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Metabolically healthy versus metabolically unhealthy obesity

Carla Iacobini^{a,1}, Giuseppe Pugliese^{a,1}, Claudia Blasetti Fantauzzi^a, Massimo Federici^b, Stefano Menini^{a,*}

^a Department of Clinical and Molecular Medicine, "La Sapienza" University, Rome, Italy

^b Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy

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ABSTRACT

Obesity-related disease complications reduce life quality and expectancy and increase health-care costs. Some studies have suggested that obesity not always entails metabolic abnormalities and increased risk of cardiometabolic complications. Because of the lack of universally accepted criteria to identify metabolically healthy obesity (MHO), its prevalence varies widely among studies. Moreover, the prognostic value of MHO is hotly debated, mainly because it likely shifts gradually towards metabolically unhealthy obesity (MUO). In this review, we outline the differential factors contributing to the metabolic heterogeneity of obesity by discussing the behavioral, genetic, phenotypical, and biological aspects associated with each of the two metabolic phenotypes (MHO and MUO) of obesity and their clinical implications. Particular emphasis will be laid on the role of adipose tissue biology and function, including genetic determinants of body fat distribution, depot-specific fat metabolism, adipose tissue plasticity and, particularly, adipogenesis. Finally, the emerging role of gut microbiota in obesity and adipose tissue dysfunction as well as the search for novel biomarkers for the obesity-related metabolic traits and associated diseases will be briefly presented. A better understanding of the main determinants of a healthy metabolic status in obesity would allow promotion of this favorable condition by targeting the relevant pathways.

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Abbreviations: BMI, body mass index; CVD, cardiovascular disease; MHO, metabolically healthy obesity; MUO, metabolically unhealthy obesity; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

* Corresponding author at: Department of Clinical and Molecular Medicine, "La Sapienza" University, Via di Grottarossa, 1035-1039, 00189 Rome, Italy.

E-mail address: stefano.menini@uniroma1.it (S. Menini).

¹ Carla Iacobini and Giuseppe Pugliese contributed equally to this work.

1. Introduction

The worldwide prevalence of obesity has nearly tripled in the last 40 years and continues to increase in many parts of the world [1]. Recent estimates report that at least 35% of men and 40% of women are obese in the US [2]. As a result, obesity and related metabolic disorders, particularly type 2 diabetes, are responsible for more deaths than undernourishment in most countries of the world [1].

Overweight and obesity are defined by body mass index (BMI), a simple calculation based on the ratio of a person's weight in kilograms divided by height in meters squared. According to the World Health Organization classification, a BMI between 25 and 29.9 is classified as overweight, and a BMI of 30 or above indicates obesity [3]. The BMI does not measure the amount of adiposity directly, though it correlates moderately with a number of direct measures of body fat content [4,5]. Importantly, BMI is associated with a reduced life expectancy [6], mainly due to an increased risk of type 2 diabetes, hypertension, dyslipidemia, cardiovascular disease (CVD) and many types of cancer [7]. Therefore, the purpose of classifying subjects according to BMI levels is to standardize terminology and clinical severity based on the evidence that there is a dose-dependent relationship between BMI and adverse health outcomes (Fig. 1) [7]. However, at an individual level, BMI cannot distinguish between fat and lean tissue and is not diagnostic of the body fatness, health status and associated risks [8]. This may partly explain the observation that a substantial proportion of overweight (~50%) and obese (~30%) individuals are free from any obvious sign of metabolic and cardiovascular complications [9]. The healthier phenotype displayed by some obese patients has been called metabolically healthy obesity (MHO) and is more often observed in young, physically active patients with a better nutritional status and low levels of ectopic and visceral fat storage [10]. Unlike subjects with metabolic unhealthy obesity (MUO), individuals with MHO display no metabolic abnormalities such as insulin resistance, hypertension and dyslipidemia. Despite similar total fat mass, MHO is also characterized by a lower degree of systemic inflammation and a favorable immune and liver function profile (Fig. 2) [11–13].

Actually, the overall prevalence of MHO is a matter of debate [11,14–16] and its very existence has been even questioned [17]. In particular, the prognostic value of MHO was recently challenged by

a study showing that MHO individuals are still at higher risk of coronary heart disease, cerebrovascular disease, and heart failure than normal weight metabolically healthy individuals [17]. In addition, in MHO individuals, cardiovascular risk may not improve upon weight loss interventions [18–20], a finding with implications for obesity management.

Many factors may be called into question to explain inconsistency among results from different studies, including the failure to account for all confounders, the lack of generally accepted criteria to define MHO [11,12], the inability of the BMI to differentiate between different fat depots and, particularly, the evidence indicating that MHO is probably a transient condition [21,22]. Long-term studies have indeed shown that the prevalence of MHO in a population of obese subjects gradually decreased over time [21] and that both severity and duration of obesity were associated with the incidence of the metabolic syndrome during the observation period [23,24]. However, even if MHO was a “fixed-term” health condition destined, sooner or later, to shift towards MUO, it would still be worthwhile to understand the biological mechanisms underlying MHO. Like the honeymoon period of type 1 diabetes, in fact, MHO may be a “honeymoon phase” of obesity, with no standard time for it to last, and no guarantee that each person will experience this phenomenon. Understanding the nature of the factors which determine MHO would allow promoting and extending the “honeymoon phase” of obesity by targeting the relevant pathways. Nevertheless, the priority should be to encourage and facilitate weight loss, since metabolic and cardiovascular complications are not the only aspects of health that obesity can detrimentally affect. Respiratory problems, sleep apnea, and musculoskeletal disorders (especially osteoarthritis) [25] as well as several types of cancer [26,27] are all established obesity-related disease complications.

In this review, we outline the differential factors contributing to the metabolic heterogeneity of obesity by discussing the environmental/behavioral, genetic, phenotypical, and biological features that have been associated with the more favorable obesity phenotype and with the associated reduced risk for CVD. Along with this, we also summarize the state of the art of our understanding of MHO biology and provide an outlook on the future clinical and experimental challenges for MHO diagnosis and research, which will hopefully lead to a more cost-effective treatment and management of obese patients.

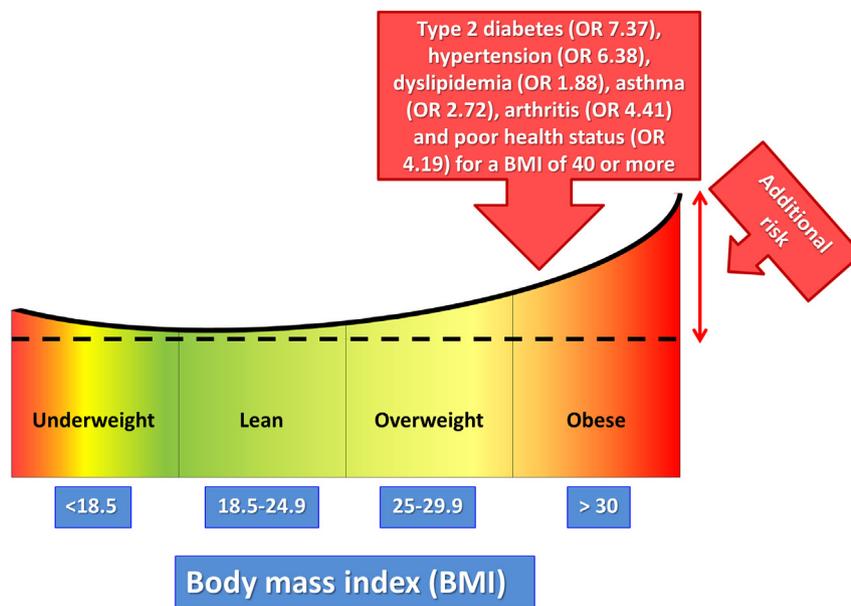


Fig. 1. Relationship between BMI and adverse health outcomes. Overweight and obesity are associated with several disease conditions. Compared with lean adults, those with a BMI equal or greater than 40 have an increased chance of being diagnosed with type 2 diabetes, hypertension, hypercholesterolemia, asthma, arthritis, and a poor general health status [7]. OR = odds ratio.

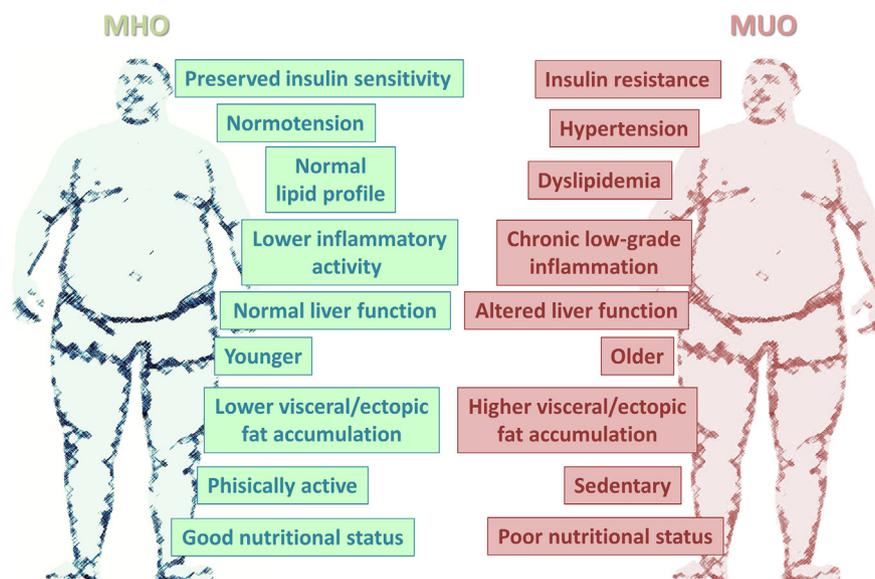


Fig. 2. Clinical and behavioral characteristics of MHO versus MUO. Despite similar total fat mass, individuals with MHO show preserved insulin sensitivity, normal blood pressure and serum lipid profile, as well as a lower degree of inflammation and liver function abnormalities compared with MUO patients [11–13]. MHO individuals are usually younger, more physically active and have a better nutritional status than their BMI-matched counterparts with MUO, from which they also differ in body fat distribution [10]. MHO = metabolically healthy obesity; MUO = metabolically unhealthy obesity; BMI = body mass index.

2. Defining MHO

Obesity commonly entails a higher risk of metabolic disorders such as insulin resistance, impaired fasting glucose and/or tolerance, dyslipidemia, and hypertension. Together with abdominal obesity, these are all risk factors for type 2 diabetes mellitus and CVD. The metabolic syndrome is a cluster of three or more of these abnormalities listed above which confers an additional risk for future CVD, over and above the sum of the individual components [28]. However, not all the individuals with high BMI exhibit abdominal obesity or its metabolic complications; in fact, the metabolic syndrome affects only some obese patients.

Starting from these widely accepted concepts, the first step is MHO definition. In fact, there are currently no universally accepted criteria for identifying MHO [12]. Generally speaking, a person with MHO has a BMI high enough to be classified as obese but without the metabolic abnormalities that are usually linked to obesity. However, this definition has some pitfalls. First, BMI cannot distinguish between fat and lean tissue or provide information on body fat distribution. Consequently, a person may have a high BMI but be muscular and physically fit; or may be obese but have little accumulation of visceral adipose tissue (VAT). Second, this definition of MHO does not clarify which metabolic complications should be considered and how these derangements should be assessed. A study showed that 30% of BMI-defined obese subjects classified as MHO according to fasting plasma glucose exhibited impaired glucose tolerance or even type 2 diabetes when subjected to an oral glucose tolerance test [29], indicating that one out of three obese patients considered to be metabolically healthy actually has glucose metabolism abnormalities. Moreover, body fat percentage was shown to be superior to BMI in identifying subjects with disturbed glucose tolerance [30]. Misclassification may explain the variability in both prevalence (3 to 57% of obese patients) [31–34] and cardiometabolic profile of MHO among studies. In fact, MHO subjects showed cardiometabolic and inflammatory profiles as well as VAT expansion comparable to those of MUO individuals when obese status and metabolic phenotype were defined based on BMI and fasting glucose measures [29]. A further argument along this line is that body composition analysis and oral glucose tolerance test enable a better identification of patients at risk of developing type 2 diabetes and CVD [35].

To eliminate subjective classification bias, Stefan et al. suggested that six parameters should be considered: waist circumference, insulin

resistance, blood sugar levels, blood pressure, cholesterol levels and physical fitness [12]. Though an agreement on the parameters defining MHO has not been reached yet, most of the authors agree that MHO is characterized by three clinical features: a reduced accumulation of VAT and ectopic fat for equal total adiposity [12,13,34], preserved insulin sensitivity, and a lower degree of systemic and adipose tissue inflammation compared to MUO patients [9,37–42]. However, the role of inflammation remains controversial [43].

3. Modifiable Lifestyle Factors

MHO patients appear to have a lower risk for CVD. Despite the strong link between high BMI/metabolic syndrome and CVD, Dhana et al. have recently demonstrated that obesity without metabolic abnormalities does not confer a higher CVD risk in the elderly population [44]. Evidence exists that lifestyle habits might partly explain the heterogeneity of obesity in terms of metabolic abnormalities. A study reports that adopting four healthy habits – i.e., moderate alcohol intake, not smoking, 30 min of exercise daily, and eating five or more servings of vegetables and fruits daily – even individuals classified as overweight and obese by BMI can have the same overall mortality risk as normal weight people [45]. Similar conclusions come from a cross-sectional study analyzing the prevalence and clinical characteristics of MHO versus MUO phenotype in a working population in Spain. Besides the observation that the MHO phenotype occurred more frequently in younger and female adults, the authors emphasize that MHO individuals were more likely to exercise and were less likely to smoke or drink heavily [46]. Mechanistically, lifestyle might modulate whole-body energy metabolism (Fig. 3), as suggested by the evidence that concurrent physical activity increases fatty acid oxidation during high calorie intake [47]. Notably, MHO individuals were shown to have a lower fasting Respiratory Quotient – i.e., higher fat utilization – than MUO individuals, particularly those with type 2 diabetes. In addition, the same study showed that insulin sensitivity is positively associated with the ability to extract energy from fat [48].

Finally, an additional distinction between MHO and MUO individuals regarding the quality of sleep has been recently identified. In addition to sleep duration [49], selected sleep quality factors, such as having trouble falling asleep and feeling unrested or overly sleepy during the day were associated with higher odds of MUO [50]. Further

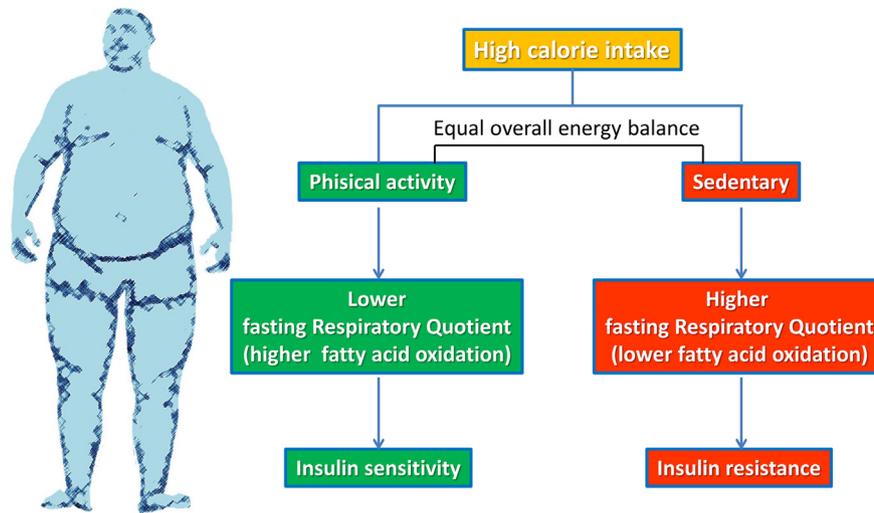


Fig. 3. Relationships between lifestyle factors, whole-body energy metabolism and insulin sensitivity. For equal overall energy balance (energy consumption versus energy expenditure), physical activity fosters the reduction of the fasting Respiratory Quotient, which is indicative of increased fatty acid oxidation [47]. It is notable that insulin sensitivity has been positively associated with the ability to produce energy from fat [48].

studies are needed to address the link between sleep disturbances and cardiometabolic health and, particularly, to assess whether sleep quality is a contributory cause or simply an indicator of MHO.

Altogether, these studies provide arguments in favor of the implementation of lifestyle modification programs to prevent or delay transition from MHO to MUO.

4. Fat Distribution and Adipose Tissue (Dys)Function

Several factors regulate fat distribution in various adipose tissue compartments. It is now generally accepted that central body fat distribution and an impaired adipose tissue function are better predictors of obesity-related metabolic abnormalities than total fat mass per se. VAT and ectopic fat accumulation, inflammation, impaired adipose tissue expandability and adipogenesis, as well as hypertrophy and altered lipid metabolism of fat cells, are anatomic and functional derangements of adipose tissue contributing to metabolic diseases and increased CVD risk [51]. A new scientific term, adiposopathy (clinically, “sick fat”), has been coined to describe this set of alterations that characterize adipose tissue dysfunction [52] and likely contribute to the cardiometabolic syndrome [51].

4.1. Genetic Determinants of Regional Fat Distribution

Considerable inter-individual variation exists in the response to excess energy intake. How much and where fat is stored is under the control of several factors. The main predictors of body fat distribution are age, sex and total body fat content [53]. However, substantial evidence indicates that fat distribution for a given BMI is regulated also by genetic factors. Consistently, several studies have attested that the waist-hip ratio, a surrogate measure of regional fat distribution, is a heritable trait with estimates of up to 60% [54,55]. The first evidence in favor of a genetic predisposition to store energy excess in visceral versus subcutaneous adipose tissue comes from the studies on identical twins conducted by Bouchard and colleagues more than 25 years ago [56]. Recently, genome-wide association studies identified a set of loci harboring genes possibly controlling both body extra fat distribution and the metabolic profile of excess adiposity (i.e., MHO or MUO). To date, a total of 14 genetic variants have been associated to a lower risk to develop metabolic abnormalities, such as dyslipidemia, hypertension, and type 2 diabetes, and consequent CVD, despite a BMI in the obese range [57–60]. Paradoxically, a study conducted in a population of 118,012 individuals from the UK Biobank [57] showed that 11 of these

genetic variants are more common in individuals with higher BMI and body fat mass percentage, thus indicating that the genetic score characterizing the MHO phenotype is associated with higher, not reduced, adiposity (Fig. 4) [57–60]. Consistent with a role of these risk alleles in the regulation of adiposity and glucose metabolism, the same 11 genes were previously identified by a hierarchical clustering analysis as sharing features of monogenic lipodystrophic insulin resistance, including lower BMI, higher VAT-to-subcutaneous adipose tissue (SAT) ratio, impaired insulin sensitivity and propensity to develop type 2 diabetes [59]. Interestingly, at least two of these variants lie near genes involved in adipocyte differentiation (*PPARG*) and insulin signaling (*IRS1*), and other two variants, those in *LYPLAL1* and *GRB14*, were previously associated to waist-to-hip ratio [60]. Variant-related changes in the expression of these genes and in the level or function of their protein products may lead to variability in fat distribution, thus linking obesity to metabolic abnormalities. A proof of concept in this regard comes from the

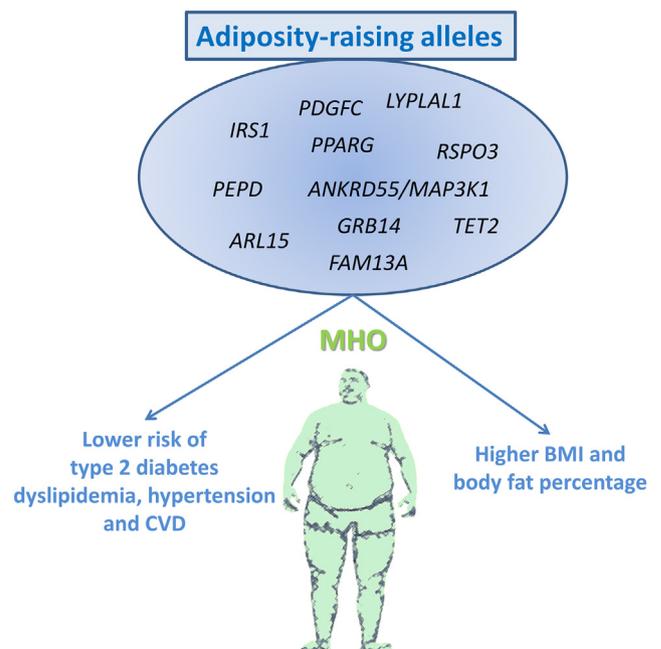


Fig. 4. Genetic variants related to MHO are associated with higher BMI and body fat percentage. MHO = metabolically healthy obesity; BMI = body mass index.

observation that the gene variant *SHOX2*, whose expression levels in human SAT positively correlate with visceral obesity, is involved in lipolysis regulation, thus confirming its role as modulator of adipocyte function and molecular determinant of fat topography (Fig. 5) [61].

In addition, there is genetic evidence that developmental genes differentially expressed in various fat compartments might play a role in the regulation of fat distribution and obesity-related metabolic traits. Importantly, the depot-specific gene expression signature appears to be intrinsic, since it is also found in pre-adipocytes derived from the adipose depots and persists during *in vitro* differentiation [62,63]. This may suggest that SAT and VAT depots derive from different mesodermal regions which confer them distinctive biological features.

Taken together, these findings suggest that the difference between the MHO and MUO phenotype may be partly attributed to specific genetic traits modulating body fat distribution in different regional fat depots which hold diverse biological properties and functions [57].

4.2. Different Adipose Tissue Depots, Different Metabolic Risk

Adipose tissue is not a single and functionally uniform organ passively responding to stimuli implicated in metabolic disorders [64]. Not all fat depots are equally hazardous for health. A different anatomical location (i.e., visceral versus subcutaneous) and type of fat (i.e., white versus brown) make a difference. SAT may not be a risk factor for metabolic diseases, whereas VAT and ectopic fat accumulation in or around the liver, heart and muscles is causally related to insulin resistance, impaired glucose homeostasis and CVD [65–67]. Regarding the type of fat, while white adipose tissue is involved in the pathogenesis of obesity-related metabolic disorders, the energy-burning potential of brown adipose tissue confers to this type of fat a potential protective role for metabolic and cardiovascular health [68]. However, the extent of the contribution of brown adipose tissue to whole-body glucose homeostasis in humans has not yet been fully elucidated. The interested reader may refer to a recent review on the subject [69].

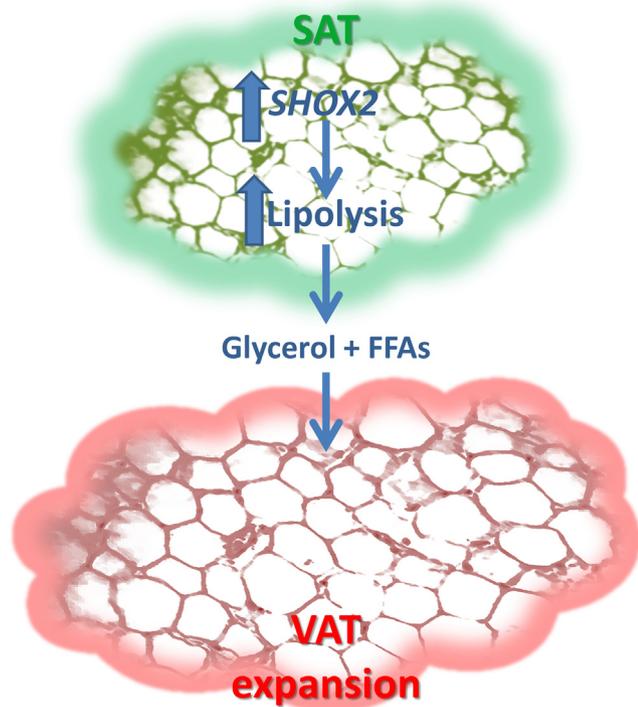


Fig. 5. *SHOX2*, an example of a genetic determinant of regional fat distribution. *SHOX2* expression in SAT is positively associated with both lipolysis activity of SAT adipocytes and visceral adiposity. SAT = subcutaneous adipose tissue; VAT = visceral adipose tissue; FFAs = free fatty acids.

MHO individuals are characterized by more SAT and less VAT, as well as lower fat accumulation in liver and skeletal muscle as compared to MUO subjects matched for BMI and fat mass [12,14,36]. Moreover, epidemiological studies have demonstrated that, for a given amount of total fat, greater fat accumulation in the lower body's SAT (i.e., gluteofemoral body fat) is a determinant of MHO [70] and is associated with lower risk of incident CVD [71]. Conversely, in equally obese individuals, prevalent fat accumulation in VAT, as determined by computed tomography, is associated with a MUO phenotype, characterized by hyperinsulinemia, glucose intolerance and atherogenic dyslipidemia [72]. Finally, a study demonstrated that obese individuals matched for VAT had comparable glucose tolerance irrespective of the amount of abdominal SAT, whereas those matched for SAT had different glucose tolerance depending on the magnitude of fat accumulation in VAT [73].

Therefore, the reason why MHO subjects are relatively protected against cardiometabolic diseases may lie in part in the divergent regulatory functions of VAT and SAT depots. Consistently, previous studies have shown that, in the presence of positive calorie balance, SAT expansion prevents the risk of lipid overflow and fat deposition in the abdominal VAT depot and in non-adipose tissue (i.e., ectopic) depots [74]. Thus, at the level of fat-specific depots, the difference between MHO and MUO seems to be reflected by the "fitness" of SAT: that is, an "healthy SAT" in the MHO and a dysfunctional SAT (adiposopathy) in the MUO.

4.3. Different Adipose Tissue Depots, Different Functions?

Adipocyte size reflects the balance between triglyceride storage and mobilization and is an important morphological characteristic to be considered in metabolic disorders [75]. Along with adipocyte hypertrophy, recruitment and proliferation of adipocyte precursors (i.e., adipocyte hyperplasia) are key mechanisms underlying adipose tissue expandability in response to surplus energy [76]. The need for adipose tissue expandability has been seen in experimental studies connecting the improved ability to increase total fat mass in response to overfeeding with metabolic improvement [77], and in epidemiological studies linking increased SAT, but not VAT expansion, with protection from type 2 diabetes risk (i.e., MHO) [78]. Consistently, the limited expandability of SAT was associated with insulin resistance (i.e., MUO) [79]. Mechanistically, a reduced lipid handling capacity of SAT, not adequate to the magnitude of the positive calorie balance, would favor diversion of lipids to VAT. In the visceral depot, hypertrophied adipocytes are characterized by a hyperlipolytic state not sensitive to the anabolic action of insulin, thus producing larger amounts of circulating free fatty acids [80] and favoring lipid overspill towards other tissues where fat is not physiologically stored, such as liver, heart, skeletal muscle, and pancreas [79]. Consistent with this scenario, adipocyte hypertrophy in VAT appears to be more closely linked to insulin resistance than adipocyte hypertrophy in SAT. In fact, a positive correlation was found between VAT adipocyte diameter and the degree of insulin resistance in obese women with metabolic syndrome, whereas a weaker, nonsignificant association was found between SAT adipocyte size and metabolic parameters [81]. Another study demonstrated that, though SAT adipocytes were larger than VAT adipocytes in severe obesity, only the size of VAT adipocytes correlated positively with insulin levels, fasting glucose and insulin resistance [82]. Finally, the surgical removal of abdominal SAT (liposuction) does not improve insulin sensitivity in obese individuals [83] and adipose transplantation of SAT into the abdominal cavity results in improved glucose metabolism [84]. These findings suggest that the different risk factor profiles for metabolic abnormalities might be due to the different biological properties of adipocytes from SAT and VAT, rather than the location of the fat depot itself.

Overall, there is substantial evidence that SAT is a safer place where to accommodate the excess energy intake than VAT. This paradigm helps to explain why some obese individuals do not develop

obesity-related metabolic abnormalities but cannot help to explain why some lean subjects are at higher risk for metabolic disorders. In fact, we must take in account that of the overall type 2 diabetes population, around one-fifth are normal weight [85].

4.4. Adipose Tissue Plasticity and Adipogenesis

Though VAT is considered a unique pathogenic depot that confers risk beyond its contribution to overall adiposity, also SAT volume and quality, as assessed by computed tomography imaging, have been associated with incident metabolic risk factors [86]. This observation suggests that both VAT and SAT volume and their qualitative features, such as lipid density and vascularity, may play a role in determining cardiometabolic risk among obese individuals. Changes of fat depots reflect the functional and expansion capacity of adipose tissue, which depends also on the ability to increase the number (i.e., hyperplasia), not only the size (i.e., hypertrophy) of adipocytes. Consistently, reduced adipose tissue expandability has been linked to inability of adipocytes to differentiate properly [87]. The beneficial effects of an increased hyperplastic capacity of adipose tissue has been experimentally demonstrated in adipose-specific mitoNEET transgenic mice in which augmented adipose tissue expandability, mainly due to adipocyte hyperplasia, is associated with preserved insulin sensitivity and glucose tolerance [88]. Several clues suggest a strict relationship between adipogenic capacity and metabolic health also in humans. Clinical observations suggest that pharmacological treatment with peroxisome proliferator activated receptor- γ agonists (thiazolidinediones) improves insulin sensitivity despite their adipogenic properties and fat-storing activity [89]. At the same time, lipodystrophies, which are disorders characterized by defective adipose tissue formation and/or inability to maintain a healthy fat, are often associated with variable degree of metabolic complications [59,90]. Patients suffering from familiar partial lipodystrophy, a group of usually monogenic, autosomal dominant disorders, are characterized by progressive adipocyte degeneration and loss of adipose tissue in extremities, hips and trunk, which are commonly accompanied by severe insulin resistance, type 2 diabetes, dyslipidemia, and non-alcoholic fatty liver disease [90–92]. These findings argue in favor of a role for adipose tissue dysfunction in metabolism dysregulation and offer compelling argument in support of the hypothesis that MHO, as well as other apparent paradoxical associations between obesity and health might be better explained if we consider adipose tissue function rather than simply the amount of fat mass.

Adipocyte/adipose tissue dysfunction, or adiposopathy, is a biological and clinical entity characterized by several anatomic, pathophysiological, and clinical manifestations [93]. The main pathophysiological features characterizing adiposopathy are impaired adipogenesis, reduced adipose tissue expandability, adipocyte hypertrophy, altered lipid metabolism, and adipose inflammation [94,95]. Among these features, increasing evidence suggests that defective adipogenesis may be the upstream factor leading to adipose tissue dysfunction and related metabolic disorders. Human findings indicate that defective adipogenesis may be involved in the development of obesity-related systemic insulin resistance and inflammation, two major hallmarks of MUO [78,96]. In obese individuals, systemic insulin resistance is associated with an increased ratio of small-to-large adipocytes and decreased levels of adipogenic genes, which is suggestive of a reduced adipogenic potential of fat tissue [87,97]. Noteworthy, these studies also demonstrate that, at the adipose tissue level, inflammation is related to the presence of a distinct sub-population of small, likely immature, adipocytes, thus linking impaired adipogenesis to adipose inflammation [95]. These data are interesting because there is experimental evidence that adipocyte inflammation is essential for healthy adipose tissue remodeling and expansion [98]. Pro-inflammatory signals from enlarging adipocytes may represent an adaptive response that favors safe storage of excess energy intake by stimulating adipogenesis and/or terminal differentiation of immature adipocytes [98]. This may imply that, besides restricting

accommodation of the chronic caloric excess in the mature adipocytes, a defective adipogenic potential would ramping up the inflammatory response of the increasingly hypertrophic adipocytes, in the attempt to further stimulate adipogenesis and the hyperplastic expansion of adipose tissue. This sequence of events is also consistent with the recent demonstration that impaired adipogenesis is positively associated with adipose secretion of interleukin-6 and insulin resistance in obese individuals [99].

To conclude, the metabolic health of obese individuals may ultimately depend on their adipogenic potential. An adequate adipogenic capacity would favor the energy-buffering activity of adipose tissue, ensuring metabolic health protection (i.e., MHO) (Fig. 6). However, for each individual, there is a finite capacity for the adipogenic process to operate and a finite limit to which adipose tissue can expand. Once the threshold is exceeded, a sequence of critical events begins, leading to metabolic complications and MUO. An impaired adipogenesis would anticipate and accelerate these events by restraining adipose tissue expandability and favoring adipocyte hypertrophy, ectopic fat accumulation and adipose/systemic inflammation, which instigate insulin resistance and abnormal glucose regulation (Fig. 6). Noteworthy, the hypothesis of defective adipogenesis as a key element of the obesity-related metabolic disorders may also explain the existence of metabolically unhealthy lean subjects.

5. Gut Microbiota and Adipose Tissue

Research in the past decade has shown that the composition of the gut microbiota and its activity are relevant to preserve metabolic homeostasis [100].

Provided with an extensive gene catalogue, the gut microbiome profoundly affects human metabolism. First, gut microbiota assists human metabolic pathways via the production of essential vitamins (especially the B group); second, gut microorganisms, through fermentation of indigestible carbohydrates, provide the human body with compounds such as short chain fatty acids that either are source of energy for the host or signal through membrane receptors to integrate metabolic functions [101].

Hence, it is not unexpected that the gut microbiome has been proposed both to interact with the immune system to harmonize individual responses to environmental factors and to portray a part in the penetrance of certain disorders not fully explained by genetics [102]. Recently, it has been suggested that loss of the microbiota-host equilibrium may result in the onset of “pre-disease” states (pre-diabetes, pre-hypertension, etc.), thus explaining the explosive development of chronic metabolic/inflammatory disorders [103].

Experimental studies have suggested that alterations in gut microbial and metabolic composition trigger inflammatory pathways in several tissues, thus boosting glucose intolerance and CVD [104,105]. In experimental models both the dysregulation of the microbiota-host co-metabolism of bile acids and metabolic endotoxemia promote metabolic dysfunction in liver and adipose tissue resulting in glucose intolerance [106,107]. Western style diets, that are typically enriched in saturated fats and deprived of fibers, have been proved to alter the gut microbiota structure ensuing adverse effects on intestinal permeability and fat colonization by intestinal bacteria, systemic inflammation and metabolic dysfunction [108,109].

Conversely, triggering the adaptive immune system with commensal gut bacteria protects against insulin resistance and glucose intolerance, implying a potential therapeutic role for probiotics to propel intestinal immunity and protect against dysbiosis [110].

Common microbiome changes observed in human obesity and type 2 diabetes initially included a higher Gram-positive *Firmicutes* to Gram-negative *Bacteroidetes* (F/B) ratio, although a recent meta-analysis showed no change of F/B ratio between lean and overweight people suggesting that the involvement of the microbiome in obesity might not be based on the taxonomic information provided by 16S rRNA

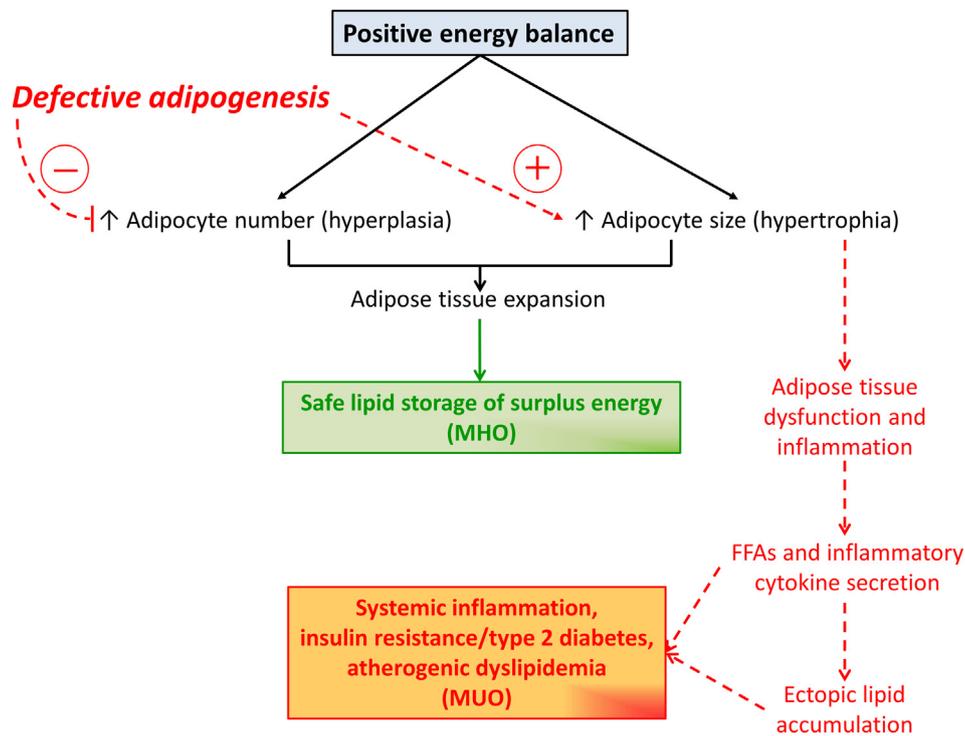


Fig. 6. Defective adipogenesis as the main instigator of obesity-related cardiometabolic disorders. MHO = metabolically healthy obesity; MUO = metabolically unhealthy obesity; FFAs = free fatty acids.

gene sequence but rather on common set of gene transcripts or metabolites that can result from diverse components of the microbiome [111].

Similarly, there is evidence for an overabundance or depletion of certain species, such as *Prevotella copri* and *Akkermansia muciniphila* [112–115]. However, two recent analyses found that *Prevotella copri* was associated with either deterioration or an improvement of insulin sensitivity [116,117]. Furthermore, most of the studies employing metagenomics methods found that obesity is associated with reduced measures of bacterial gene count or richness [118,119]. In obese subjects, deviation in microbial tryptophan metabolism with a specific induction of kynurenine and reduction in indoles is related to glucose intolerance, a phenomenon that is in part regulated in the intestine via interleukin 22 [120]. Gut microbiota composition has been also shown to be involved in adipose tissue browning and insulin action in morbidly obese subjects, possibly through circulating acetate [121]. Finally, in a randomized clinical trial, *Bifidobacterium animalis* ssp. *lactis* 420 (B420) was shown to positively affect markers of intestinal permeability such as serum zonulin 1 as well metabolic inflammation in parallel to improvement in abdominal obesity [122].

6. Metabolic-biochemical Profiling of MHO Versus MUO: The VAT and Plasma Sphingolipidome

By encompassing a significant fraction of a category of biomolecules, an “-omic” analysis helps to accelerate the understanding of the role of a biological system in physiology and disease [123,124]. Recently, a wide metabolomic approach was used to investigate differences among MHO, MUO and lean healthy controls in the metabolic profile of VAT [125]. Besides confirming substantial alterations of oxidative stress and glucose metabolism markers, this study revealed that VAT derived from MUO individuals is characterized by significant dysregulation of several lipid metabolic pathways. Specifically, higher levels of the sphingolipids ceramides and sphingomyelins were observed in VAT from MUO as compared to VAT from MHO and lean healthy subjects [125]. A recent study analyzed the plasma sphingolipidome profile of MHO and MUO subjects to check for specific signatures associated to

the metabolic status [126]. Specific molecular composition variations, such as altered serum levels of sphingomyelin and ceramide species with distinct saturated acyl chains, were associated with obesity and correlated with insulin sensitivity, liver function and atherogenic dyslipidemia [126]. Unfortunately, the clinical relevance of the plasma sphingolipidome still remains to be elucidated.

7. Conclusion and Perspectives

Obesity, as defined by BMI and total fat mass, is a risk factor for CVD, regardless of the associated metabolic health status. Therefore, reducing fat mass with lifestyle or surgical interventions remains the first therapeutic goal to be achieved.

With respect to the obesity-related metabolic dysfunctions, the first issue to be addressed is the lack of universally accepted criteria to define MHO. In this regard, the identification of individual metabolites and discrete pathways able to distinguish between MHO and MUO would help to establish diagnostic and prognostic criteria for the obesity-related cardiometabolic complications. Although evidence of obesity-related variations of lipidome composition is available, the clinical relevance of such associations is largely speculative at this time and much effort should be put in this direction. Clearer and more consistent diagnostic criteria would have obvious clinical implications, such as improvement of risk stratification and more appropriate, cost-effective forms of treatment.

With respect to the body fat distribution, VAT and ectopic fat accumulation is a major contributor to metabolic complications, over and above the BMI. Conversely, the relatively higher body fat accrual in SAT is a trait associated with MHO. Therefore, the challenge of implementation in clinical practice of fat distribution assessment for risk evaluation refinement should be a priority. At the same time, future research should focus efforts on clarifying the genetic and behavioral factors that influence the body fat distribution profile and the associated cardiometabolic risk. In addition, the molecular mechanisms underlying the genetic susceptibility to obesity-related metabolic traits need to be elucidated. In this regard, the role of adiposopathy, particularly

defective adipogenesis, deserves to be thoroughly investigated using suitable animal models.

Advancement in the understanding of the molecular mechanisms that foster a favorable body fat distribution profile and a healthier adipose tissue would help to identify new diagnostic targets to improve the ability of discriminating between MHO and MUO individuals and would hopefully enrich the pharmacological armamentarium against cardiometabolic complications of obesity.

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Disclosure statement

The Authors declare no conflicts of interest.

Author contributions

Drs. Menini, Iacobini and Pugliese drafted the article, except the paragraph entitled "Gut microbiota and adipose tissue", which was written by Dr. Federici. Dr. Blasetti Fantauzzi critically revised the article for important intellectual content.

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