REVIEW

Metabolomic Signature Between Metabolically Healthy Overweight/Obese and Metabolically Unhealthy Overweight/Obese: A Systematic Review

Dihe Cheng
Xue Zhao
Shuo Yang [ID]
Haiying Cui
Guixia Wang

Department of Endocrinology and Metabolism, The First Hospital of Jilin University, Changchun, 130021, People's Republic of China

Abstract: The clinical manifestations of overweight/obesity are heterogeneous and complex. In contrast to metabolically unhealthy overweight/obese (MUO), a particular sub-group of obese patients who are considered as metabolically healthy overweight/obese (MHO), display favorable metabolic profiles characterized by high levels of insulin sensitivity, normal blood pressure, as well as favorable lipid, inflammation, hormone, liver enzyme, and immune profiles. While only a few available studies focused on the metabolic files underlying the obese phenotypes, the current review aimed to perform a systematic review of available studies focusing on describing the metabolomic signature between MUO and MHO. We did the systematic search for literature on MEDLINE (PubMed), the Cochrane Library, EMBASE, and searched for the references of relevant manuscripts from inception to 29 May 2020. After critical selection, 20 studies were eligible for this systematic review and evaluated by using QUADOMICS for quality assessment. Eventually, 12 of 20 studies were classified as "high quality". Branched-chain amino acids (isoleucine, leucine, and valine), aromatic amino acids (phenylalanine and tyrosine), lipids (palmitic acid, palmitoleic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid), and acylcarnitines (propionyl carnitine) levels might be elevated in MUO. The current results suggested that MHO showed a favorable trend in the overall metabolic signature. More longitudinal studies are needed to elaborate deeply on the metabolic pathway and the relationship between metabolic patterns and the occurrence of the disease.

Keywords: metabolically unhealthy overweight/obese, metabolically healthy overweight/obese, metabolomics, amino acid, lipid

Introduction

Obesity is defined as an excess of body fat and alters the state of metabolism and physiology leading to an increased risk of all-cause mortality, especially cardiovascular disease and type 2 diabetes mellitus.[1,2] Additionally, obese individuals are more likely to suffer cancer, asthma, gallbladder disease, osteoarthritis, and chronic pain.[3] It is estimated that more than 1.9 billion adults are overweight and 650 million are obese throughout the world, and approximately 2.8 million deaths were reported as a result of overweight or obese.[4] Hence, overweight/obesity represents a major public health concern in both developing and developed countries.

However, the clinical manifestations of overweight/obesity are heterogeneous and complex. In contrast to metabolically unhealthy overweight/obese (MUO), a particular sub-group of obese patients who are considered as metabolically healthy

Correspondence: Guixia Wang
Department of Endocrinology and Metabolism, The First Hospital of Jilin University, Changchun, 130021, People's Republic of China
Tel +15843081103
Email gwang168@jlu.edu.cn

overweight/obese (MHO), display favorable metabolic profiles characterized by high levels of insulin sensitivity, normal blood pressure, as well as favorable lipid, inflammation, hormone, liver enzyme, and immune profiles.[5] Currently, there are still no unique criteria to define metabolic health, resulting in the prevalence of the MHO phenotype widely varying. The prevalence estimates (6 to 75%) depends on which criteria are used and some socio-demographic variables such as gender, age, and race/ethnicity.[6] Several previous studies have indicated that the MHO group is at a lower risk of type 2 diabetes mellitus (T2DM), cardiovascular disease, and mortality compared with the MUO group.[7,8] Paradoxically, there are also studies reporting inconsistent results that the MHO phenotype is not protected from obesity-related metabolic complications relative to other phenotypes.[9–11]

Modern metabolomics is a comprehensive and systematic identification and quantification of small molecular weight metabolites (<1500 Da) in biological samples at a given point of time.[12] The metabolome represents a set of all metabolites present in cell, tissue, organ, or organism at a given time and can capture the dynamic physiological conditions corresponding to the clinical outcomes of interest. Nuclear magnetic resonance (NMR) and mass spectrometry (MS), coupled with different separation techniques, dominate in metabolomics research.[12] The potential use of metabolomics in the biomedical field is the discovery of new biomarkers for prognosis and/or diagnosis of diseases, such as obesity, diabetes, metabolic syndrome.[13–15] Our previous work showed metabolomics profiles might be useful biomarkers and predictors for childhood obesity and diabetic kidney disease.[16,17]

Even though the concept of MHO remains controversial, a profound understanding of the underlying metabolic regulation between the MHO and the MUO is necessary to enhance the current knowledge of the development and regulation pathways of different overweight/obesity-related metabolic profiles, and to optimize prevention and treatment strategies. Recent literature on metabolic signature in overweight/obesity mainly focused on the comparisons between normal weight and overweight/obese individuals and only a few studies focused on the metabolic profiles underlying the different overweight/obese phenotypes. Thus, the current work aimed to perform a systematic review of available studies focusing on describing the metabolomic signature between MUO and MHO.

Methods
Literature Search Strategy

We did the systematic search for papers on MEDLINE (PubMed), the Cochrane Library, EMBASE and searched for the references of relevant manuscripts. Studies were identified in the databases by applying a publication date of 29 May 2020, using the following relevant keywords: "metabolic healthy" OR "metabolically healthy" OR "metabolic benign" OR "metabolically benign" OR "metabolic syndrome" AND "body mass index" OR "overweight" OR "obesity" OR "adiposity" OR "obese" AND "metabolomics" OR "metabolome" OR "mass spectrometry" OR "magnetic resonance spectroscopy" OR "metabolic signature" OR "metabolomic profiles".

During this process, two independent investigators (Dihe Cheng and Xue Zhao) finished this work to reduce selection bias. If there were disputes, a third investigator (G. Wang) joined in to resolve the disagreements.

Inclusion and Exclusion Criteria

Studies were considered eligible for the following inclusion criteria: (1) were conducted in the human population; (2) focused on biomarkers for metabolic healthy overweight/obese and metabolic unhealthy overweight/obese; (3) metabolic health overweight/obese was used as the control group, and there were two ways to define metabolically healthy overweight/obese: (i) metabolically healthy overweight/obese was clearly defined in the literature included or (ii) the included literature did not define metabolic health overweight/obese, but did define overweight/obese without metabolic syndrome. Overweight/obesity without metabolic syndrome was also considered as metabolically healthy overweight/obesity in the current systematic review; (4) stratified subjects according to body mass index (BMI) categories or defined overweight/obesity; (5) utilized a metabolomics approach, such as MS, NMR spectroscopy, or ultrahigh pressure liquid chromatography mass spectrometry (UPLC-MS); (6) the types of studies were observational, including cross-sectional, cohort, and case-control studies; (7) only papers published in English were included. Studies were excluded if they were categorized as animal studies, editorials, literature reviews, case reports, and conference abstracts.

Data Extraction and Analysis

From each study, we extracted the following information: authors, year of publication, country, population (tissue),

study design, platform, sample size, participant characteristics, and significant findings. During this process, two independent investigators (Dihe Cheng and Shuo Yang) finished this work to reduce selection bias. Due to the high heterogeneity in study population characteristics, platforms, analysis methods, and outcomes, a quantitative meta-analysis could not be implemented.

Quality Assessment

We evaluated the quality of the studies independently and in duplicate based on QUADOMICS.[18,19] QUADOMICS is an adaptation of QUADAS to the special nature of '-omics'-based diagnostic research including in systematic reviews. Points were summed (up to 1 point per criterion), and studies with scores 0–10 were considered as "low quality", while scores 11–16 were considered as "high quality".

Results
Literature Search and Study Characteristics

The process for the selection of studies according to the PRISMA statement was shown in Figure 1. Initially, among the 2746 abstracts and titles reviewed, 2723 were excluded. After retrieved 23 full-text articles, 19 of them were included in our present systematic review.

A total of 19 articles (20 studies) were included,[13,20–37] 5 of which were cross-sectional studies,[20,23,33,36,37] 13 of which were case-control studies,[13,21,22,24–29,31–32,34–35] and 1 article described both cross-sectional and cohort studies.[30] 2 of them used the same population, but used different metabolomic analysis method.[32,35] (Table 1). All the 20 studies were published between 2013 and 2020. The definitions of metabolic healthy overweight/obese were different in Table 1. Overweight/obesity in one study was defined by body fat or BMI,[29] and in others was defined by BMI but with different cut off point.[13,20–28,30–37] Sample types were multiple, as 11 studies used plasma,[13,20,21,23,25–27,29,31,36,37] 7 studies used serum,[22,28,30,32,33,35] 1 study used visceral adipose tissue,[24] 1 study used both cultured human adipocytes and plasma.[34] Only 1 study focused on children or adolescents,[25] and the subjects of rest studies were adults.[13,20–24,26–37] 19 studies included both males and females,[13,20–32,34–37] and 1 study only focused on females.[33] 14 studies used targeted methods,[13,20,23,26–30,32–34,36,37] 4 studies used untargeted methods,[21,22,24,25] and 2 study used both targeted and untargeted methods.[31,35]

Comparison of the Metabolomic Signature Between Metabolically Healthy Overweight/Obese and Metabolically Unhealthy Overweight/Obese

Table 2 summarized the different metabolomic signatures between MHO and MUO. By summarizing the metabolites identified, branched-chain amino acids (isoleucine, leucine, and valine), aromatic amino acids (phenylalanine and tyrosine), lipids (palmitic acid, palmitoleic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid), and acylcarnitines (propionyl carnitine) levels might be elevated in MUO.

Quality Assessment

According to QUADOMICS,[18,19] we conducted a quality assessment process (see the score in Table 1). 12 of 20 studies were classified as "high quality". The general characteristics of the selected studies and methodological quality assessment were independently checked by Dihe Cheng and Haiying Cui.

Discussion

To the best of our knowledge, this is the first systematic review focusing on describing the metabolomic signature between MUO and MHO. MHO is different from the metabolomic characteristics of MUO and has drawn much attention. MHO group is described at a lower risk of type 2 diabetes, cardiovascular disease, and mortality compared with the MUO group.[7,8] Although the results are controversial, the metabolomic signature in different overweight/obese phenotypes may help to identify the risk of developing metabolic diseases, which may lead to an appropriate treatment strategy.

Metabolomic Signature Between Metabolically Healthy Overweight/Obese and Metabolically Unhealthy Overweight/Obese
Amino Acid

Branched-chain amino acids (BCAAs), composed of isoleucine, leucine, and valine, levels of which were higher in MUO compared with MHO in many of the included studies, however, Kim and Badoud[21,35] did not find significant differences in BCAAs between MHO and MUO. Such inconsistencies may result from a different definition of MHO and a lack of a unified research platform. BCAAs
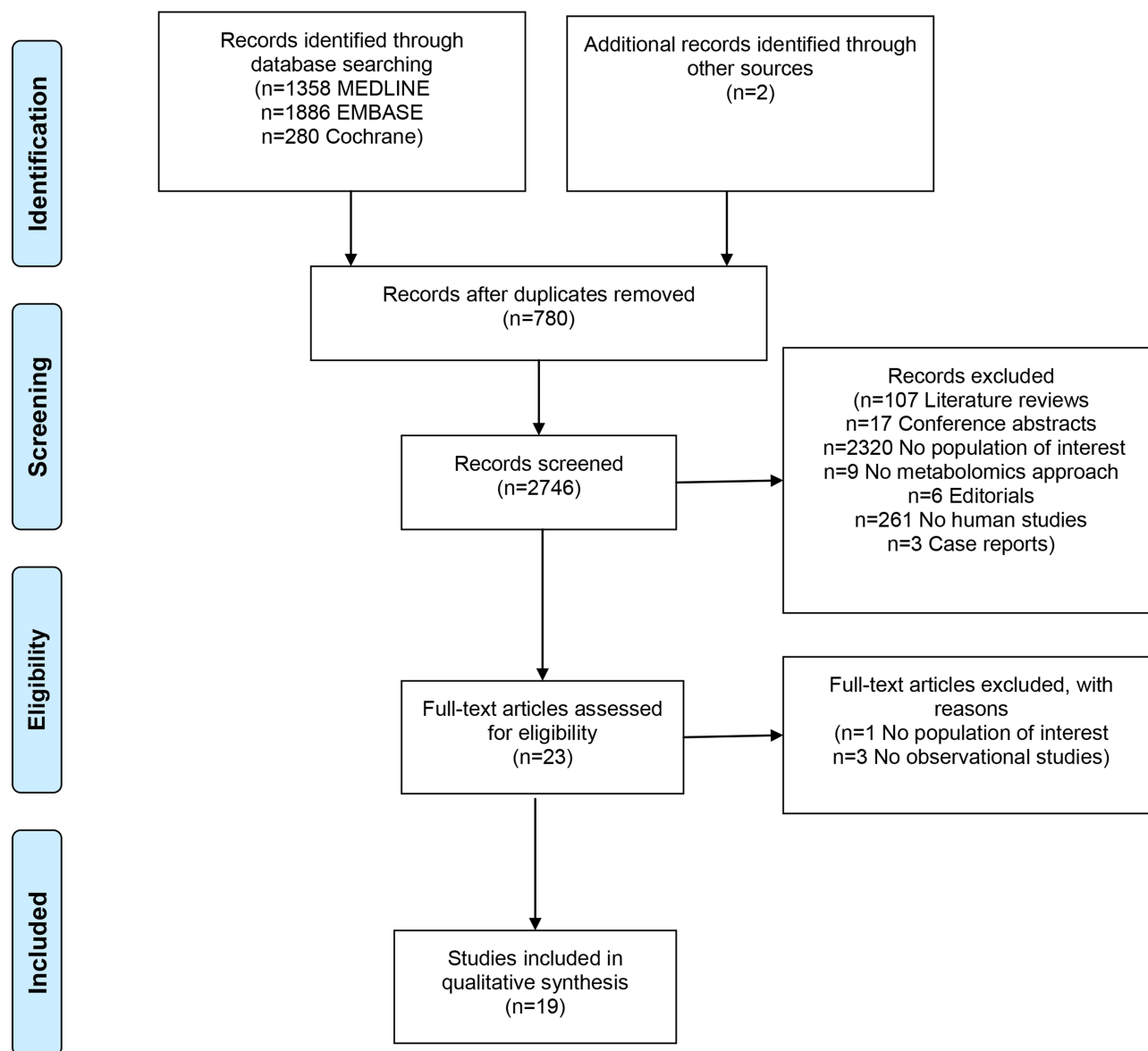
Figure 1 PRISMA flow diagram of this systematic review.
Note: Adapted from Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *Preferred Reporting Items for Systematic Reviews and Meta-Analyses*: The PRISMA Statement. PLoS Med 6(7): e1000097.

are often associated with some metabolically unfavorable indicators, especially lipid metabolism. Petri K Wiklund found factor which composed of BCAAs, aromatic amino acids, and orosomucoid was associated with very low density lipoprotein, and inversely with high density lipoprotein.[33] Telle-Hansen also discovered that there was a strong positive correlation between BCAAs and triglyceride and a negative correlation between BCAAs and high density lipoprotein cholesterol.[20] Results showed BCAAs were significantly reduced in plasma of MUO when compared with MHO in children and adolescents.[25]

This finding may indicate that compared with adults, metabolic regulation mechanisms may be different in children and adolescents. The increased levels of BCAAs are associated with obesity and the risk of T2DM.[14,15,38] Defective BCAAs oxidative metabolism might lead to a further accumulation of BCAAs and toxic intermediates in general obese subjects.[39] A hypothesized mechanism linked the increased levels of BCAAs and T2DM involved leucine-mediated activation of the mammalian target of rapamycin complex 1 (mTORC1), which results in uncoupling of insulin signaling at an early stage.[40] Higher

Table 1 Characteristics of Studies Identifying Metabolomic Signature Compared Between Metabolically Unhealthy Overweight/Obese and Metabolically Healthy Overweight/Obese

Study (Year)	Country	Study Design	Population (Tissue)	Platform	Criteria for Definition of Metabolic Health	BMI Categories	Sample Size (n)	Mean Age	Male (%)	BMI (kg/m²)	Score
1. Telle-Hansen et.al (2020)[20]	Norway	Cross-sectional study	Adult (plasma)	GC-MS; GC-FID; NMR (targeted)	≥3 of the following criteria: HOMA-IR index ≤ 1.95, TG ≤ 1.7 mmol/L, TC ≤ 5.2 mmol/L, LDL-cholesterol ≤ 2.6 mmol/L, and HDL-cholesterol ≥ 1.3 mmol/L.	Normal weight BMI ≤ 25 kg/m²; obese BMI ≥ 30 kg/m²	MHO (9)	49 (42–63)	5 (55)	33 (30–37)	10
							MUO (10)	52 (43–59)	9 (90)	32 (30–34)	
2. Kim et.al (2020)[21]	Korea	Case-control study	Adult (plasma)	LC-MS (untargeted)	Metabolic syndrome: SBP ≥ 130 mmHg or DBP ≥ 85 mmHg, TG ≥ 150 mg/dL, TC ≥ 200 mg/dL, HDL cholesterol < 40 mg/dL in males or < 50 mg/dL in females, LDL cholesterol ≥ 130 mg/dL, fasting glucose ≥ 100 mg/dL, and visceral fat area at L4 ≥ 100 cm². Metabolically health have normal status in all of the above indices, except in the waist circumference.	Obese 25 kg/m² ≤ BMI < 30 kg/m²	MHO (34)	35.7 ± 1.62	6 (17.6)	27.0 ± 0.20	12
							MUO (34)	37.3 ± 1.41	6 (17.6)	27.1 ± 0.22	
3. Chashmniam et.al (2020)[22]	Iran	Case-control study	Adult (serum)	1H-NMR (untargeted)	Meeting all the following criteria: SBP < 140 mmHg and DBP<90 mmHg, no antihypertensive drug use, FBS < 126 mg/dl, no hypoglycemic agents use, and serum levels of HDL cholesterol ≥ 40 mg/dL in men or ≥ 50 mg/dL in women.	Non-obese BMI < 30 kg/m²; obese BMI ≥ 30 kg/m²	MHO (21)	29 (25.0–35.0)	8 (38.1)	31.7 (30.45–32.60)	13
							MUO (21)	26 (23–33)	10 (47.6)	31.2 (30.5–33.3)	

(Continued)

Table 1 (Continued).

Study (Year)	Country	Study Design	Population (Tissue)	Platform	Criteria for Definition of Metabolic Health	BMI Categories	Sample Size (n)	Mean Age	Male (%)	BMI (kg/m²)	Score
4. Ojwang et.al (2019)[23]	South Africa	Cross-sectional study	Adult (plasma)	GC-MS/MS (targeted)	Metabolic syndrome (JIS recommendations) ≥ 3 criteria: FPG levels ≥ 5.6 mmol/l or the use of oral hypoglycaemic medication, serum TG ≥ 1.7 mmol/l, serum HDL ≤ 1.0 mmol/l for men and ≤ 1.3 mmol/l for women, BP ≥ 130/85 mmHg or the use of BP medication, and WC of ≥ 94 cm for men and ≥ 80 cm for women. Metabolically health was defined as without metabolic syndrome.	Underweight and healthy weight BMI < 25 kg/m², overweight and obesity BMI ≥ 25 kg/m²	MHO[a] (120)	49 (43–56)	Unclear	30.4 (26.9, 34.1)	9
							MUO[a] (167)	55 (48–65)	Unclear	31 (27.6, 34.5)	
5 Rousseau et.al (2019)[37]	Canada	Cross-sectional study	Adult (plasma)	MS (targeted)	Metabolic syndrome: ≥3 of the following criteria: WC > 88 cm for women and 102 cm for men, fasting plasma TG ≥ 1.7 mmol/L, HDL-cholesterol levels ≤ 1.29 mmol/L for women and 1.03 mmol/L for men, glucose levels ≥ 5.6 mmol/L and resting SBP/DBP ≥ 130/85 mmHg. Metabolically health was defined as without metabolic syndrome.	Normal weight BMI < 25kg/m²; overweight BMI > 25kg/m²	MHO[a] (84)	35.7 ± 10.4	41(48.9)	31.4 ± 4.2	10
							MUO[a] (48)	38.4± 10.1	31(64.6)	34.3 ± 4.8	

Study	Country	Study type	Sample (source)	Method	Metabolic syndrome criteria	Weight criteria	Group (n)	Age	n (%)	BMI	#
6. Libert et.al (2018)[13]	USA	Case-control study	Adult (plasma)	HPLC-UV; LC-MS/MS (targeted)	Metabolic syndrome: ≤1 of the ATP III criteria: BP ≥ 130/≥ 85 mmHg, fasting glucose ≥ 110 mg/dL, HDL < 40 mg/dL for men, < 50 mg/dL for women, TG ≥ 150 mg/dL, and WC > 40 in. for men, > 35 in. for women	Normal weight BMI < 25 kg/m², obese BMI > 30 kg/m²	MHO (26)	37.5 ±10.5	12 (46)	35.0 (33.0, 39.7)	10
							MUO (20)	38.7 ± 11.7	11 (55)	37.4 (32.6, 40.0)	
7. Candi et.al (2018)[24]	Italy	Case-control study	Adult (visceral adipose tissue)	GC/MS;LC/MS/MS (untargeted)	Metabolic syndrome: ≥ 3 of the ATP III criteria: WC > 40 inches (men) or 35 inches (women), BP > 130/85 mmHg, fasting TG level > 150 mg/dl, fasting HDL cholesterol < 40 mg/dl (men) or 50 mg/dl (women) and fasting blood sugar > 100 mg/dl. Metabolically health was defined as without metabolic syndrome.	Obese BMI > 40 kg/m²	MHO (18)	Unclear	4 (22.2)	43.16 ± 1.57	11
							MUO (18)	Unclear	8 (44.4)	48.59 ± 1.72	
8. Bervoets et.al (2018)[25]	Belgium	Case-control study	Children and adolescent (plasma)	¹H NMR (untargeted)	Metabolic syndrome: ≥ 2 of the International Diabetes Federation (IDF) criteria: HDL- cholesterol <40mg/dl (females 16 years or older: HDL- cholesterol < 50 mg/dl), TG ≥ 150 mg/dl, SBP ≥ 130 mm Hg or DBP ≥ 85 mm Hg, fasting plasma glucose ≥ 100 mg/dl. Metabolically health was defined as without metabolic syndrome.	Normal-weight, overweight or obese according to IOTF BMI criteria	MHO[a] (18)	13.4 ± 2.0	10 (55.6)	31.4 (23.6–36.0)	12
							MUO[a] (17)	13.2 ± 2.0	10 (58.8)	33.4 (27.3–44.1)	

(Continued)

Table 1 (Continued).

Study (Year)	Country	Study Design	Population (Tissue)	Platform	Criteria for Definition of Metabolic Health	BMI Categories	Sample Size (n)	Mean Age	Male (%)	BMI (kg/m²)	Score
9. Bagheri et.al (2018)[26]	Iran	Case-control study	Adult (plasma)	LC-MS (targeted)	≤1 of the IDF criteria, except the WC: Hypertension (characterized as SBP ≥ 130 mmHg or DBP ≥ 85 mmHg or a history of taking anti-hypertensive medication), impaired FBS ≥ 100 mg/dL and < 126 mg/dL, TG ≥ 150 mg/dL and HDL-cholesterol < 40 mg/dL in men or < 50 mg/dL in women.	Normal weight 18.5 kg/m² ≤ BMI < 25 kg/m²; obese BMI ≥ 30 kg/m²	MHO (107)	35.0 (30.5–41.5)	39.0 (36.0)	33.9 (31.8–36.4)	12
							MUO (100)	36.0 (31.8–43.0)	49.0 (49.0)	35.0 (32.8–38.4)	
10. Zhong et.al (2017)[27]	USA	Case-control study	Adult (Plasma)	HPLC-MS/MS (targeted)	Metabolic syndrome: ≥3 of the following criteria: WC ≥ 102 cm for men and ≥ 88 cm for women, fasting TG ≥ 150 mg/dL, fasting glucose ≥ 100 mg/dL, resting systolic ≥ 130 mmHg and diastolic ≥ 85 mmHg blood pressure, and HDL-cholesterol < 40 mg/dL for men and < 50 mg/dL for women. Metabolically health was defined as without metabolic syndrome.	Obese BMI ≥ 30kg/m²	MHO (43)	29.3 ± 10.3	12 (27.9)	35.2 ± 6.8	12
							MUO (26)	27.4 ± 9.8	17 (65.4)	35.2 ± 6.8	

Study	Country	Study type	Sample	Method	Definition	Group (n)	Age	Female (%)	BMI	Ref
11. Allam-Ndoul et.al (2016)[29]	Canada	Case-control study	Adult (plasma)	MS (targeted)	Overweight and obese BMI ≥ 25 kg/m², obese Body fat ≥ 25% of total body fat mass in men and ≥ 30% in women	Metabolic syndrome: ≥3 of the following criteria: WC > 88 cm for women and 102 cm for men, fasting plasma TG ≥ 1.7 mmol/L, HDL- cholesterol levels ≤ 1.03 mmol/L for men and 1.29 mmol/L for women, glucose levels ≥ 5.6 mmol/L and resting blood pressure ≥ 130/85 mmHg. Metabolically health was defined as without metabolic syndrome.	MHO[a] (83)	35.7 ± 10.4	Unclear	31.4 ± 4.2	8
						MUO[a] (46)	37.9 ± 10.0	Unclear	34.3 ± 4.9	
12. Gao et.al (2016)[28]	Canada	Case-control study	Adult (Serum)	LC-MS/MS (targeted)	Normal weight 18 kg/m² < BMI < 25 kg/m², obese BMI ≥ 27.2 kg/m²	HOMA-IR < 4.27, HDL-cholesterol level ≥ 1.03 mmol/L in men and ≥ 1.30 mmol/L in women, fasting blood glucose < 5.6 mmol/L, and WC ≤ 102 cm in men and ≤ 88 cm in women.	MHO (30)	43.4 ± 11.8	15 (50)	30.1 ± 1.4	11
						MUO (29)	49.3 ± 11.4	14 (48.3)	31.6 ± 3.2	
13. Ni et.al (2015)[30]	China	Cross-sectional study	Adult (serum)	UPLC-QTOF-MS (targeted)	Normal weight < 24 kg/m², overweight ≥ 24 kg/m²; obesity ≥28 kg/m²	Meeting all the following criteria: FPG ≤ 6.1 mmol/L, OGTT ≤ 7.8 mmol/L and no previous history of diabetes, SBP/DBP < 140/90 mmHg and no previous history of high blood pressure, fasting plasma TG < 1.7 mmol/L and fasting plasma HDL ≥ 0.9 mmol/L (men) or ≥ 1.0 mmol/L (women), and no previous history of high cholesterol (TC < 5.18mmol/L), no cardiovascular or endocrine disease history.	MHO[a] (107)	46.269 ± 0.788	39 (36.5)	27.11 ± 0.18	10
						MUO[a] (73)	57.111 ± 0.846	37 (50.7)	27.68 ± 0.3	

(Continued)

Table 1 (Continued).

Study (Year)	Country	Study Design	Population (Tissue)	Platform	Criteria for Definition of Metabolic Health	BMI Categories	Sample Size (n)	Mean Age	Male (%)	BMI (kg/m²)	Score
14. Chen et.al (2015)[31]	China (Taiwan)	Case-control study	Adult (plasma)	LC-MS; GC-MS (targeted and untargeted)	Metabolic syndrome (ATP III criteria for Asian): fasting blood glucose > 100 mg/dL, TG > 150 mg/dL, HDL cholesterol < 40 mg/dL in males or 50 mg/dL in females, SBP > 130mmHg or DBP > 85mmHg. Metabolically health was defined as having normal status in all of the above indexes, except in the WC.	Obese BMI > 25 kg/m²	MHO (34)	32.12 ± 7.92	17 (50)	31.53 ± 6.03	11
							MUO (34)	34.68 ± 8.73	17 (50)	34.16 ± 6.92	
15. Böhm et.al (2014)[34]	Germany	Case-control study	Adult (culture of human adipocytes; plasma)	GC-MS; LC-MS/MS; HILIC-ESI-MS/MS (targeted)	Based on insulin sensitivity index, metabolically health was defined as insulin-sensitive.	Obese BMI > 40 kg/m²	MHO (10)	45 ± 9	4 (40)	52.5 ± 8.9	11
							MUO (10)	38 ± 13	4 (40)	51.1 ± 6.9	
16. Badoud et.al (2014)[35]	Canada	Case-control study	Adult (serum)	GC-MS; CE-MS (targeted and untargeted)	≥3 of the following criteria: HDL-cholesterol > 1.0 mmol/L for males and > 1.3 mmol/L for females, TG < 1.7 mmol/L without use of lipid-lowering drugs, TC < 5.2 mmol/L; LDL cholesterol < 2.6 mmol/L, and HOMA-IR < 1.95 without use of antidiabetic drugs.	Normal weight BMI < 28 kg/m² for males and <24 kg/m² for females; obese ≥ 28 kg/m² for males and ≥ 24 kg/m² for females	MHO (10)	50 ± 4	3 (30)	30.6 ± 1.1	13
							MUO (10)	48 ± 2	3 (30)	33.0 ± 1.9	

Study	Country	Study design	Sample (type)	Method	Criteria	Group	Age	Male n (%)	BMI	Ref
17. Perreault et. al (2014)[32]	Canada	Case-control study	Adult (serum)	GC (targeted)	Normal weight BMI < 28 kg/m² for males and < 24 kg/m² for females, obese BMI ≥ 28 kg/m² for males and < 24 kg/m² for females	MHO (10)	50 ± 4	3 (30)	30.6 ± 1.1	11
					≥3 of the following criteria: HDL- cholesterol >1.0 mmol/L for males and > 1.3 mmol/L for females, TG < 1.7 mmol/L without use of lipid-lowering drugs, TC < 5.2 mmol/L, LDL-cholesterol < 2.6 mmol/L, and HOMA-IR < 1.95 without use of anti-diabetic drugs	MUO (10)	48 ± 2	3 (30)	33.0 ± 1.9	
18. Wiklund et. al (2014)[33]	Finland	Cross-sectional study	Adult (serum)	NMR (targeted)	BMI between 25 and 40 kg/m²	MHO^a (42)	39.7 ± 7.6	0	28.9 ± 3.2	12
					Metabolic syndrome: ≥ 3 of the following criteria: WC ≥ 88 cm, fasting serum TG ≥ 1.7 mmol/L, HDL-cholesterol < 1.30 mmol/L, glucose ≥ 5.6 mmol/L and resting blood pressure ≥ 130/85 mmHg. Metabolically health was defined as having normal status in all of the above indexes, except in the WC.	MUO^a (36)	44.1 ± 6.1	0	30.6 ± 3.4	
19. Batch et.al (2013)[36]	USA	Cross-sectional study	Adult (plasma)	MS/MS (targeted)	Normal weight BMI < 25 kg/m², overweight 25kg/m² ≤ BMI < 30 kg/m², obese BMI ≥ 30 kg/m²	MHO (114)	55.84 ± 11.45	39 (34)	34.63 ± 4.14	10
					≤1 of Cardiometabolic abnormalities: impaired fasting glucose ≥ 100 mg/dL and ≤ 126 mg/dL, hypertension (defined as a self-reported diagnosis of hypertension or taking a blood pressure medication), TG ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL in men or < 50 mg/dL in women, and HOMA-IR > 5.13.	MUO (738)	57.14 ± 10.72	384 (52)	36 ± 4.96	

Table 1 (Continued).

Study (Year)	Country	Study Design	Population (Tissue)	Platform	Criteria for Definition of Metabolic Health	BMI Categories	Sample Size (n)	Mean Age	Male (%)	BMI (kg/m²)	Score
20. Ni et al (2015)[30]	China	Cohort study	Adult (serum)	UPLC-QTOF-MS (targeted)	Meeting all the following criteria: FPG ≤ 6.1 mmol/L, OGTT ≤ 7.8 mmol/L and no previous history of diabetes, SBP/DBP <140/90 mmHg and no previous history of high blood pressure, fasting plasma TG < 1.7 mmol/L and fasting plasma HDL ≥ 0.9 mmol/L (men) or ≥ 1.0 mmol/L (women), and no previous history of high cholesterol (TC < 5.18mmol/L), no cardiovascular or endocrine disease history.	Normal weight < 24 kg/m²; overweight ≥ 24 kg/m², obesity ≥ 28 kg/m²	MHOª (12)	39.92 ± 3.66	1 (8.3)	26.88 ± 0.47	10
							MUOª (50)	43.94 ± 1.83	15 (30)	26.89 ± 0.37	

Notes: ªInclude overweight and obese, not marked with ªinclude only obese.
Abbreviations: MUO, metabolically unhealthy overweight/obese; MHO, metabolically healthy overweight/obese; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; FPG, fasting plasma glucose; BP, blood pressure; WC, waist circumference; OGTT, oral glucose tolerance test.

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Table 2 Significant Findings Compared Between Metabolically Unhealthy Overweight/Obese and Metabolically Healthy Overweight/Obese

Study (Year)	Significant Findings
1.Telle-Hanse et.al (2020)[20]	Apolipoproteins: ApoB (↑), ApoA-I (↓), ApoB/ApoA-I (↑) Cholesterol: Remnaut-C (↑), VLDL-C (↑), HDL-C (↓), HDL2-C (↓) Triglycerides: TG (↑), VLDL-TG (↑), LDL-TG (↑), HDL-TG (↑) Phospholipids: TG-PG ratio (↑) Amino acids, branched-chain: Isoleucine (↑), Leucine (↑), Valine (↑) Short-chain FA: Propionate (↓) SFA: C16:0 (↑), C20:0 (↓), C22:0 (↓), C23:0 (↓) MUFA: MUFA (↑), C16:1 (↑), C18:1 c9 (↑), C18:1 c11 (↑) PUFA, total and n6: PUFA (↓), C18:2 n6 (↓) PUFA, n3: C22:5 n3 (↓) Ratio: 16:1/16:0 ratio (↑), 18:1/18:0 ratio (↑)
2. Kim et.al (2020)[21]	Glycolic acid (↓) lysoPEs (↑): 16:0, 18:2, 18:1, 20:5, 20:4, 22:6 lysoPCs (↑): 14:0, 16:1, 16:0, 18:4, 18:3, 18:2, 18:1, 20:5, 20:4, 20:3, 22:6, 22:5
3. Chashmniam et.al (2020)[22]	Alanine (↓), Glutamine (↓), Proline (↓), Asparagine (↓), L-Glutathione reduced (↓), Betaine (↓), Taurine (↓), Choline (↓), 2-Aminobutyrate (↓), Tagatose (↓), 2-Oxoglutarate (↓), L-alpha-phosphatidylinositol (↑), D-Sphingosine (↑)
4. Ojwang et.al (2019)[23]	Plasma phospholipid fatty acids: Saturated: C16:0 (↑), C18:0 (↓), C20:0 (↓), C22:0 (↓), C24:0 (↓) Monounsaturated: C16:1 n7 (↑), C18:1 n9 (↑), C20:1 n9 (↓), C22:1 n9 (↓), C24:1 n9 (↓) PUFA, n3: C20:5 n3 (↑) PUFA, n6: C18:3 n6 (↑), C20:2 n6 (↓), C22:2 n6 (↓), Total n-6 (↓) Trans-fatty acid: C18:1 n9T (↓)
5. Rousseau et.al (2019)[37]	BCAAs (↑): Valine, Leucine and Isoleucine Acylcarnitines (↑): C3, C5
6. Libert et.al (2018)[13]	Alanine (↑), Alloisoleucine (↑), Alpha-aminoadipic acid (↑), Cystine (↑), Isoleucine (↑), Leucine (↑), Lysine (↑), Phenylalanine (↑), Propionylcarnitine (↑), Tyrosine (↑), Valine (↑) Essential AAs (↑) (Histidine+ Isoleucine+ Leucine+ Lysine+ Methionine+ Phenylalanine+ Threonine+ Tryptophan+ Valine) Ketogenic AAs (↑) (Leucine+Lysine) Determines brain serotonin synthesis (↓): Tryptophan/(Tyrosine +Phenylalanine +Leucine +Isoleucine +Valine) BCAA metabolism (↑): (C3+C5)/Total carnitine
7.Candi et.al (2018)[24]	Glutathione Metabolism: glutathione, oxidized (GSSG) (↑), 5-oxoproline (↑), 2-hydroxybutyrate/2-hydroxyisobutyrate (↑), ophthalmate (↑) Gamma-glutamyl Amino Acid: gamma-glutamylglutamine (↑), gamma-glutamylthreonine (↑), gamma-glutamylvaline (↑) Glycolysis, Gluconeogenesis, and Pyruvate Metabolism: glucose (↑) Fructose, Mannose and Galactose Metabolism: mannose (↑) Aminosugar Metabolism: N-acetyl-glucosamine 1-phosphate (↑), N-acetylneuraminate (↑), erythronate (↑) TCA Cycle: alpha-ketoglutarate (↑) Polyunsaturated Fatty Acid (n3 and n6): eicosapentaenoate (EPA; 20:5 n3) (↑), docosahexaenoate (DHA; 22:6 n3) (↑) Fatty Acid, Dicarboxylate: 2-hydroxyglutarate (↑), 3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF) (↑) Fatty Acid Metabolism(Acyl Carnitine): acetylcarnitine (C2) (↑), hexanoylcarnitine (C6) (↑), palmitoylcarnitine (C16) (↑) Phospholipid Metabolism: glycerophosphorylcholine (GPC) (↑), glycerophosphoethanolamine (↑), 1, 2-dipalmitoyl-GPC (16:0/16:0) (↑), 1-stearoyl-2-arachidonoyl-GPC (18:0/20:4) (↑), 1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6) (↑), 1-stearoyl-2-arachidonoyl-GPI (18:0/20:4) (↑), 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) (↑), 1-palmitoyl-2-stearoyl-GPC (16:0/18:0) (↑), 1-stearoyl-2-oleoyl-GPG (18:0/18:1) (↑), 1-stearoyl-2-linoleoyl-GPS (18:0/18:2) (↑)Phosphatidylserine (PS): 1-stearoyl-2-arachidonoyl-GPS (18:0/20:4) (↑), 1-stearoyl-2-oleoyl-GPS (18:0/18:1) (↑) Lysolipid: 1-palmitoyl-GPC (16:0) (↑), 1-stearoyl-GPC (18:0) (↑), 1-palmitoyl-GPE (16:0) (↑), 1-stearoyl-GPE (18:0) (↑), 1-linoleoyl-GPE (18:2) (↓), 1-stearoyl-GPI (18:0) (↑), 1-stearoyl-GPS (18:0) (↑) Plasmalogen: 1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0) (↑), 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4) (↑), 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P-16:0/20:4) (↑), 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4) (↑) Lysoplasmalogen: 1-(1-enyl-palmitoyl)-GPE (P-16:0) (↑), 1-(1-enyl-oleoyl)-GPE (P-18:1) (↑), 1-(1-enyl-stearoyl)-GPE (P-18:0) (↑) Glycerolipid Metabolism: glycerophosphoglycerol (↑) Diacylglycerol: oleoyl-arachidonoyl-glycerol (18:1/20:4) (↑), stearoyl-arachidonoyl-glycerol (18:0/20:4) (↑) Sphingolipid Metabolism: N-palmitoyl-sphingadienine (d18:2/16:0) (↑), N-behenoyl-sphingadienine (d18:2/22:0) (↑), behenoyl sphingomyelin (d18:1/22:0) (↑), tricosanoyl sphingomyelin (d18:1/23:0) (↑), lignoceroyl sphingomyelin (d18:1/24:0) (↑), sphingomyelin (d18:2/16:0, d18:1/16:1) (↑), sphingomyelin (d18:1/20:0, d16:1/22:0) (↑), sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1) (↑), sphingosine (↑), glycosyl-N-palmitoyl-sphingosine (d18:1/16:0) (↑), hexadecasphingosine (d16:1) (↑), sphingadienine (↑) Ceramides: ceramide (d14:1/22:0, d16:1/20:0) (↑), ceramide (d18:1/14:0, d16:1/16:0) (↑), ceramide (d18:1/17:0, d17:1/18:0) (↑), ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0) (↑) Purine Metabolism, (Hypo)Xanthine/Inosine containing: xanthosine (↑) Pyrimidine Metabolism, Uracil containing: uridine (↑), uracil (↑), pseudouridine (↑) Nicotinate and Nicotinamide Metabolism: N1-Methyl-2-pyridone-5-carboxamide (↑), N1-Methyl-4-pyridone-3-carboxamide (↑) Drug: oxypurinol (↑) Chemical: N-methylpipecolate (↑)

(Continued)

Table 2 (Continued).

Study (Year)	Significant Findings
8. Bervoets et.al (2018)[25]	L-Histidine (↓), L-Isoleucine (↓), L-Histidine + -N$^+$(CH$_3$)$_3$ of choline head group in SM/PC (↓), Lipids: -CH$_2$-C=O and -CH$_2$-CH=CH- in fatty acid chain (↑), L-Glutamine (↓), L-Lysine + L-Leucine (↓), L-Lactate (↑), L-Valine + L-Isoleucine (↓)
9. Bagheri et.al (2018)[26]	comparing MUO to MHO, MUO phenotype was not associated with any of the assessed metabolites.
10. Zhong et.al (2017)[27]	(↑) Alanine, Trans-4-hydroxyproline, N-alpha-acetyl-L-lysine, Leucine/isoleucine, Glutamic acid, Creatine, Cysteine, Kynurenine, Inosine-5′-diphosphate, Urate, Inosine, Tryptophan, Methionine, D-glucosamine-6-sulfate, (2R,3R)-(-)-2,3-butanediol
11. Allam-Ndoul et.al (2016)[29]	Acylcarnitines: C0 (↑), C3 (↑) C14:1-OH (↓) Phosphatidylcholines diacyl: C32:1 (↑), C34:1 (↑), C34:2 (↑), C34:3 (↑), C36:0 (↓), C36:4 (↑), C38:0 (↓), C38:3 (↑), C40:1 (↓), C40:3 (↓), C40:4 (↑), C40:5 (↑), C42:0 (↓), C42:1 (↓), C42:6 (↓) Phosphatidylcholines acyl-alkyl: C34:2 (↓), C34:3 (↓), C36:2 (↓), C36:3 (↓), C40:1 (↓), C40:2 (↓), C40:3 (↓), C40:4 (↓), C40:5 (↓), C40:6 (↓), C40:2 (↓), C42:3 (↓), C42:4 (↓), C42:5 (↓), C44:5 (↓), C44:6 (↓) SM(OH): C16:1 (↓), C22:1 (↓), C22:2 (↓), C24:1 (↓) Sphingomyelins: C16:0 (↓), C24:0 (↓), C24:1 (↓) Amino acids: Leucine (↑), Isoleucine (↑), Valine (↑)
12. Gao et.al (2016)[28]	Leucine (↑), Isoleucine (↑), Valine (↑), Alpha-aminoadipic acid (↑), Propionylcarnitine (↑)
13. Ni et.al (2015)[30]	FFAs: TFA (↑), SFA (↑), C8:0 (↑), C10:0 (↑), C12:0 (↑), C14:0 (↑), C15:0 (↑), C16:0 (↑), C17:0 (↑), C18:0 (↑), C19:0 (↑), C20:0 (↑), C22:0 (↑), C24:0 (↑), C14:0 iso (↑), C15:0 iso (↑), C16:0 iso (↑), C17:0 iso (↑), C18:0 iso (↑), MUFA (↑), C14:1 n5 (↑), C14:1 t9 (↑), C16:1 n9 (↑), C16:1 n7 (↑), C16:1 t9 (↑), C17:1 n7 (↑), C18:1 n9 (↑), C18:1 t9 (↑), C19:1 n9 (↑), C20:1 n9 (↑), C24:1 n9 (↑), n3PUFA (↑), C18:3 n3 (↑), C20:5 n3 (↑), C22:3 n3 (↑), C22:5 n3 (↑), C22:6 n3 (↑), n6PUFA (↑), C18:2 n6 (↑), C18:3 n6 (↑), C20:2 n6 (↑), C20:3 n6 (↑), C20:4 n6 (↑), C22:2 n6 (↑), C22:4 n6 (↑), C22:5 n6 (↑)
14. Chen et.al (2015)[31]	L-Kynurenine (↑), Glycerophosphocholine (↓), N-Acetylserine (↑), Decanoylcarnitine (↑), L-Alloisoleucine/L-Norleucine/L-Isoleucine (↑), Hexanoylcarnitine (↑), 5, 6-Dihydrothymine (↑), Glycerol 1-phosphate (↓), Glycolic acid (↓), Tagatose (↓), Methyl palmitate (↑), Uric acid (↑), Dioctyl phthalate (↑), Phosphoglycolic acid (↑), L-Threonine (↓), Palmitic acid (↑), Stearic acid (↓), L-Valine (↑), Benzoic acid (↓), N-Methyl-DL-glutamic acid (↑), L-(+) Lactic acid (↑), L-Tyrosine (↑), Isopropyl beta-D-1-thiogalactopyranoside (↑)
15. Böhm et.al (2014)[34]	Intracellular differences: Aspartate (↓), Hexose (↑), Glutamine (↑), Histidine (↑), Spermidine (↑), lysophosphatidylcholine C18:0 (↑), Phosphatidylcholine acyl: C32:3 (↑), C34:4 (↑), C36:4 (↑), C36:5 (↑) Sphingolipid C16:1 (↑) Extracellular milieu: Stearic acid (↓), Linoleic acid (↑), Arachidonic acid (↑), 15-hydroxy-eicosatetraenoic acid (↑), Docosahexaenoic acid (↑), Serotonin(↑), Phosphatidylcholine acyl: C32:0 (↑), C32:3 (↓), C36:6 (↓) Phosphatidylcholine ether side chain: C34:3 (↓), C42:2 (↑) Sphingolipids: C20:2 (↓), C22:3 (↑) Plasma: Acylcarnitine: C16 (↓), C18:1 (↓) Adrenic acid (↓), Cervonic acid [=DHA] (↓), Lysophosphatidylcholine: C16:0 (↓), C16:1 (↓), C18:1 (↓), C20:3 (↓), C20:4 (↓) Phosphatidylcholine ether side chain: 36:4 (↓), 36:5 (↓), 38:5 (↓),38:6 (↓) Sphingolipids: C26:0 (↓), Glutamic acid (↑), Hexosephosphate (↓)
16. Badoud et.al (2014)[35]	Lysine (↓), Hydroxyproline (↓) metabolites and/or metabolite ratios: glutamic acid-to-lysine ratio, glutamic acid-to-ornithine, the glutamic acid-to-carnitine ratio, the glutamic acid-to-hydroxyproline ratio, the tyrosine-to-hydroxyproline ratio, the glutamic acid-to-cystine ratio, and the glutamic acid-to-serine ratio, hydroxyproline
17. Perreault et.al (2014)[32]	Fatty acid: Saturated: TG-Myristic Acid (↑), TG-Stearic Acid (↓) Polyunsaturated: PL-Arachidonic Acid (↓)
18. Wiklund et.al (2014)[33]	Factor (↑) (leucine, Isoleucine, Valine, Tyrosine, Phenylalanine, Orosomucoid); Factor (↑) (Total fatty acids, Omega-6 fatty acids, Omega7 and 9 fatty acids, Linoleic acid, Mono-Unsaturated fatty acids, Total Phosphoglycerides, Total Phosphocholines)
19. Batch et.al (2013)[36]	BCAA related (↑) (Phenylalanine, Leucine/Isoleucine, Valine, Tyrosine, Methionine, Alanine, Histidine) Various metabolites (↑) (C5:1, Non Esterified Fatty Acids, Glutamate/glutamine, Ornithine, Arginine (-), Histidine (-)) Short chain acylcarnitines (↑) (C4/Ci4, C3, C5S) Various metabolites (↑) (Proline, Citruline, C22 Acylcarnitine)
20. Ni et.al (2015)[30]	FFA: SFA: C24:0 (↑) MUFA: C16:1 n7 (↑), C18:1 n9 (↑) PUFA, n3:C22:5 n3 (↑) PUFA, n6:C20:2 n6 (↑), C20:3 n6 (↑)

Abbreviations: ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; HDL-C, total cholesterol in HDL; HDL2-C, total cholesterol in HDL2; LDL-TG, triglycerides in LDL; LDL, low-density lipoprotein; Remnant-C, remnant cholesterol (non-HDL, non-LDL-cholesterol); TG-PG ratio, ratio of triglycerides to phosphoglycerides; TG, total triglycerides; VLDL-C, total cholesterol in VLDL; VLDL-TG, triglycerides in VLDL; HDL-TG, triglycerides in HDL; lysoPEs, lysophosphatidylethanolamines; lysoPCs, lysophosphatidylcholines; FA, fatty acids; TFA, total fatty acids; SFA, saturated FAs; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; PC, phosphatidylcholine; SM, sphingomyelin; BCAA, branched-chain amino acids; PL, phospholipid; FFAs, free fatty acids.

BCAAs were usually considered as a consequence rather than a cause of insulin resistance, reflecting an abnormal protein breakdown. However, strong evidence suggests that elevations of BCAAs contribute to insulin resistance. Infusions of BCAAs in humans acutely elevated stimulate secretion of insulin and glucagon, and worsen insulin sensitivity.[41] The elevation of plasma BCAAs may last more than 10 years before the development of diabetes.[42] A locus near *PPMIK* that was associated with BCAAs levels was correlated with the incidence of diabetes, suggesting that the BCAAs related genetic factors lead to diabetes.[43] Given that BCAAs have been extensively studied, BCAAs may be the most promising biomarker for distinguishing obesity phenotypes and may be a contributing factor to phenotypical deleterious development.

Aromatic amino acids (AAAs) are another important class of amino acids. Evidence has shown that AAAs were associated with a higher risk of T2DM after a median follow-up of 3.8 years.[44] Among AAAs, phenylalanine, and tyrosine may serve as biomarkers to distinguish MUO from MHO.[13,31,33,36] Phenylalanine was higher in obese pregnant women compared to their overweight pregnant counterparts.[45] Tyrosine is a hydroxylation product of phenylalanine metabolism and is metabolized in the liver. In Chinese adults, tyrosine over 46 μmol/L was associated with increased odds of T2DM.[46] Thus, the elevated levels of AAAs, especially phenylalanine and tyrosine, are parallel to metabolic disorders, indicating that they may serve as biomarkers of MUO.

Besides BCAAs and AAAs, other related amino acids have also been identified. Plasma amino acid concentrations increased in obese people compared with non-obese people, likely as a consequence of increased insulin resistance and protein catabolism.[47] The Lower glutamine-to-glutamic acid ratio might serve as an indicator for metabolic unhealthy,[35] and glutamine level was higher in MHO compared with MUO.[22,25] Since glutamine indeed is thought to have a beneficial effect on cardiometabolic risk,[48] it's not surprising that it appeared in a heart-protected obesity phenotype. Besides, among these studies, one showed that glutamic acid was positively associated with glycosylated hemoglobin (HbA1c), whereas the glutamine-to-glutamic acid ratio was inversely associated with HbA1c.[35] This may explain that balanced levels of glutamine and glutamic acid may be important for blood glucose control. A Higher level of alanine was observed in the MHO group compared with MUO in an Iran study.[22] However, in other studies included, high levels of alanine were found in MUO.[13,27,36] Alanine previously has been shown to increase glucagon secretion in humans[49] and related to higher levels of homeostasis model assessment of insulin resistance (HOMA-IR).[50] Methionine, an essential amino acid, levels were found higher in the MUO group.[27,36] In the general obese population, the plasma concentration of methionine can track insulin resistance and/or predict risk toward the development of T2DM.[51] Alpha-aminoadipic acid is a product of lysine degradation and its levels were found lower in MHO.[13,28] Since insulin sensitizer therapy significantly reduced αlpha-aminoadipic acid,[52] the lower level of alpha-aminoadipic acid in MHO may be a result of increased insulin sensitivity. Levels of kynurenine were higher in MUO.[27,31] Kynurenine pathway metabolites have been proposed as biomarkers for the initiation and progression of atherosclerosis and diabetes.[53] Although results were inconsistent, histidine levels may also be elevated in the MUO group,[13,34,36] consistent with its changes in diabetes progression.[54] The above amino acids have been identified, but few studies were focusing on them, which may easily lead to bias conclusions. More metabolomic studies are needed to better identify the amino acids mentioned above.

Lipid

In the included studies, it seemed that saturated fatty acids (SFAs) especially long-chain SFAs did not show strong correlations with MUO compared with unsaturated fatty acids, except the most abundant fatty acid, palmitic acid (C16:0).[20,23,30] Blood concentration of palmitic acid was elevated in obese patients compared with non-obese people, leading to inflammatory responses through toll-like receptors (TLR), TLR2, and TLR4.[55] It is also reported that palmitic acid is associated with metabolic syndrome,[56] and contributes to the development of metabolic syndrome through white adipocyte dysregulation and death, chronic low-grade inflammation, insulin resistance, leptin resistance, and adipokine release dysregulation.[57] Among SFAs, only stearic acid (C18:0) was most associated with MHO.[23,31,32,34] Interestingly, higher baseline stearic acid/palmitic acid was found to be associated with a greater probability of diabetes remission after bariatric surgery.[58] It possibly indicates that an increase in stearic acid/palmitic ratio may deliver a favorable change.

Most of the time, unsaturated fatty acids (UFAs) are generally considered beneficial as opposed to SFAs. However, a systematic review and meta-analysis suggested that an increase of omega-3, omega-6, or total

polyunsaturated fatty acids (PUFAs) showed little or no effect on the prevention and treatment of T2DM.[59] In another meta-analysis, monounsaturated fatty acids (MUFAs) levels were associated with future cardiovascular events.[60] Among the studies we included, the only cohort study showed that fasting concentrations of a panel of UFAs were elevated up to 10 years before the onset of metabolic syndrome.[30] This may explain a linkage between cause and result while the other studies are cross-sectional or case-control studies.

The difference of UFAs was common between MUO and MHO groups, but the results were inconsistent. On the whole, among MUFAs, palmitoleic acid (C16:1 n7) and oleic acid (C18:1 n9) seemed to be associated with the MUO group.[23,30] Shreds of evidence have shown that palmitoleic acid has been associated with an increased risk of metabolic syndrome,[61,62] and oleic acid may predispose to obesity and obesity-related disorders.[63] Among PUFAs, levels of and eicosapentaenoic acid (EPA, C20:5 n3),[23,24,30] and docosahexaenoic acid (DHA, C22:6 n3)[24,30,34] increased in the MUO group. EPA and DHA are the most important n-3 PUFAs, and they are associated with a lower risk of developing metabolic syndrome.[64] Moderate supplementation with EPA and DHA improved the insulin resistant condition in patients with chronic renal failure on maintenance hemodialysis.[65] Besides, EPA and DHA supplements also are beneficial to reducing and preventing cardiovascular disease.[66,67] The mechanism underlying why these two PUFAs levels were increased in the MUO group is unclear. It is undemonstrable to conclude that high levels of EPA and DHA contribute to developing MUO. A possible explanation of the increased levels of EPA and DHA in MUO might be a result of barriers to utilization or consumption, which may be unique manifestations of obesity combined with metabolic unhealthy. There are also other fatty acids, for example, C20:0, C22:0, C24:0, C22:5 n3, C18:2 n6, C18:3 n6, C20:2 n6, C20:3 n6, levels of which may have an increased or decreased trend in MUO compared with MHO. However, due to the limited number of studies, more researches are needed to further explore whether these fatty acids can be used as biomarkers to distinguishing overweight/obese phenotypes. In addition to the differences in metabolomics studies, more attention should be paid to differences in dietary intake, for example, many studies used plasma or serum and blood fatty acids are closely related to diet.

Acylcarnitine

Acylcarnitines (ACs) are intermediate oxidative metabolites formed intracellularly from carnitine during the metabolism of long-chain fatty acids and BCAAs. We observed some differences in acylcarnitine levels in the collected literature between the MUO group and the MHO group.[29,34,36,37] Results showed that propionylcarnitine, a product of mitochondrial BCAAs (especially isoleucine and valine) catabolism,[68] has been identified the most frequently. The associations of increased levels of acylcarnitines in diabetes[69,70] and general obesity[16] have been investigated. Excessive accumulation of acylcarnitine may indicate β-oxidation dysfunction, mitochondrial stress, and insulin resistance,[71] and these may be related to the metabolic characteristics of MUO.

Relevance and Significance to Other Systematic Reviews of Obesity Metabolomics

In the current systematic review, the difference of metabolites between the MHO group and the MUO group showed more information about metabolic effects, since both groups were overweight/obese. However, other systematic reviews of metabolomics in overweight/obesity showed more information about obesity effects,[14,16,72] since the difference of metabolites were identified between normal weight and overweight/obese. In previous systematic reviews, metabolites that different between obese and normal people, such as BCAAs, were also found different between MUO and MHO in our study. Perhaps elevated and reduced levels of these metabolites indicate unfavorable metabolic signature. The current results suggested that MHO showed a favorable trend in the overall metabolic signature. A study showed that obese individuals with no metabolic abnormalities had a higher risk of coronary heart disease, cerebrovascular disease, and heart failure compared with normal weight healthy individuals during a mean follow-up of 5.4 years. Meanwhile, these risks increased with an increasing number of metabolic abnormalities.[73] A meta-analysis demonstrated that the pooled adjusted relative risk (RR) for the incident of T2DM was 4.03 (95% CI=2.66–6.09) in healthy obese adults and the risk among unhealthy obese subjects was approximately two times that of healthy obese individuals.[74] The above studies have approved that compared with patients with MUO, patients with MHO have a lower risk of developing metabolic diseases. The favorable metabolic signature is consistent with a lower risk of metabolic disease in MHO patients.

Significance of Metabolomic Signature of Overweight/Obesity Phenotype

The metabolic phenotype of overweight/obesity is not immutable, while a subject's status can switch from metabolically healthy to metabolically unhealthy. During an 8-year follow-up, a prospective community-based cohort study showed several healthy obese individuals moved to unhealthy groups.[75] Similarly, in one of the studies included, 50 subjects out of 62 metabolic healthy people turned metabolically unhealthy and only 12 maintained metabolic health, about 12 years later.[30] Correspondingly, a transition from metabolically unhealthy to healthy obesity can be achieved. A study observed that 25% of women from the MUO group changed to MHO after a 12-week energy-restricted diet intervention.[76] Thus, the health condition of obesity is more of a dynamic change. MHO can serve as an appropriate primary goal that helps motivate patients toward the long-term goals of obesity treatment, and MHO can also warn that the patient's health condition is moving in a progressive direction. Therefore, it is essential for clinical treatment and prevention. However, clinical significance alone does not seem to support the importance of MHO's presence. Some health care professionals still suggest that the definition of MHO may be considered meaningless as a transitional state or as a "honeymoon period". The current systematic reviews focused on the metabolic signature between MUO and MHO and noted that underlying metabolic mechanisms might be different in these two obesity phenotypes, all of which add support to the importance of MHO's existence. Moreover, metabolomic signature as a biomarker of MHO and MUO may contribute to weight loss, treatment, and prevention of overweight/obesity.

Limitations and Prospects

Despite significant findings that explain differences between the MUO and MHO groups, this systematic review has limitations. Firstly, there is still no consensus about the criteria applied to define metabolically healthy and unhealthy overweight/obese in the current literature. However, rigorous criteria would have a significant impact on the types and numbers of metabolites that might be detected. Secondly, there is only one prospective study, and the rest are non-prospective studies. These non-prospective studies did not show the differences in metabolites have occurred before the progression to an unhealthy status in obese patients and therefore causal relationship is merely a hypothesis. Thirdly, levels of physical activity and calorie intake may critically attribute to the levels of various metabolites, it should be considered that the different levels of confounding factor control may lead to differences in conclusions. Fourthly, in this systematic review, more than half of the studies had a total sample size of less than 100 and small sample sizes also may reduce the credibility of the results.

It has been previously shown that MHO subjects have a potential risk to become metabolically unhealthy. However, how the progression happened remained unknown. The underlying metabolic pathways and metabolic mechanisms are still not yet identified. Because the disease itself is dynamic, more longitudinal studies are needed to elaborate more deeply on the metabolic pathway and the relationship between metabolic patterns and disease occurrence.

Conclusion

Metabolomics is a tool with the potential for a better understanding of the disease progression and metabolic pathways. The present systematic review presents the first study to show valuable information on specific metabolite patterns on signatures of the metabolically healthy and unhealthy phenotypes in patients with overweight/obesity. Branched-chain amino acids (isoleucine, leucine, and valine), aromatic amino acids (phenylalanine and tyrosine), lipids (palmitic acid, palmitoleic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid), and acylcarnitines (propionyl carnitine) levels might be elevated in MUO. The current results suggested that MHO showed a favorable trend in the overall metabolic signature. Due to the limited literature, more research on the metabolomic signature already identified is needed.

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Disclosure

The authors report no conflicts of interest in this work.

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