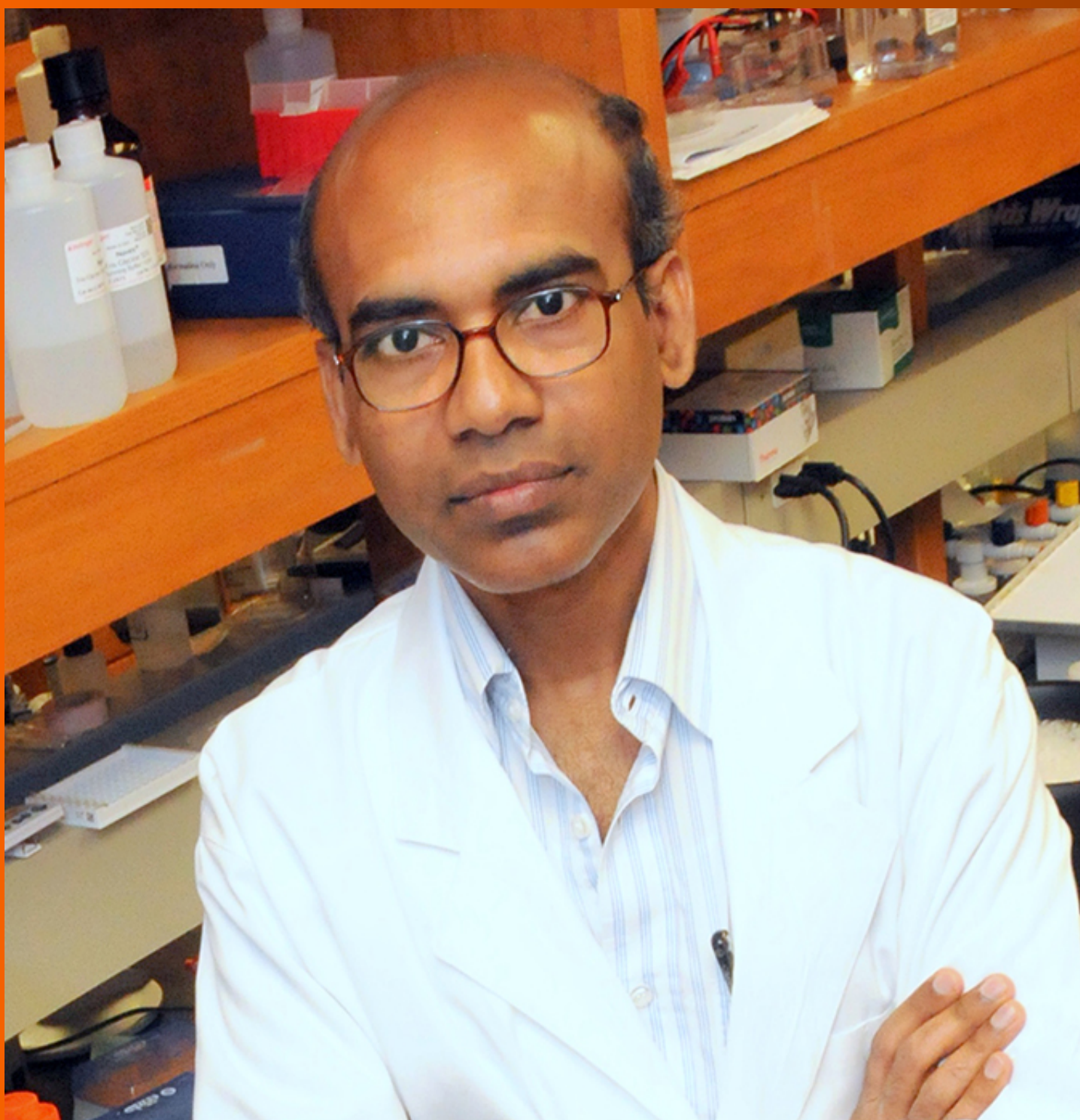


# World Journal of *Diabetes*

*World J Diabetes* 2021 April 15; 12(4): 306-513



**THERAPEUTIC AND DIAGNOSTIC GUIDELINES**

- 306** Feasibility of large experimental animal models in testing novel therapeutic strategies for diabetes  
*Nagaya M, Hasegawa K, Uchikura A, Nakano K, Watanabe M, Umeyama K, Matsunari H, Osafune K, Kobayashi E, Nakauchi H, Nagashima H*

**REVIEW**

- 331** Exercise intervention under hypoxic condition as a new therapeutic paradigm for type 2 diabetes mellitus: A narrative review  
*Kim SW, Jung WS, Chung S, Park HY*
- 344** Diagnosis, treatment and prevention of type 2 diabetes mellitus in children and adolescents  
*Serbis A, Giapros V, Kotanidou EP, Galli-Tsinopoulou A, Siomou E*
- 366** Maternal obesity as a risk factor for developing diabetes in offspring: An epigenetic point of view  
*Lecoutre S, Maqdasy S, Breton C*
- 383** Diabetic heart disease: A clinical update  
*Rajbhandari J, Fernandez CJ, Agarwal M, Yeap BXY, Pappachan JM*

**MINIREVIEWS**

- 407** Alphabet strategy for diabetes care: A checklist approach in the time of COVID-19 and beyond  
*Upreti R, Lee JD, Kotecha S, Patel V*
- 420** Obesity, metabolic health and omics: Current status and future directions  
*Paczowska-Abdulsalam M, Kretowski A*
- 437** Malfunction of outer retinal barrier and choroid in the occurrence and progression of diabetic macular edema  
*Țălu Ș, Nicoara SD*

**ORIGINAL ARTICLE****Basic Study**

- 453** Effect of oligofructose on resistance to postoperative high-fat diet-induced damage of metabolism in diabetic rats after sleeve gastrectomy  
*Zhong MW, Li Y, Cheng YG, Liu QR, Hu SY, Zhang GY*
- 466** Elevated retinol binding protein 4 levels are associated with atherosclerosis in diabetic rats *via* JAK2/STAT3 signaling pathway  
*Zhou W, Ye SD, Wang W*

- 480** Vascular endothelial growth factor B inhibits insulin secretion in MIN6 cells and reduces  $\text{Ca}^{2+}$  and cyclic adenosine monophosphate levels through PI3K/AKT pathway  
*Jia JD, Jiang WG, Luo X, Li RR, Zhao YC, Tian G, Li YN*
- 499** Three-dimensional-arterial spin labeling perfusion correlation with diabetes-associated cognitive dysfunction and vascular endothelial growth factor in type 2 diabetes mellitus rat  
*Shao JW, Wang JD, He Q, Yang Y, Zou YY, Su W, Xiang ST, Li JB, Fang J*

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## Obesity, metabolic health and omics: Current status and future directions

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### Abstract

The growing obesity epidemic is becoming a major public health concern, and the associated costs represent a considerable burden on societies. Among the most common complications of severe obesity are the development of hypertension, dyslipidemia, type 2 diabetes, cardiovascular disease, and various types of cancer. Interestingly, some obese individuals have a favorable metabolic profile and appear to be somehow protected from the detrimental effects of excessive adipose tissue accumulation. These individuals remain normoglycemic, insulin sensitive, and hypotensive with proper blood lipid levels, despite their high body mass index and/or waist circumference. Multiple independent observations have led to the concept of the metabolically healthy obese (MHO) phenotype, yet no consensus has been reached to date regarding a universal definition or the main mechanism behind this phenomenon. Recent technological advances and the use of high-throughput analysis techniques have revolutionized different areas of biomedical research. A multi-omics approach, which is used to investigate changes at different molecular levels in an organism or tissue, may provide valuable insights into the interplay between the molecules or pathways and the roles of different factors involved in the mechanisms underlying metabolic health deterioration. The aim of this review is to present the current status regarding the use of omics technologies to investigate the MHO phenotype, as well as the results of targeted analyses conducted in MHO individuals.

**Key Words:** Metabolically healthy obesity; Cardiovascular diseases; Genomics; Transcriptome profiling; Proteomics; Metabolomics

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**Core Tip:** Multiple independent observations have led to the concept of the metabolically healthy obese (MHO) phenotype, in which individuals, despite a high body mass index, remain normoglycemic, insulin sensitive, and hypotensive with proper blood lipid levels. Even though this issue is of great interest to the scientific community, no consensus has been reached to date regarding the main mechanism behind this phenomenon. The aim of this review is to present the current status regarding the use of omics technologies to investigate the MHO phenotype at different molecular levels, as well as the results of targeted analyses conducted in MHO individuals.

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## INTRODUCTION

Over the past several decades, the incidence of obesity has tripled worldwide<sup>[1]</sup>. The high amounts of excess or abnormal adipose tissue, together with all the related comorbidities, are imposing a considerable burden on societies, and obesity has become a major public health concern. Among the most common complications of severe obesity are the development of hypertension, dyslipidemia, type 2 diabetes, cardiovascular disease (CVD), and various types of cancer<sup>[2]</sup>. There are, however, some obese individuals who exhibit a favorable metabolic profile. These individuals remain normoglycemic, insulin sensitive (IS), and hypotensive with proper blood lipid levels, despite their high body mass index (BMI) and/or waist circumference (WC). Based on multiple independent observations, the concept of the metabolically healthy obese (MHO) phenotype<sup>[3,4]</sup> has been proposed, yet no consensus has been reached to date regarding a uniform definition or the main mechanism behind this phenomenon.

### Definition and prevalence

More than 30 sets of criteria for the MHO phenotype have been used in different clinical studies over the years, and most have included parameters such as BMI, blood pressure, fasting plasma glucose, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), WC, and homeostasis model assessment of insulin resistance<sup>[5]</sup>. The inconsistencies between studies are mostly related to different cutoff values for the parameters or the number of criteria that have to be met to define the MHO phenotype<sup>[6,7]</sup>. Furthermore, some researchers have applied metabolic syndrome (MetS) definitions, while others have defined metabolic health based on insulin sensitivity<sup>[8]</sup> or liver fat<sup>[9]</sup>. The variabilities in the criteria used in studies represent a significant limitation, complicating the interpretation and comparison of research results. This issue may also, in part, explain the discrepancies in the reported prevalence of the MHO phenotype, which has differed between studies depending on the type of population studied and definition used. For example, Liu *et al*<sup>[10]</sup> reported a prevalence that ranged from 4.2% to 13.6%, whereas that reported by Rey-López *et al*<sup>[11]</sup> ranged from 6% to 75%. A recent meta-analysis of data from 12 cohort and 7 intervention studies found that almost one-third of obese individuals were metabolically healthy<sup>[12]</sup>. In general, the prevalence of the MHO phenotype has been found to be higher in women and younger study participants<sup>[11,13]</sup>. Metabolically healthy individuals have also been observed to be less sedentary and more physically active than their unhealthy counterparts<sup>[14]</sup>.

### MHO phenotype and associated risks

The lack of clearly defined diagnosis criteria for the MHO phenotype has also led to inconsistent results among studies evaluating the association between the metabolic health status and the risk of CVD, type 2 diabetes development, and mortality. Some epidemiological studies with short-term follow-ups have reported that MHO individuals have a similar risk of incident CVD as metabolically healthy normal weight (MHNW) individuals. On the other hand, more recent studies with long follow-ups have found that MHO individuals have a higher risk of CVD and

cardiovascular mortality than healthy non-obese subjects<sup>[15]</sup>. A meta-analysis by Eckel *et al*<sup>[16]</sup> revealed that study participants with the MHO phenotype had a higher risk of developing CVD than MHNW individuals and a lower risk than metabolically unhealthy normal weight subjects, regardless of the MHO definition. Similar results were obtained by Bell *et al*<sup>[17]</sup>, who investigated the incidence of type 2 diabetes among MHO individuals and found that their risk of developing diabetes mellitus was approximately half that of metabolically unhealthy obese (MUO) subjects; however, the risk was significantly higher in MHO individuals than in MHNW individuals. The most recent meta-analysis, which included 23 studies, found an increased risk of CVD in the MHO group compared with healthy lean subjects<sup>[18]</sup>. Interestingly, in this meta-analysis, the risk of CVD in MHO individuals was similar even when different numbers of risk factors were used to define good metabolic health and remained high even when none of the metabolic abnormalities were present. The MHO group was found to have a significantly increased risk of all-cause mortality, but not cardiovascular mortality, when compared to the MHNW group<sup>[18]</sup>.

### **Mechanisms underlying the MHO phenotype**

Although MHO individuals have a favorable metabolic profile, it remains unclear whether this protection from the detrimental consequences of obesity is permanent or temporary. A recent meta-analysis including 5914 MHO individuals revealed that half of them experienced a deterioration of their metabolic health over time<sup>[12]</sup>. The Multi-Ethnic Study of Atherosclerosis, which was based on data from 6809 individuals, found that the MHO phenotype was not significantly associated with the incidence of CVD; however, half of the population in the study developed MetS during the follow-up period. The conversion from the MHO to MUO phenotype was found to result in a substantially increased CVD risk. Interestingly, study participants with a stable MHO phenotype were found to have a comparable risk as MHNW subjects<sup>[19]</sup>. Therefore, maintaining a healthy metabolic status appears to be a valid approach for preventing cardiometabolic diseases.

Despite the great interest of the research community, little is known about the exact mechanism responsible for a favorable metabolic profile in the presence of obesity. Several factors and phenotypic traits have been described, including normal adipose tissue function, low liver fat content, low visceral and ectopic fat volume, preserved insulin sensitivity, and low levels of inflammation<sup>[20]</sup>. Several studies have also suggested that genetic predisposition<sup>[21]</sup>, circulating micro-RNAs (miRNAs)<sup>[22]</sup>, or gut microbiota<sup>[23]</sup> may play a role (Figure 1).

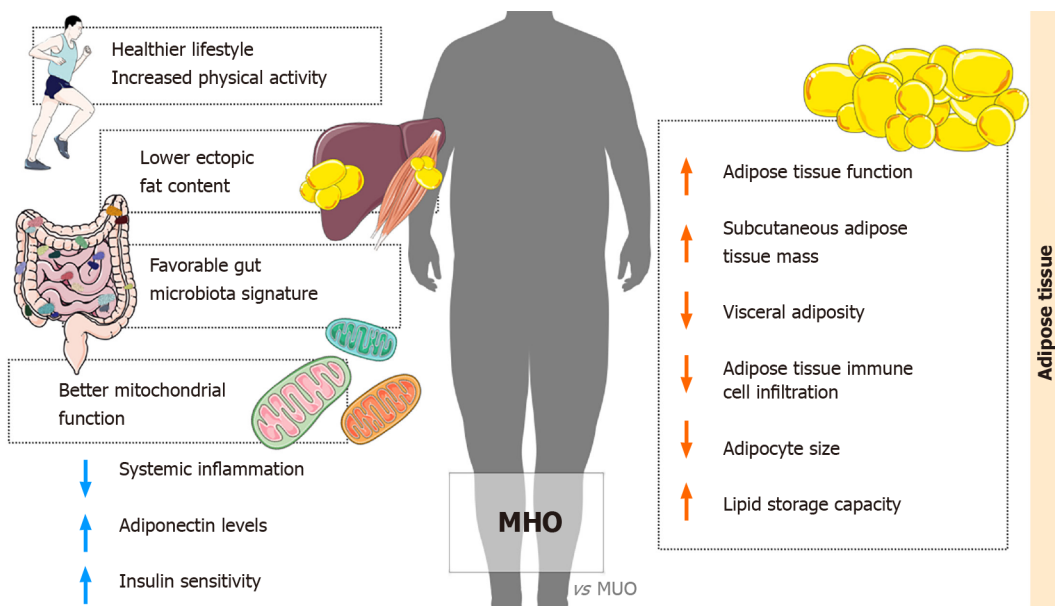
### **Aim of the review**

Given all these discrepancies and the rather unclear understanding of the underlying mechanisms, this review article aims to provide an overview of the current state of knowledge regarding the results of different omics studies that have investigated the MHO phenotype. In some cases, because of the lack of genome-wide analyses, the results of targeted studies are presented. We believe that this multi-level approach will help to better understand the mechanisms behind healthy obesity. With the recent progress in technology, it is now possible to study processes on different molecular levels in detail and on a wide scale (Figure 2). Therefore, we performed extensive searches in the PubMed database for articles that were published between 2010 and 2020. The following search terms in a variety of combinations were used: 'healthy obesity', 'MHO', 'metabolically healthy obese', 'genomics', 'transcriptomics', 'gene expression', 'proteomics', 'metabolomics', 'gut microbiota', 'epigenomics', and 'miRNA'.

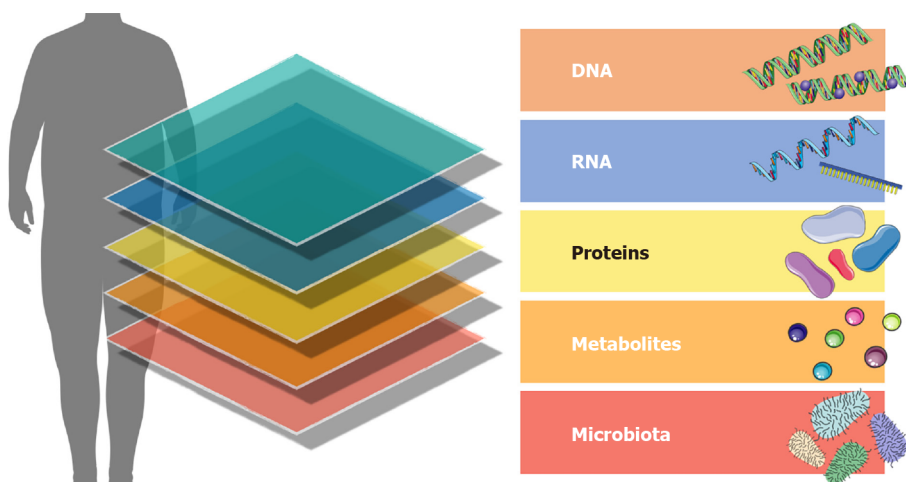
## **GENOMICS**

It is well established that genetic factors contribute to the development of obesity<sup>[24]</sup>; however, their role in metabolic health deterioration in obese individuals is still being clarified<sup>[21]</sup>. Even though genetic predisposition cannot be changed, being aware of the presence of certain variants allows the modification of environmental factors, which can delay or prevent the development of a disease or condition. Genome-wide association studies help to identify the link between a single nucleotide polymorphism (SNP) and a particular disorder<sup>[25]</sup>.

In a study by Schlauch *et al*<sup>[26]</sup>, 89 out of 905027 investigated SNPs showed an association with the MHO phenotype, which was defined by following characteristics: BMI  $\geq 35$  kg/m<sup>2</sup>, normal blood pressure, normal fasting plasma glucose (less than 100



**Figure 1** Differences in characteristics between metabolically healthy obese and metabolically unhealthy obese individuals. MHO: Metabolically healthy obese; MUO: Metabolically unhealthy obese.



**Figure 2** Different molecular levels targeted by a multi-omics approach.

mg/dL), and a desirable fasting lipid panel based on the National Cholesterol Education Panel Adult Treatment Panel III (NCEP ATP III) recommendations. Interestingly, all 89 SNPs identified in this study were located within intergenic regions, or non-coding regions, of their respective genes. Further analysis revealed that 12 genes associated with significant SNPs had been previously linked to obesity or cardiometabolic disease: *DPYD* to syndromic obesity, *KLHL6* to acute insulin response to glucose, *RTP4* to future incidence of hypertension, *PCDH7* to WHR, *FSTL4* to increased risk of stroke, *CSMD1* to MetS, *ITIH5* to BMI, *KCNQ1* to type 2 diabetes, *NBEA* to increased diastolic BP, *BBX* to type 1 diabetes, and *NTRK2* to obesity and depression. Furthermore, 31 polymorphisms were observed to be located close to at least one other SNP within the same gene or intergenic region, suggesting their special relevance according to the authors. A different study reported a significant association of *FTO* (rs1121980) and *TCF7L2* (rs7903146) genetic variants with maintaining metabolic health in obese women, who did not develop MetS according to joint interim statement criteria<sup>[27]</sup>. In addition, a genetic variant of *SLC39A8* (rs13107325) was found to have a statistically significant association with metabolic health status in a subgroup of postmenopausal women. The *TCF7L2* and *SLC39A8* genetic markers were shown to have a protective effect and help preserve metabolic health status, while the other marker was found to increase the risk of transitioning from the MHO



to MUO phenotype. The same *FTO* genetic marker (rs1121980) was demonstrated to have a significant association with the MUO but not MHO phenotype in a previous genotyping study, which also applied joint interim statement MetS definition<sup>[28]</sup>.

Gao *et al.*<sup>[29]</sup> investigated the association of SNPs of the *MC4R* gene with the MHO and MUO phenotypes in a Chinese population and found that rs2331841 increased the genetic risk of the MUO phenotype. Metabolically healthy subjects were defined as having less than two of the metabolic abnormalities in the NCEP ATP III criteria, while metabolically abnormal patients had at least two abnormalities. Based on the results of their study, the authors suggested that the rs2331841 SNP was associated with not only obesity but also metabolic abnormalities in obese subjects. Another group of researchers focused on the lactoferrin (*LTF*) gene-related polymorphisms and observed significant differences in the genotype frequencies of *LTF* rs2239692 between MHO and MUO individuals<sup>[30]</sup>. The CT variant of *LTF* rs2239692 was found to significantly decrease the risk of MetS development in obese individuals. Two other studies have reported an association of the adiponectin rs2241766 genetic variant with metabolic health phenotypes<sup>[31,32]</sup>. One reported more than a two-fold increase in the risk of developing metabolic abnormalities in the presence of the T45T genotype, while the second one found that the adiponectin T45G polymorphism was associated with the progression from the MHO to MUO phenotype. Both studies used International Diabetes Federation (IDF) MetS criteria to identify metabolically healthy individuals. The genetic variants associated with the MHO phenotype are presented in [Table 1](#).

## TRANSCRIPTOMICS

### Gene expression

In recent years, transcriptome profiling has become one of the most common approaches for investigating diseases on a molecular level<sup>[33]</sup>. Gene expression profiling provides valuable information about any alterations in biological processes, and this information is crucial for not only understanding the underlying mechanisms but also making a molecular diagnosis and selecting a clinical therapy for the condition under study. The methods most widely used in gene expression studies are real-time reverse transcription polymerase chain reaction (RT-PCR), microarrays, and next-generation sequencing. Only a few research groups have applied a genome-wide approach to identify gene expression alterations in the MHO phenotype ([Table 2](#)). Gaye *et al.*<sup>[34]</sup> performed whole blood transcriptome sequencing on samples from 8 MHO and 21 MUO individuals. The top 10 of the 149 enriched GO terms were related to mRNA translation processes. The canonical pathway analysis conducted in QIAGEN's Ingenuity Pathway Analysis (IPA) revealed significant enrichment in EIF2 signaling, regulation of eIF4 and p70S6K signaling, and mTOR signaling pathways. Of note, the top differentially expressed genes were ribosomal protein genes. The authors stated that the MUO subjects exhibited higher levels of ribosomal and endoplasmic reticulum (ER) stress and inflammation than the MHO individuals. They also noted that the TG/HDL-C ratio was possibly the major driver of the altered expression of ribosomal protein genes in these two groups. The authors proposed controlling ER and ribosomal stress and keeping the TG/HDL-C ratio in the proper range as potential strategies to prevent the development of an unfavorable metabolic profile in obese individuals.

We found that similar pathways were enriched in our recent transcriptomic study of different metabolic health phenotypes<sup>[35]</sup>. The IPA core analysis of differentially expressed genes between MHO and MHNW subjects revealed significant enrichment in EIF2 signaling, regulation of eIF4 and p70S6K signaling, oxidative phosphorylation, mitochondrial dysfunction, and mTOR signaling canonical pathways. Moreover, a vast majority of the genes within each of the identified pathways were upregulated. We also compared gene expression patterns between MUO and MHO individuals and found enrichment in canonical pathways associated with processes of inflammation, coagulation and thrombosis, hepatic fibrosis, and atherosclerosis signaling. Another study used whole genome microarrays to evaluate gene expression in the peripheral blood of obese individuals with and without metabolic disturbances<sup>[36]</sup>. The data analysis revealed enrichment in genes involved in lipid metabolism, carbohydrate metabolism, protein synthesis, and the activation, signaling, and function of cells. Another microarray study analyzed gene expression in abdominal subcutaneous adipose tissue samples from MHO and MUO subjects<sup>[37]</sup>. The authors performed unsupervised hierarchical clustering of 1595 obesity-associated transcripts and identified two obesity subtypes among the study participants. Differentially expressed



**Table 1** Single nucleotide polymorphisms associated with metabolically healthy obese phenotype

Nearest gene	SNPs	Ref.
ADIPOQ	rs2241766	Berezina <i>et al</i> <sup>[31]</sup> and Chang <i>et al</i> <sup>[32]</sup>
MC4R	rs2331841	Gao <i>et al</i> <sup>[29]</sup>
LTF	rs2239692	Jamka <i>et al</i> <sup>[30]</sup>
FTO	rs1121980	Gharooi Ahangar <i>et al</i> <sup>[27]</sup>
TCF7L2	rs7903146	Gharooi Ahangar <i>et al</i> <sup>[27]</sup>
SLC39A8	rs13107325	Gharooi Ahangar <i>et al</i> <sup>[27]</sup>
RTP4	rs9028, rs9843429	Schlauch <i>et al</i> <sup>[26]</sup>
CDH18	rs1022207, rs2967027	Schlauch <i>et al</i> <sup>[26]</sup>
FST/LOC257396	rs37785, rs10461563	Schlauch <i>et al</i> <sup>[26]</sup>
FSTL4/WSPAR	rs7719102, rs4246020	Schlauch <i>et al</i> <sup>[26]</sup>
LOC107986637	rs11753543, rs9384860	Schlauch <i>et al</i> <sup>[26]</sup>
TRPS1	rs2245221, rs2737214, rs2737215	Schlauch <i>et al</i> <sup>[26]</sup>
DLG2	rs7131460, rs12275254	Schlauch <i>et al</i> <sup>[26]</sup>
TOX2	rs766622, rs6065690, rs6093921	Schlauch <i>et al</i> <sup>[26]</sup>
FAM19A2/SLC16A7	rs4143650, rs6581305	Schlauch <i>et al</i> <sup>[26]</sup>
LOC101927367/LOC105371833	rs11079177, rs12601773	Schlauch <i>et al</i> <sup>[26]</sup>
LOC105371989	rs206549, rs206547	Schlauch <i>et al</i> <sup>[26]</sup>
LOC107986666	rs9295227, rs9458896, rs6928576, rs6902153, rs10945918, rs7748991, rs9356148	Schlauch <i>et al</i> <sup>[26]</sup>

SNPs: Single nucleotide polymorphisms.

genes between the two groups were mainly enriched in CVD pathways (occlusion of artery, vascular disease, peripheral arterial occlusive disease) and immune and inflammatory response pathways (complement system, TREM1 signaling, interleukin-8 signaling).

Targeted gene expression analyses of the MHO phenotype have been much more common. In one study, five out of six investigated mitophagy genes showed no significant differences in expression between MHO and metabolically healthy non-obese subjects<sup>[38]</sup>. The authors concluded that unaltered mitophagy allowed the control of oxidative stress and inflammation, leading to improved mitochondrial function and preserved insulin sensitivity in MHO subjects. An earlier study reported lower levels of oxidative and ER stress in MHO individuals based on the results of a gene expression experiment focusing on genes involved in the regulation of the unfolded protein response<sup>[39]</sup>. Another research group investigated a set of proinflammatory genes and found no significant differences between abdominally obese MHO and MUO subjects<sup>[40,41]</sup>. The two groups were matched for abdominal fat, and because of this matching, the authors suggested that the expression levels of Toll-like-receptor (TLR) 2, TLR4, TRIF, MyD88, and nuclear factor- $\kappa$ B (NF- $\kappa$ B) were related more to abdominal obesity than to metabolic health status. Telle-Hansen *et al*<sup>[42]</sup> analyzed the expression of selected genes involved in lipid uptake, transport, lipolysis, lipogenesis, and fatty acid oxidation and found reduced gene expression of *UCP2*, *LIPE*, and *PPARG* in the MUO group compared to that in the MHO group, while no differences were observed between MHO and MHNW individuals. A different research group analyzed the expression of inflammatory and matrix remodeling genes in visceral adipose tissue (VAT) and liver tissue samples from MHO and MUO subjects and found similar gene expression profiles between the groups in both tissues<sup>[43]</sup>.

### miRNAs

miRNAs are short non-coding RNA molecules consisting of around 22 nucleotides<sup>[44]</sup>. They have been proven to be important gene expression regulators, and most human protein-coding genes are regulated by at least one miRNA<sup>[45]</sup>. They exert their function

**Table 2 Studies investigating genome-wide gene expression levels in metabolically healthy obese individuals**

Biological material	Method	Study design	Sample size	Metabolic health definition	Main findings	Ref.
Whole blood	RNA-seq	MHO <i>vs</i> MUO	8/21	All conditions must be met: BP $\leq$ 130/85 mmHg, no medication; FPG $\leq$ 100 mg/dL, no medication; HOMA-IR $\leq$ 5.1; TG/HDL $\leq$ 1.65 for males, TG/HDL $\leq$ 1.32 for females; hsCRP $\leq$ 0.3 mg/dL	Enrichment in pathways: EIF2 signaling, eIF4 and p70S6K signaling, mTOR signaling. Enrichment in GO terms related to mRNA translation processes. Ribosomal protein genes among top differentially genes	Gaye <i>et al</i> <sup>[34]</sup>
Whole blood	RNA-seq	MHO <i>vs</i> MUO	8/8	No MetS, according to harmonized MetS definition	Enrichment in pathways: granulocyte/agranulocyte adhesion and diapedesis, coagulation system, intrinsic prothrombin activation pathway, atherosclerosis signaling, integrin signaling, binding and aggregation of blood cells	Paczkowska-Abdulsalam <i>et al</i> <sup>[35]</sup>
Whole blood	RNA-seq	MHO <i>vs</i> MHNW	8/8	No MetS, according to harmonized MetS definition	Enrichment in pathways: EIF2 signaling, eIF4 and p70S6K signaling, mTOR signaling, oxidative phosphorylation, mitochondrial dysfunction, vascular/arterial disease	Paczkowska-Abdulsalam <i>et al</i> <sup>[35]</sup>
PBMCs	Microarray	MHO <i>vs</i> MHNW	17/15	No MetS, according to NCEP ATP III MetS definition	Enrichment in pathways: carbohydrate metabolism, lipid metabolism, protein synthesis, amino acid metabolism, cell morphology, death and survival, cell-to-cell signaling and interaction, cellular development, movement, growth and proliferation	de Luis <i>et al</i> <sup>[36]</sup>
aSAT	Microarray	MHO <i>vs</i> MUO	16/14	MHO group identified through unsupervised hierarchical clustering of 1595 obesity-associated transcripts	Enrichment in pathways: complement system, TREM1 signaling, IL-8 signaling, actin cytoskeleton signaling, vascular disease, occlusion of artery	Das <i>et al</i> <sup>[37]</sup>

aSAT: Abdominal subcutaneous adipose tissue; PBMCs: Peripheral blood mononuclear cells; NCEP ATPIII: National Cholesterol Education Program Adult Treatment Panel III; BP: Blood pressure; FPG: Fasting plasma glucose; HOMA-IR: Homeostatic model assessment for insulin resistance; TG: Triglycerides; HDL: High-density lipoprotein; CRP: C-reactive protein; MHO: Metabolically healthy obese; IL: Interleukin.

through binding to target mRNAs, which results in mRNA translational repression or transcript degradation, depending on the degree of base-pairing complementarity<sup>[46]</sup>. Specific miRNA signatures have been described in many diseases, including obesity, type 2 diabetes, and CVD<sup>[22]</sup>. To date, however, only a few studies have investigated miRNAs in the MHO phenotype. The molecules that have been identified in MHO subjects by different research groups are presented in [Table 3](#).

In a recent study, Yang *et al*<sup>[47]</sup> used microarrays to analyze miRNA levels in plasma samples from six young MHO individuals and six healthy controls. Individuals with diabetes mellitus, hypertension, hyperlipidemia, coronary artery disease, valvular or congenital heart disease, cardiomyopathy, atrial fibrillation, and heart failure were excluded from the study. A distinct miRNA profile was identified for the MHO phenotype. Gene ontology analysis revealed the highest enrichment in the following terms: cell-cell adhesion, translational initiation, and apoptotic process among biological processes; nucleoplasm and nucleus among cellular components; and protein binding, DNA binding, and protein serine/threonine kinase activity among molecular functions. The expression levels of six of the top ten up- or downregulated miRNAs were further confirmed in a group of 600 MHO subjects in a RT-PCR experiment. MiRNA-500, miRNA-454, and miRNA-320a were found to be downregulated in MHO individuals, while miRNA-21, miRNA-148a, and miRNA-126 were found to be upregulated. The authors reported that high circulating miRNA-21 levels were associated with impaired diastolic function as well as increased cardiac fibrosis markers *via* the TGF- $\beta$ 1/Smad signaling pathway. A different set of miRNAs was identified in another study, which screened 179 serum miRNAs in a group of MHO and MUO individuals<sup>[48]</sup>. The authors applied a stringent inclusion criteria of three distinct definitions of MHO: the basic definition; the modified Wildman definition; and the most stringent definition which adds an inflammation marker to all parameters included in the modified Wildman definition. One of the eight differentially expressed miRNAs, identified in this study, remained significant after validation in an independent sample of 98 obese subjects with/without metabolic abnormalities. According to the authors, the differences in miRNA-374-5p expression between the MHO and MUO phenotypes were related to the TG/HDL-C ratio. Based

**Table 3** Different expression of micro-RNAs identified in metabolically healthy obese individuals

miRNA	Upregulation/ Downregulation	Sample size (MHO/MHNW)	Biological material	Ref.
MHO <i>vs</i> MHNW				
hsa-miR-500 <sup>1</sup>	↓	6/6	Plasma	Yang <i>et al</i> <sup>[47]</sup>
hsa-miR-454 <sup>1</sup>	↓			
hsa-miR-142	↓			
hsa-miR-320a <sup>1</sup>	↓			
hsa-miR-107	↓			
hsa-miR-34a	↑			
hsa-miR-21 <sup>1</sup>	↑			
hsa-miR-99b	↑			
hsa-miR-148a <sup>1</sup>	↑			
hsa-miR-126 <sup>1</sup>	↑			
MHO <i>vs</i> MUO				
hsa-miR-223-3p	↓	10/10	Serum	Doumatey <i>et al</i> <sup>[48]</sup>
hsa-miR-374a-5p <sup>1</sup>	↑			
hsa-miR-10b-5p	↑			
hsa-miR-26b-5p	↑			
hsa-let-7d-3p	↑			
hsa-miR-29a-3p	↑			
hsa-miR-342-3p	↑			
hsa-miR-16-2-3p	↑	34/21	Serum	Yue <i>et al</i> <sup>[49]</sup>
hsa-miR-503	↑			

<sup>1</sup>Validated with real-time reverse transcription polymerase chain reaction in an independent cohort. miRNA: Micro-RNAs; MHO: Metabolically healthy obese; MHNW: Metabolically healthy normal weight; MUO: Metabolically unhealthy obese.

on an analysis in an independent cohort, they also stated that miRNA-374-5p may modulate CCL2 expression, which is a pro-inflammatory marker, upstream of the pathway leading to dyslipidemia in obesity.

A more recent study applied a targeted RT-PCR approach and found significantly higher serum miRNA-503 Levels in MHO and MHNW subjects than in MUO individuals<sup>[49]</sup>. It was found that miRNA-503 negatively correlated with metabolic abnormalities in obese subjects, and the authors concluded that miRNA-503 can serve as a biomarker to distinguish between the MHO and MUO phenotypes, which were defined according to the 2007 Joint Committee for Developing Chinese Guidelines. Another study defined good metabolic health as being IS despite concurrent obesity<sup>[50]</sup>. Fifteen miRNAs were observed to be differentially expressed in subcutaneous white adipose tissue between IS and insulin resistant (IR) obese women. In further functional *in vitro* studies, the authors concluded that miRNA-143-3p and miRNA-652-3p might enhance insulin-stimulated glucose incorporation into lipids in human fat cells. Jones *et al*<sup>[51]</sup> analyzed 175 miRNAs in the plasma of IS and IR obese women and healthy controls. MiRNA-335 and miRNA-423-5p were found to be differentially expressed between the IR and IS groups. The authors also compared the miRNA profiles of the IS obese and healthy control groups and reported the top ten up- and downregulated molecules with different expression levels in each of the groups.

## DNA METHYLATION

DNA methylation is an epigenetic mechanism involving the covalent addition of methyl groups to nucleotide bases, usually cytosines in CpG dinucleotide sequences. It

affects gene expression levels by regulating interactions with transcriptional activators and repressors, as well as chromatin remodeling enzymes<sup>[52]</sup>. This heritable genetic marker is considered to play a major role in the epigenetic suppression of the transcription process. Differentially methylated regions have been described in a variety of conditions, including type 2 diabetes, cancers, and autoimmune and neurological disorders<sup>[53]</sup>. Only a few studies, however, have investigated this type of DNA modification and its association with the MHO phenotype.

A group led by Turcot analyzed the methylation levels of long interspersed nuclear element 1 (*LINE-1*) repetitive elements in VAT samples from obese individuals with and without MetS<sup>[54]</sup>. As *LINE-1* is considered to be a marker of genome-wide methylation, the authors concluded that lower global DNA methylation levels were associated with a greater risk of MetS in the presence of obesity. A year later, the same group reported that they found no association between dipeptidyl peptidase-4 (*DPP4*) methylation levels and metabolic health status<sup>[55]</sup>. In their study, Guénard *et al.*<sup>[56]</sup> applied a genome-wide approach to analyze altered biological pathways in VAT samples from obese men with and without metabolic abnormalities, which were defined by NCEP ATP III MetS definition. Using differential methylation analysis, they identified 8578 CpG sites with significant differences between the groups. Some of them were located within genes previously described as being related to processes of cellular growth and proliferation (*PRKCA*, *PPP2R2B*), lipid metabolism (*FASN*, *LRP1B*, *PLA1A*), or inflammation (*IL17RA*). Of note, the *RPTOR* gene, which regulates cell growth in response to nutrient and insulin levels, was found to contain 28 differentially methylated sites<sup>[56]</sup>. The subsequent enrichment analysis revealed pathways related to hepatic cholestasis, renin-angiotensin signaling, cell cycle regulation (cdc42 signaling, inositol phosphate metabolism), inflammation and immunity (antigen presentation pathway, autoimmune diseases signaling), and structural components of the cell membrane (glycerophospholipid/phospholipid metabolism).

## PROTEOMICS

Proteomics enables the genome-wide identification and quantification of all proteins present at a certain moment in a cell or tissue of interest. It can provide valuable insight into the molecular basis of a studied condition, disease, or phenotype at the protein level. Proteomics-based approaches are widely applied in different research areas, and they often lead to the discovery of novel biomarkers or drug targets<sup>[57]</sup>. The proteome of the MHO phenotype has also been studied by a few research groups (Table 4). In one study, a serum analysis of 20 African-American women was performed, and 20 differentially expressed proteins were identified between the MHO and MUO groups<sup>[58]</sup>. Metabolically healthy subjects fulfilled criteria of all three distinct definitions of the MHO phenotype: the basic definition; the modified Wildman definition; and the most stringent definition which adds an inflammation marker to all parameters included in the modified Wildman definition. Pathway analysis revealed enrichment in inflammatory and lipid pathways, including LXR/RXR (liver X receptor/retinoid X receptor) and FXR/RXR (farnesoid X receptor/retinoid X receptor) activation, atherosclerosis signaling, acute phase response signaling, and the complement system. Overall, lower levels of pro-inflammatory and higher levels of anti-inflammatory markers were observed in the MHO status than in the MUO status. A more recent study investigated changes in the urinary proteome of 18 obese individuals with different metabolic statuses defined by the IDF MetS criteria<sup>[59]</sup>. The authors detected 54 proteins with altered expression, and most were related to the NF-κB and p38 mitogen-activated protein (MAP) kinase pathways. The MUO study participants were found to have a higher abundance of proteins involved in inflammation (FIBA, TRFE, KNG1) and insulin resistance (ARL15, RET4) than the MHO individuals. In another study, 28 differentially expressed proteins were identified in VAT samples obtained from 18 patients undergoing bariatric surgery who had previously been divided into MHO and MUO groups according to the IDF MetS definition<sup>[60]</sup>. The differentially expressed proteins belonged to three functional categories: protein and lipid metabolism, cytoskeleton, and regulation of other metabolic processes. The top overrepresented IPA canonical pathways included death receptor signaling, coagulation system, acute phase response signaling, Rho GDI signaling, and NRF2 (nuclear factor erythroid 2-related factor 2)-mediated oxidative stress response. The proteins that were found to be upregulated in MHO individuals were mainly cytoskeletal and antioxidant proteins, as well as proteins involved in

**Table 4 Proteins with altered expression in metabolically healthy obese compared to metabolically unhealthy obese individuals**

Biological material	Over-expressed	Under-expressed	Top enriched pathways	Ref.
Serum	APOB, AHSG, SERPINC1, APOA4, SERPING1, RBP4, ITIH2, GSN, HRG, ITIH1, GC, C7	HBA1, HPR, HBB, CFB, ITIH4, CRP, PON1, C4A	LXR/RXR activation, FXR/RXR activation, acute phase response signaling, complement system, atherosclerosis signaling, IL-12 signaling and production in macrophages, production of nitric oxide and reactive oxygen species in macrophages, clathrin-mediated endocytosis signaling, extrinsic prothrombin activation pathway, intrinsic prothrombin activation pathway, coagulation system	Doumatey <i>et al</i> <sup>[58]</sup>
Urine	RASN, IGHG2, K1C10, VTDB	ACOT2, ARL15, APC4, APC7, APOA1, DYH3, FIBA, C1GLT, HIX, ITIH4, KNG1, P3H2, AMBP, COO33, RET4, TRFE, ZFP2, ZN568, ZN655	LXR/RXR activation, FXR/RXR activation, acute phase response signaling, clathrin-mediated endocytosis signaling, atherosclerosis signaling, IL-12 signaling and production in macrophages, coagulation system, intrinsic prothrombin activation pathway, production of nitric oxide and reactive oxygen species in macrophages, systemic lupus erythematosus signaling	Benabdelkamel <i>et al</i> <sup>[59]</sup>
VAT	ANXA5, ACTG, ACTB, LEG1, GPDA, APOA1, CO6A1, SBP1, CATA, TO20L, BRE1A, RNA58, SOX21	POTEE, SPTN4, GDIR1, TTHY, HSP1, PPIA, UPAR, PAI1, BLVRB, ERI2, YQ019	death receptor signaling, coagulation system, acute phase response signaling, RhoGDI signaling, NRF2-mediated oxidative stress response	Alfadda <i>et al</i> <sup>[60]</sup>

IL: Interleukin; VAT: Visceral adipose tissue; LXR: Liver X receptor; RXR: Retinoid X receptor; FXR: Farnesoid X receptor; NRF2: Nuclear factor erythroid 2-related factor 2; RhoGDI: Rho GDP-dissociation inhibitor.

mitochondrial import and transcriptional activity. The MUO individuals, on the other hand, were found to exhibit the upregulation of proteins that increase metabolic dysfunction in the ECM, mitochondria, and lipid droplets. The authors noted that the differentially expressed proteins were involved in complex pathways linked to insulin dysregulation. Doulamis *et al*<sup>[61]</sup> used a targeted proteomic approach to measure 30 potential biomarkers in serum and VAT samples from 28 obese patients undergoing bariatric surgery. The metabolic health status was defined as the presence (MUO) or absence (MHO) of comorbidities such as hypertension, dyslipidemia and diabetes mellitus. Compared to the levels in MUO individuals, six downregulated proteins in VAT (TWEAK, TRAIL, GDF-15, RETN, MMP-9, ICTP) and four downregulated proteins in serum (interleukin-20, PROK-1, TWEAK, CCL-3) were identified in MHO individuals. Those with the MUO phenotype were found to exhibit increased inflammation and higher levels of pro-inflammatory markers not only locally in adipose tissue but also systemically in the peripheral blood.

## METABOLOMICS

Metabolomics is an emerging technology used to analyze all small molecule compounds that are products or substrates of chemical reactions within a biological system (cell, tissue, or organism)<sup>[62]</sup>. It provides comprehensive information on the activity and status of cellular and organismal metabolism and is therefore widely applied in different settings to identify metabolic disturbances underlying a disease, novel biomarkers, or new therapeutic targets<sup>[63]</sup>. The analysis of the molecular composition of a sample is carried out through the use of nuclear magnetic resonance (NMR) or mass spectrometry (MS). Both liquid and gas chromatography are applied to separate metabolites<sup>[62]</sup>.

Several research groups that have investigated the MHO phenotype have performed metabolomic profiling experiments using NMR technology. A study on overweight and obese women with and without MetS in Finland found significant differences in branched-chain amino acids (BCAAs), aromatic amino acids (AAAs), orosomucoid, several species of fatty acids, and phospholipids between metabolically healthy and MetS study participants<sup>[64]</sup>. Of note, orosomucoid, BCAAs, and AAAs were associated with all MetS risk factors. A more recent study identified higher levels of alanine, glutamine, proline, asparagine, L-glutathione reduced, betaine, taurine, choline, 2-aminobutyrate, tagatose, and 2-oxoglutarate in MHO individuals than in MUO individuals<sup>[65]</sup>. Additionally, lower levels of L-alpha-phosphatidylinositol and D-sphingosine were observed in MHO subjects. A pathway enrichment analysis that



compared MUO and MHO subjects revealed alterations in the urea cycle, ammonia recycling, aspartate metabolism, and glycine and serine metabolism, among other pathways (Table 5).

A research group led by Telle-Hansen *et al.*<sup>[42]</sup> reported that the concentrations of very low-density lipoprotein, intermediate-density lipoprotein, and low-density lipoprotein subclasses were, overall, significantly higher in MUO individuals than in MHO individuals, with the MHNW group having the lowest values. In addition, the levels of HDL subclasses were lower in MUO and higher in MHNW individuals than in the MHO group; together, these results indicate that MHO subjects have an intermediate CVD risk profile compared to those of MUO and MHNW individuals. Similarly, an earlier study that used gas chromatography-MS analysis reported that the MHO group had an intermediate serum amino acid profile compared to those of MHNW and MUO subjects<sup>[66]</sup>. Even though most individual amino acids did not differ significantly between the MHO and MUO groups, several of them, including glycine, reflected the intermediate cardiometabolic profile of the MHO phenotype. Overall, the results suggested improved insulin sensitivity in the MHO group compared to that in the MUO group, as well as differences between the two groups in the availability of metabolites that enter the TCA cycle. Hydroxyproline concentration together with the ratios of other amino acids were proposed as biomarkers for distinguishing between the MHO and MUO phenotypes.

In their study, Chen *et al.*<sup>[67]</sup> identified a group of serum metabolites, including L-kynurenine, glycerophosphocholine, glycerol 1-phosphate, glycolic acid, tagatose, methyl palmitate, and uric acid, that differed significantly between the MHO and MUO groups. In addition, they found that several metabolic pathways, such as fatty acid biosynthesis, phenylalanine metabolism, propanoate metabolism, and valine, leucine, and isoleucine degradation, were altered between the two phenotypes. The authors reported an association of liver and mitochondria functions with metabolic disturbances in obese subjects. Another metabolomics study reported that the plasma metabolic profiles of MetS obese individuals correlated with the fulfillment of a number of criteria used to diagnose MetS<sup>[68]</sup>. Fifteen metabolites, including nucleosides, amino acids and derivatives, amino sugars, purine derivatives, and polyols, that could differentiate between metabolically healthy and unhealthy individuals were identified. These metabolites with altered concentrations belonged to numerous physiologically significant pathways, such as purine metabolism; valine, leucine, and isoleucine degradation; aminoacyl-tRNA biosynthesis; and tryptophan metabolism. A case control study that included over 100 MHO individuals reported that BCAAs, glutamic acid, tyrosine, and a specific pattern of lysophosphatidylcholines were associated with both the MHO and MUO phenotypes<sup>[69]</sup>. Interestingly, when the MUO and MHO phenotypes were directly compared, no significant differences in metabolites were found. Another study highlighted the importance of body fat distribution and performed a metabolomic analysis of serum samples from metabolically healthy peripherally obese and metabolically unhealthy centrally obese individuals<sup>[70]</sup>. The authors found that significantly higher levels of BCAAs (leucine, isoleucine, valine), propionylcarnitine (C3 acylcarnitine), and alpha-amino adipic acid could distinguish metabolically unhealthy central obesity from metabolically healthy peripheral obesity.

To better understand the role of visceral fat in the development of metabolic abnormalities, Candi *et al.*<sup>[71]</sup> analyzed the metabolomic profiles of VAT samples from MHO and MUO subjects. They found that the state of pathological obesity was associated with the increased metabolism of  $\gamma$ -glutamyl amino acids, which are involved in glutathione metabolism and the response to oxidative stress, and plasmalogens, which contribute to insulin resistance and hypertension<sup>[72]</sup>.

## MICROBIOME

Increasing evidence suggests that the human gut microbiota has a significant impact on maintaining immune and metabolic homeostasis and protecting against pathogens<sup>[73]</sup>. Changes in the composition of intestinal bacteria have been reported in a variety of disorders, including neurological, cardiovascular, and respiratory illnesses<sup>[74]</sup>. The most popular method for studying the human endogenous microbial community is 16S rRNA sequencing, which can effectively distinguish between different taxa<sup>[75]</sup>. To date, only a few research groups have investigated the association between gut microbiota and the MHO phenotype. Kim *et al.*<sup>[76]</sup> profiled fecal microbiota from over 700 overweight and obese Korean individuals who had no metabolic

**Table 5 Differentially regulated pathways between metabolically healthy obese and metabolically unhealthy obese groups identified by metabolomics studies**

Study	Chen <i>et al.</i> <sup>[67]</sup> , 2015	Zhong <i>et al.</i> <sup>[68]</sup> , 2017	Candi <i>et al.</i> <sup>[71]</sup> , 2018	Chashmian <i>et al.</i> <sup>[65]</sup> , 2020
Biological material	Plasma	Plasma	VAT	Serum
Method	LC-MS, GC-MS	LC-MS/MS	LC-MS/MS	NMR
MHO definition	No MetS, according to NCEP ATP III MetS definition	No MetS, according to harmonized MetS definition	No MetS, according to NCEP ATP III MetS definition	No MetS, according to NCEP ATP III MetS definition
MHO (n)	34	43	18	21
MUO (n)	34	26	18	21
Affected pathways	Fatty acid biosynthesis; Phenylalanine metabolism; Propanoate metabolism; Valine, leucine and isoleucine degradation; Pyrimidine metabolism; Citrate cycle (TCA cycle); Galactose metabolism; Glyoxylate and dicarboxylate; and Tryptophan metabolism	Purine metabolism ( <i>i.e.</i> , urate); Valine, leucine and isoleucine degradation; Aminoacyl-tRNA biosynthesis; Tryptophan metabolism; Cysteine and methionine metabolism; Lysine degradation; Pyrimidine metabolism; Arginine and proline metabolism; Glycine, serine and threonine metabolism; Taurine and hypotaurine metabolism; Alanine, aspartate and glutamate metabolism; Pantothenate and CoA biosynthesis	Ceramide metabolism; Phosphatidylserine; Fatty acid, dicarboxylate; Glutathione metabolism; Lysoplasmalogen; Lysolipid; Aminosugar metabolism; Gamma-glutamyl amino acid; Pyrimidine metabolism, uracyl containing; Plasmalogen; Glycerolipid metabolism; Sphingolipid metabolism; Phospholipid metabolism; Fructose, mannose, and galactose metabolism	Urea cycle; Ammonia recycling; Aspartate metabolism; Glycine and serine metabolism; Glucose-alanine cycle; and Arginine and proline metabolism

LC: Liquid chromatography; GC: Gas chromatography; MS: Mass spectrometry; NCEP ATP III: National Cholesterol Education Program Adult Treatment Panel III; TCA: Citric acid cycle; MetS: Metabolic syndrome; NMR: Nuclear magnetic resonance; MHO: Metabolically healthy obese; MUO: Metabolically unhealthy obese.

abnormality (metabolically healthy) or had at least one metabolic abnormality (metabolically unhealthy). To define metabolic abnormalities the NCEP ATP III criteria were used. Genera such as *Oscillospira* and *Clostridium* were found to be significantly more abundant in metabolically healthy individuals, and the results were similar for the family Coriobacteriaceae within *Actinobacteria* and the family Leuconostocaceae within Firmicutes. On the other hand, metabolically unhealthy individuals were found to have an increased abundance of Fusobacteria. Interestingly, no differences in the Firmicutes/Bacteroidetes ratio between the two groups were observed. Of note, the microbial profile of metabolically healthy overweight/obese subjects was closer to that of lean individuals than to that of metabolically unhealthy overweight/obese individuals. A study on obese Mexican women with and without MetS reported that three genera and families were significantly enriched in the obese without MetS group: *Roseburia*, *Succinivibrio*, and S24-7 (all were three-fold more abundant than in the MetS obese and control groups)<sup>[77]</sup>. Another study, in which the NCEP ATP III MetS definition was applied to define the metabolic status of older Irish individuals, reported no differences in the diversity, richness, or taxonomy between the MHO and MUO groups<sup>[78]</sup>. A different approach was applied by Kashtanova *et al.*<sup>[79]</sup>, who analyzed the association of the gut microbiota composition with individual cardiovascular risk factors. An increase in the abundance of certain genera was observed depending on the type of metabolic abnormality: *Blautia* in cases of impaired carbohydrate metabolism and *Prevotella* in individuals with elevated BP and obesity. The abundance of *Serratia* increased together with a number of cardiovascular risk factors, whereas an inverse relationship was observed between the abundance of *Oscillospira* and abdominal obesity.

## CONCLUSION

As evidenced by the findings discussed in this review, over the past several years, enormous progress has been made regarding the molecular characterization of different metabolic health phenotypes. The studies conducted to date are a source of valuable information on the protective mechanisms underlying the MHO phenotype or the mechanisms responsible for metabolic health deterioration and the transition from the MHO to MUO phenotype. Genomic studies have identified genetic variants

that are related to increased adiposity with a favorable metabolic profile. Many of the identified loci are located near genes previously reported to be involved in insulin signaling, insulin resistance, fat distribution, and adipogenesis. The results of transcriptomic analyses suggest that mitochondrial dysfunction, ER and oxidative stress, pathological changes in the liver, and activated atherosclerotic processes contribute to the development of metabolic abnormalities. Moreover, miRNA profiling studies have identified several molecules that are characteristic of the MHO phenotype, some of which are involved in processes such as cell-cell adhesion, translational initiation, and the apoptotic process. Epigenomic analyses have revealed differences in the methylation of sites within genes involved in lipid metabolism, inflammation, and cellular growth. Similar pathways have been found to be altered within the proteomes of different metabolic health phenotypes. Metabolomics has been used to confirm the important role of liver and mitochondria functions in metabolic disturbances, while significant differences in the gut microbiota composition have been found between MHO and MUO individuals.

Nevertheless, multiple issues still need to be addressed in the near future to take full advantage of all the research done in this field. The inconsistent or even conflicting results among some studies underscore the need for a unique metabolic health definition that is agreed upon by everyone in the research community. There must be consensus regarding not only the criteria and their cut-off values but also the number of risk factors that determine the MHO phenotype. Until then, the interpretation and comparison of the results from different study groups will remain difficult, if not impossible. Another challenge is to go beyond pilot molecular studies with small numbers of participants and develop national and international collaborative networks. Larger sample sizes should address the issues of heterogeneity among study participants, as well as difficulties in the recruitment of the most MHO individuals, who do not exhibit any of the risk factors despite their obesity. Another issue that needs to be considered is that only a few studies on metabolic health have applied the global analysis approach in the form of omics technologies, likely because of the high cost combined with the limited funds of pilot studies. Ideally, a large-scale study on universally defined metabolic health phenotypes should be carried out, implementing all omics technologies and therefore exploring metabolic function comprehensively at all possible levels: genomic, transcriptomic, epigenomic, proteomic, and metabolomic, as well as that of the gut microbiota. The markers identified with different omics methods can then be fitted into a multi-omics framework, revealing the interplay between molecules or pathways and the roles of different factors in mechanisms underlying metabolic health deterioration.

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