC) [68]. Dietary PC is indeed the main source of the TMAO's precursors (i.e., choline and betaine) that have been previously related to risk for CVDs (i.e., lower levels of choline and higher CVD risk). Together these metabolites were also described to increase the risk for future cardiovascular events, thereby unveiling an important link between dietary intake of lipids, gut microbiota, and future CVD events. Of note, high levels of TMAO were also found in the urine of T2D patients by highlighting the potential of these metabolites for alternative therapeutical approaches [87]. A further example of microbiota-derived metabolites that play a major role in the host metabolism is represented by secondary bile acids (BAs, deoxycholate, and lithocholate, among

others) which are generated in the gut by the action of the microbiota and reabsorbed from the distal ileum through the enterohepatic circulation. BAs have been described as signaling molecules through their interaction with the farsenoid X receptor and the G-protein-coupled receptor TGR5 in the liver and adipose tissue, thus involved in the lipid and glucose homeostasis of the host [88]. Besides, the altered bile acid pool has been described as an underlying condition of various disease and disease-related states. For instance, Zhao and colleagues reported high levels of glycochenodeoxycholic acid in plasma of impaired glucose-tolerant subjects [89]; Mastrangelo et al. identified in the increased of taurodeoxycholic acid and glycochenodeoxycholic acid in serum samples of obese children a marker of IR state [90]; and Shure et al. identified an altered bile acids pool (low levels of cholic and muricholic acids and increased deoxycholic acid) in diabetic patients of the KORA cohort [91] (see Sect. 10.5.3).

Together with the influence of the gut microbiota, other novel contributors to NCDs have been uncovered by metabolomics, namely, the adenylosuccinate (S-AMP) and the beta-aminoisobutyric acid (BAIBA) that have been associated with T2D and cardiometabolic risk factors, respectively [92, 93]. While Gooding et al. unveiled a novel action of S-AMP as a glucose-derived amplifying stimulus of insulin secretion, Roberts et al. showed a fascinating effect of BAIBA (by-product of the catabolism of thymine or valine) on the browning of the white fat and on the stimulation of the beta oxidation in hepatocytes via PPAR-alpha. Briefly, Gooding et al. demonstrated the effect of glucose on the production of S-AMP (intermediated of the purine/nucleotide pathway) via the pentose phosphate pathway; they have also highlighted the stimulating action mediated by S-AMP on insulin secretion from human pancreatic beta cells upon normal and diabetic conditions, thereby showing a striking ability on rescuing the T2D-impaired secretory function in beta cells and suggesting a novel target for therapies. Roberts and colleagues instead discovered a novel effect of the BAIBA on the expression of the genes coding for brown adipocytes in murine white adipocyte and in human pluripotent stem cells during the differentiation to mature adipocytes; they also found an increase of the BAIBA during physical activity and a further inverse correlation of the BAIBA to cardiometabolic risks by suggesting new metabolic pathways related to the beneficial effect of physical activity.

### *10.5.3 Metabolomics in the Epidemiological Setting*

Prospective studies are important tools in the epidemiological setting to investigate the etiology of a disorder; indeed they offer the possibility to study a large cohort of subjects (i.e., thousands of participants) over a period of time (usually for years) by allowing the determination of the disease outcomes from initially healthy subjects and the eventual association with lifestyle risk factors to which they are exposed. An overview of the typical prospective study design is depicted in Fig. 10.4. The major prospective studies developed in the last years to address the investigation of NCDs

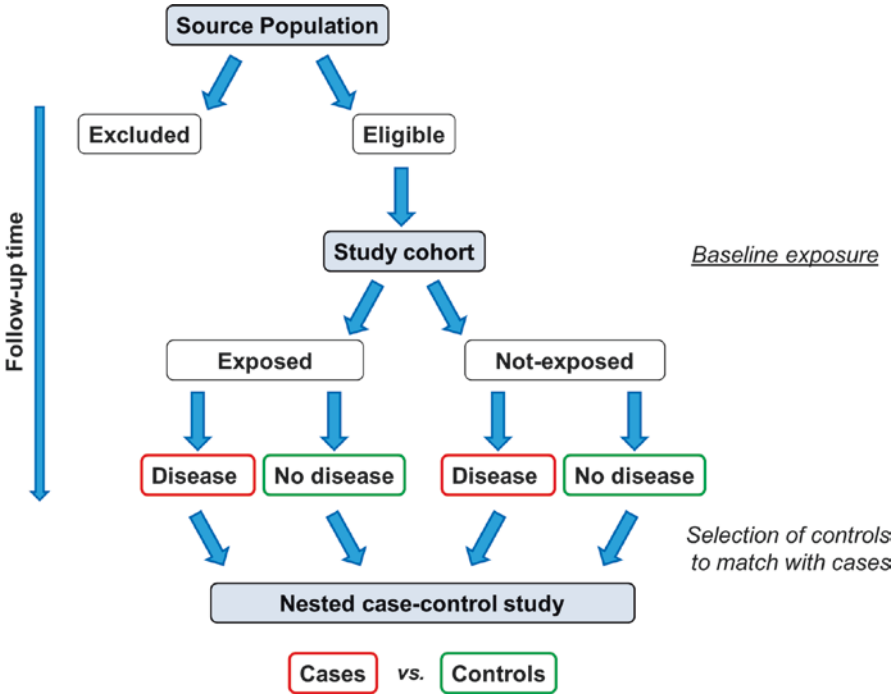


**Fig. 10.4** Flowchart of a prospective study design

and lifestyle biomarkers by a metabolomics strategy are described in the following paragraphs, and their main characteristics and findings are summarized in Table 10.1 and Fig. 10.5, respectively.

The *Framingham Heart Study (FHS)* is the first longitudinal study aimed to identify the common factors that contribute to CVD. The original cohort (5,209 men and women between the ages of 30 and 62 from Framingham, Massachusetts) was recruited in 1948 and followed up every 2 years. Further cohorts were also included (the Offspring Cohort in 1971, the Third Generation Cohort in 2002, the Omni Cohort in 1994, and the Second Generation Omni Cohort in 2003), for a total of over 15,000 participants for a study that is still ongoing [105]. The study, led by the National Heart, Lung, and Blood Institute, in collaboration with Boston University, has generated a variety of graded risk scores to estimate the risk of several cardiovascular diseases 10–30 years in advance by using a sex-specific algorithm that includes smoking habits, blood pressure levels, age, and family history of CVD events, among others [106]. Over the years, new technologies have emerged and successfully employed in the investigation of the Framingham cohorts. Among the metabolomic-based studies, accurate predictors of future cardiovascular disease, diabetes, and metabolic syndrome (including obesity, dyslipidemia, and dysglycemia) were uncovered by studying the offspring and the third-generation cohorts. Concerning the risk assessment for diabetes, in 2011,

**Table 10.1** Characteristics of the major prospective studies on NCDs and lifestyle biomarkers longitudinally investigated by a metabolomics approach

| Reference | Cohort study | Study design | *N*, follow-up time | Sample type | Analytical strategy | Outcome | Validation cohort |
|---|---|---|---|---|---|---|---|
| Wang et al. [42] | FSH offspring | Nested case–control | 378, 12 years | Plasma | Targeted LC-MS/MS | T2D | MCD-CC |
| Rhee et al. [94] | FSH offspring | Nested case–control | 378, 12 years | Plasma | Targeted LC-MS/MS | T2D | – |
| Cheng et al. [95] | FSH offspring | Nested case–control | 601, 12 years | Plasma | Targeted LC-MS/MS | T2D, CVDs | MCD-CC |
| Wang et al. [96] | FSH offspring | Nested case–control | 376, 12 years | Plasma | Targeted LC-MS/MS | T2D | MCD-CC |
| Yin et al. [97] | FSH offspring and third generation | Cohort prospective | 554, 5–7 years | Plasma | Untargeted GC-MS | Obesity, dyslipidemia | BioImage |
| Magnusson et al. [98] | MCD-CC | Nested case–control | 506, 12 years | Plasma | Targeted LC-MS/MS | CVDs | – |
| Floegel et al. [99] | EPIC-Potsdam | Case-cohort | 2282 (subcohort) and 800 (T2D cases), 7 years | Serum | Targeted FIA-MS/MS | T2D | KORA |
| Drogan et al. [100] | EPIC-Potsdam | Nested case–control | 300, 6 years | Serum | Untargeted LC-MS | T2D | – |
| Jacobs et al. [101] | EPIC-Potsdam | Case-cohort | 1,610 (subcohort) and 417 (T2D cases), 7 years | Serum | Targeted FIA-MS/MS | T2D and coffee consumption | – |

(continued)

**Table 10.1** (continued)

| Reference | Cohort study | Study design | *N*, follow-up time | Sample type | Analytical strategy | Outcome | Validation cohort |
|---|---|---|---|---|---|---|---|
| Wittenbecher et al. [102] | EPIC-Potsdam | Case-cohort | 2,681 (subcohort) and 688 (T2D cases), 7 years | Serum | Targeted FIA-MS/MS | T2D and red meat consumption | – |
| Wang-Sattler et al. [38] | KORA S4/F4 | Case-cohort | 641 (NGT) and 118 (IGT), 7 years; 876 (subcohort) and 91 (T2D), 7 years | Serum | Targeted FIA-MS/MS | Prediabetes (IGT), T2D | EPIC-Potsdam |
| Mook-Kanamori et al. [103] | KORA S4/F4 | Case-cohort | 755 (subcohort) and 121 (cases), 7 years | Serum | Targeted LC-MS/MS | Hypertriglyceridemia | – |
| Wahl et al. [104] | KORA S4/F4 | Cohort prospective | 1,658, 7 years | Serum | Untargeted LC-MS/MS$^2$ GC-MS NMR | Weight change | – |
| Wurtz et al. [37] | FINRISK | Case-cohort | 7,256 (subcohort) and 800 (CVD), 15 years | Serum | Targeted NMR LC-MS | CVDs | SABRE BWHHS |

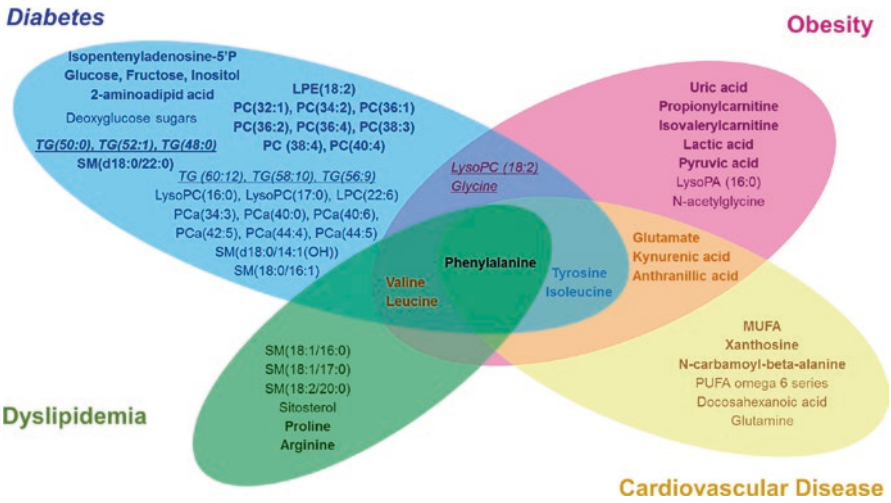


**Fig. 10.5** Venn diagram illustrating the metabolites found to be associated with diabetes, cardiovascular disease, obesity, and/or dyslipidemia by prospective studies investigated by metabolomics. *Bold* metabolites were found to be increased in one of the four conditions understudy, whereas *underlined* metabolites were found to be highly associated with insulin resistance

Wang et al. described fasted levels of five amino acids (i.e., isoleucine, leucine, valine, tyrosine, and phenylalanine), at a baseline exposure, as highly associated with future onset of diabetes, particularly in predisposed subjects (i.e., obese and with high fasting glucose levels) [42]. They further uncovered that a combination of three amino acids (i.e., isoleucine, phenylalanine, and tyrosine), the so-called diabetes-predictive amino acid score (DM-AA score), predicted future diabetes up to 12 years in advance (four- to fivefold higher risk for individuals with the highest amino acids score). The findings were also replicated in an independent cohort study, the Malmö Diet and Cancer study (MCD, see below), by demonstrating their generalizability. Notably, a further link was uncovered in the same study population between the double-bond content and the carbon chain length of lipids (mainly triglycerides) and the risk of diabetes: lipids of lower/higher carbon number and double-bond content are associated to an increased/decreased risk of future diabetes (12 years in advance) [94]. In 2012, Cheng et al. identified an association between tryptophan metabolism by-products with future CVDs; besides, they confirmed the previous findings for the DM-AA score and uncovered a further metabolite (glutamine) as inversely related to future risk of diabetes [95]. In 2013, Wang et al. unveiled a further metabolite (2-aminoadipic acid, 2-AAA) as strongly associated with future diabetes (up to 12 years in advance), both in the discovery (FHS) and replication (MCD) cohorts [96]. Subsequent studies on cell-based and animal models have suggested that the 2-AAA might be involved in the stimulation of insulin secretion in pancreatic β-cells and the modulation of glucose homeostasis in vivo, respectively [96]. Finally, in 2016, Yin et al. investigated the relationship between metabolic profiles, at the baseline level,

with risk factors of the metabolic syndrome including obesity, dyslipidemia, and dysglycemia [97]. They discovered longitudinal associations between several metabolites, such as lipids [e.g., lysoPA(16:0), sphingomyelins, and sitosterol] and organic acids (e.g., quinic acid), with one or more features of the metabolic syndrome.

The *European Prospective Investigation into Cancer and Nutrition (EPIC)* is a prospective cohort with more than 521,000 study participants (men and women, between 35 and 70 years old) enrolled from 23 centers in ten Western European countries. Originally designed to explore the association between nutrition and cancer, it has included over the years the investigation of other chronic diseases such as CVDs and T2D. At the enrolment (1992–1999), detailed information on diet, lifestyle characteristics, anthropometric measurements, and medical history was collected; blood samples were also taken and stored in liquid nitrogen at the International Agency for Research on Cancer – World Health Organization [107]. Among the NCDs investigated, the association between cancer and diet has been the most studied, whereas upon the study of CVD and T2D and their risk factors, only two cohorts were used, namely, a selection of the MDC cohort, the MDC Cardiovascular Cohort (MCD-CC, 6,103 participants), and the EPIC-Potsdam cohort (27,584 participants), respectively [108, 109]. Concerning the MCD-CC, it was predominantly employed to replicate the findings of the FSH study, thereby describing a metabolic profile for diabetes and cardiovascular diseases' prediction. In 2013, the MCD-CC was also used by Magnusson et al. to investigate the predictive capability of the DM-AA score described by Wang et al. (see above) both for the onset and the consequences of a CVD event [98]. They found that the DM-AA score was able to predict CVD events (12 years in advance) by suggesting a possible link between diabetes and CVDs. Besides, a link between the amino acid score and an increased propensity toward atherosclerosis and inducible ischemia was unveiled. In the same year, Floegel et al. described for a subcohort of the EPIC-Potsdam study (2,500 selected randomly subjects and 800 T2D cases) a significant association between serum metabolites both with increased risk of T2D (e.g., hexose, phenylalanine, and diacyl-phosphatidylcholines) and decreased risk of T2D [i.e., glycine, SM(18:0/16:1), LPC(18:2), and alkyl-phosphatidylcholines] [99]. These metabolites were further included in the predictive model of the German Diabetes Risk score (i.e., ROC AUC from 0.847 to 0.912), thereby demonstrating their value as biomarkers. The results were then successfully replicated in the prospective KORA study (see below). The EPIC-Potsdam subcohort was further employed in 2015 to generate a nested case–control study for the investigation of the pathophysiology of T2D by using an untargeted approach [100]. Alteration in serum carbohydrates (e.g., hexoses), purines (e.g., isopentanyladenosine-5-monophosphate), and phospholipids [e.g., LPC(16:0)] was found to predict the onset of T2D up to 6 years in advance. Finally, the EPIC-Potsdam cohort was investigated to evaluate the effect of the specific food consumption (i.e., coffee and red meat) and the incidence of T2D [101, 102]. Sex-specific correlations were found by showing an inverse trend between coffee and T2D risk only in men and different metabolic profiles according to sex both for coffee and red meat consumption. Concerning the coffee consumption, only phenylalanine was

found to be slightly associated to T2D, whereas ferritin, glycine, and some lipids [i.e., PC(36:4), LPC(17:0), and SM(14:1)] were found to reflect both red meat consumption and increased risk for T2D.

The *multinational monitoring of trends and determinants in cardiovascular disease (MONICA)* is a WHO-funded project aimed to monitor the common risk factors (i.e., cigarette smoking, hypertension, obesity, total cholesterol) leading to CVD; a total of 38 populations and 21 countries from all over the word were included in the project, and more than ten million of men and women (25–64 years old) were surveyed (overall period covered: 1979–1996) [110]. Although the MONICA project ended in 1996, the survey on the Augsburg cohort continued and derived into the MONICA/Cooperative Health Research in the Region Augsburg (KORA) study (18,000 participants) that added the study of diabetes to the investigation of CVDs. The MONICA/KORA study comprises of four surveys (S1 to S4, from 1996 to 2001) that were performed with a 5-year interval and followed longitudinally between 4 and 20 years [111]. In 2010 the first metabolomic-based study was performed on a subset of the KORA F3 cohort (40 cases and 60 controls, males, over 54-year-old) [91]. This pilot study replicated the findings of known biomarkers of diabetes (i.e., BCAA, sugar metabolites, ketone bodies) and identified novel metabolites (i.e., 3-idroxyl sulfate, glycerophospholipids, free fatty acids, and bile acids) related to diabetes under subclinical condition. In 2012, then Wang-Sattler et al. investigated a subset of the KORA S4/F4 cohort (876 participants) by unraveling three metabolites (i.e., glycine, LPC(18:2), and acetylcarnitine) as markers of prediabetes [38]; the findings were replicated in the EPIC-Potsdam cohort by describing a role for glycine and LPC(18:2) as marker both of prediabetes and T2D. Besides, the KORA S4/F4 cohorts (S4 $n=4{,}261$, F4 $n=3{,}080$) were further investigated to explore the relationship of the metabolic profile with risk factors for T2D and CVDs including hypertriglyceridemia and obesity: Mook-Kanamori et al. highlighted increased levels of amino acids (i.e., leucine, valine, arginine, proline, and phenylalanine) as related to high levels of triglycerides both at the baseline and at 7 years follow-up [103], whereas Wahl et al. identified in dyslipidemia (altered lipoproteins and triglycerides) and modulated amino acids metabolism (mainly BCAA) the features of a potential mitochondrial dysfunction underlying long-term weight change [104]. Finally, a further prospective study that derives from the MONICA project and employs a metabolomics approach, namely, the National FINRISK Study (7,256 participants, overall period 1972–2012) [112], was explored by Wurtz et al. by identifying serum level of phenylalanine, monounsaturated fatty acids, omega-6 fatty acids, and docosahexaenoic acid as hallmarks for future CVDs. The results were further validated in two independent UK cohorts (i.e., Southall and Brent REvisited cohort and British Women's Heart and Health Study cohort) [37].

*The Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE)* is a 3-year longitudinal study conducted at 46 centers in 12 countries. A total of 2,180 COPD patients (men and women aged 40–75, under medication) were surveyed every 3 months in order to identify predictors of the COPD progression and improve the discrimination of the COPD subtypes [113]. In 2012, Ubhi et al. employed a subset of the ECLIPSE cohort for two metabolomic-based investigations

that unraveled changes in the metabolism of several amino acids (e.g., serine, sarcosine, tryptophan, BCAAs, and 3-methylhistidine, among others) that enabled the stratification of COPD patients (i.e., smoker vs. nonsmokers, patients with and without emphysema or with and without cachexia) [73, 114]. Although these findings provided valuable information in the partially/merely explored field of respiratory chronic diseases, none of the metabolomic-based studies investigated the ECLIPSE cohort longitudinally, and markers of disease progression and patient's outcome are still lacking to date.

## 10.6 Concluding Remarks

Through this chapter we have highlighted the versatility and the striking potential of metabolomics to provide new advances in the field of chronic diseases: disease-related metabotypes were described; crucial players involved in the NCDs are unveiled; and finally, a long-term perspective on the disease's progression was pointed out. Even though several limitations still need to be addressed (i.e., the improvement of the metabolite identification, the exploiting of the synergies between different omics, and the effective use of metabolomics in clinical practice, among others), the metabolic signature of diseases that is revealed by the study of NCDs is a clear demonstration of the importance of this discipline, not only for NCDs but also in the wider context of the human health. The metabolic alterations are indeed potentially detectable, understandable, and ultimately treatable by a metabolomic-based strategy that thus holds the promise to drive a paradigm shift toward the tailoring of the therapy on the altered metabolic pathways rather than on the disease's symptomatology.

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