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Clinical, functional, behavioural and epigenomic biomarkers of obesity

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Clinical biomarkers
- *3.1. Anthropometric biomarkers of obesity*
- *3.2. Inflammatory biomarkers of obesity*
- *3.3. Gut microbiota as a biomarker of obesity*
  - *3.3.1. Akkermansia (A.)muciniphila*
  - *3.3.2. Faecalibacterium (F.) prausnitzii*
  - *3.3.3. Prevotella (P.) copri*

4. Biomarkers of skeletal muscle functioning and physical activity habits
- *4.1. Biomarkers of muscle functional impairment in obesity*
  - *4.1.1. Muscle quality*
  - *4.1.2. Muscle architecture*
  - *4.1.3. Muscle composition (intramuscular fat content)*
- *4.2. Biomarkers of sedentary behaviours associated to increased risk of obesity and metabolic abnormalities*
  - *4.2.1. Biological impact of sedentary behaviour*
  - *4.2.2. Sedentary behaviour as a risk factor for health independent from low physical activity*
  - *4.2.3. Subjective and objective measure of sedentary behaviour*
  - *4.2.4. Role of objective measure of physical activity and sedentary behaviour in obesity care and prevention*

5. Emerging epigenomic biomarkers with potential therapeutic target application
- *5.1. DNA Methylation*
- *5.2. Chromatin modifications*
- *5.3. Small Non-coding RNA*
  - *5.3.2. Circulating miRNAs*
  - *5.3.1. Non-circulating miRNAs*
- *5.4. miRNAs as metabolic syndrome biomarkers*
- *5.5. Theranostic miRNAs*

6. Conclusions
7. References

1. ABSTRACT

Overweight and obesity are highly prevalent conditions worldwide, linked to an increased risk for death, disability and disease due to metabolic and biochemical abnormalities affecting the biological human system throughout different domains. Biomarkers, defined as indicators of biological processes in health and disease, relevant for body mass excess management have been identified according to different criteria, including anthropometric and molecular indexes, as well as physiological and behavioural aspects. Analysing these different biomarkers, we identified their potential role in diagnosis, prognosis and treatment. Epigenetic biomarkers, cellular mediators of inflammation and factors related to microbiota-host interactions may be considered to have a theranostic value. Though, the molecular processes responsible for the biological phenomenology detected by the other analysed markers, is not clear yet. Nevertheless, these biomarkers possess valuable diagnostic and prognostic power. A new frontier for theranostic biomarkers can be foreseen in the exploitation of parameters defining behaviours and lifestyles linked to the risk of obesity, capable to describe the effects of interventions for obesity prevention and treatment which include also behaviour change strategies.

2. INTRODUCTION

Overweight and obesity are serious medical conditions undergoing a recent explosive surge in prevalence worldwide (1). Moreover, they involve an increased risk for death, disability and disease (2) to the point that the steady rise in life expectancy recorded in the past two centuries in US is claimed as expected to level off or even reduce in the 21st century, for the effect of the current obesity trends (3). A similar impact is expected also in the WHO European Region, where over 50% of both men and women were estimated in 2008 as overweight, and over 20% obese, one in three 11-year-old being moreover overweight or obese (4).

The overall negative outcomes of overweight and obesity for health are the result of complex and not completely understood pathological processes deriving from environmental and genetic interactions. These include and combine reduced needs for physical activity, increased engagement in sedentary pursuits and widespread availability and over-consumption of highly palatable energy-dense foods characterizing modern societies (5), together with genetic susceptibility and epigenetic factors (6). Under this perspective, the univocally urged action to tackle the obesity epidemics through societal prevention and medical management (7) appears a challenging task requiring effective and appropriate knowledge for understanding, preventing and treating this emergency, through a multidisciplinary approach encompassing different domains of human health.

Biomarkers, defined by the National Institutes of Health Biomarkers Definitions Working Group (8) as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”, seem to represent such a powerful tool available for basic and clinical research as well as for clinical practice to fight conditions related to body mass excess and the associated physiological and biochemical derangements.

Therefore, the purpose of the present paper is to review and discuss a wide spectrum of biological markers, shown to be reproducibly measurable and reliably linked to relevant outcomes of overweight and obesity, developed during the last decades through research in different domains, spanning from the clinical to the physiological and behavioural context, and to the biology of epigenomic processes. A particular focus will be put on promising findings from current research, which may possibly provide the definition of new markers with clinical impact in the next future.

In the first section, a selection of markers of common usage in the clinical practice will be presented, enlightening the issues concerning the anthropometric evaluation of body mass excess, body composition and fat distribution, and their association with cardiovascular and metabolic abnormalities. Emerging knowledge concerning inflammatory processes, adipose tissue secretory activity and modifications in gut microbiota-host interactions for the definition of new biomarkers relevant in overweight and obesity context will be also reviewed in this section.

The second section will focus on some markers related to skeletal muscle functionality in obesity and physical activity as a life style linked to the risk of body mass excess. Features of skeletal muscle composition and architecture interfering with motor performance, as they can be detected through imaging techniques, and indicators of habits, available from individual’s technological monitoring as potential markers of sedentary behaviours and active life style, will be highlighted in the section.

A last section will finally review biomarkers related to epigenomic processes which have been shown to have roles in the pathogenesis of different metabolic abnormalities and a strong link with obesity associated metabolic syndrome. The background of the epigenomic mechanisms, highlighting the importance of environmental pressure on gene expression of modified heritable phenotypic traits,

is briefly reviewed, with a particular emphasis to the biology of small non-coding RNA (miRNA), in relation to the metabolic processes involved in obesity.

3. CLINICAL BIOMARKERS

3.1. Anthropometric biomarkers of obesity

During the development of obesity, several metabolic and physiological abnormalities gradually ensue, among which insulin resistance, dyslipidaemias, systemic inflammation, changes in blood pressure, fat liver, are commonly detected (8). These alterations are considered the basis for the development of chronic degenerative diseases which accompany obesity, particularly type 2 diabetes (T2D) and cardiovascular diseases (CVD) (2).

Since these alterations are substantially linked to the increased amount of adipose tissue, anthropometric measurements provide one of the basic information to evaluate the risk of the development of these abnormalities.

In particular, body mass index (BMI) is a widely used metric recommended from the World Health Organization to classify obesity (9). This index is useful in large population studies, but it is considered inaccurate for the evaluation of individual patients. Indeed, BMI does not consider body composition, being blind to lean and fat mass contribution and their distribution. Thus, alternative methods are needed to define obesity. Several anthropometric indicators, among which waist circumference, waist to hip-ratio, waist to height-ratio and distribution of body fat, may better than BMI reflect the risk of comorbidity and mortality linked to obesity (10-12).

Since obesity has been defined as an excess of body fat, it has been considered the need to properly measure the body fat mass. This index is particularly relevant considering that visceral fat (VF) produces and releases adipokines and fatty acids responsible of metabolic abnormalities (13). It has been reported that VF and per cent of body fat are better indicators to evaluate the risk of metabolic complications of obesity than BMI (14). The principal clinical indicator of VF is the waist circumference (WC). WC is considered as an effective clinical tool to assess the risk of T2D and CVD (15) for the diagnosis of metabolic syndrome (MS). Moreover, the process that lead to WC increase beyond race-specific cut-off points, increasing the risk of pathologic adverse metabolic effects is defined as “Central obesity” (16). However these indexes are stronger when waist to hip ratio (WHR) is also used (17).This measure is suggested by the World Health Organization as an additional measure of body fat distribution (18). It is used as an indicator for the risk to develop CVD (19). Recently, a new anthropometric index of abdominal obesity, called Waist to Height ratio (WHtR), has been proposed (20). WHtR has provided supportive evidence as a better diagnostic predictor than BMI or WC for CVD risk (20, 21).

All these anthropometric variables have been proposed as strong tools for the evaluation and prediction risk of chronic diseases as a result of obesity. Moreover, the anthropometric markers characterising the grade of overweight or obesity provide the guide to prioritize the suitable dietary strategy and/or physical activity protocol, for the treatment of body mass excess at either individual or community levels, and provide the best individual health benefits in subjects with obesity by improving body composition.

However, since the clinical and molecular mechanisms of obesity are complex and not completely understood, other diagnostic and prognostic biomarkers that can evaluate and predict the outcomes of obesity, are needed.

3.2. Inflammatory biomarkers of obesity

One of the consequences of obesity is the development of a sub-clinical inflammatory condition that promotes the production of pro-inflammatory factors involved in the pathogenesis of the metabolic syndrome and insulin resistance (IR) (19). In this section we will describe several adipokines proposed as potential biomarkers of some chronic diseases associated to obese status.

Adiponectin is a circulating plasma molecule linked with visceral adiposity, IR and atherosclerosis (22, 23). It controls many functions such as insulin sensibility, lipid oxidation enhancement, and vasodilation effects (24). The different forms of adiponectin include low molecular weight trimmer, middle molecular weight hexamer, and high molecular weight form (HMW). HMW is the more active form, and it has the most favourable metabolic effects on insulin sensibility and protection against diabetes. The reduction of adiponectin levels has been observed in obese subject, a condition that favours the suppression of adiponectin promoter activity suppressing adiponectin expression (25, 26). High levels of adiponectin are associated with an increased risk of all-causes of mortality including CVD (27).

Leptin is a hormone specific of adipose tiso sue (AT), known as a key molecule that regulates appetite, energy expenditure, food behaviour and glucose metabolism, and also inhibits neuropeptide Y neurons (28). Leptin-resistant mice develop obesity and a diabetic phenotype (29). Human plasma leptin concentrations increase in proportion to body FM (30). Obese individuals have, in most cases, high levels of circulating leptin. The failure of these high levels to

control body weight suggested the development of a leptin resistance (LR) process to the hormone partly responsible of disturbances on body weight regulation (31). Hyperleptinaemia, is associated with LR, and it is a precursor of IR (32).

Resistin is a peptide hormone produced by the white adipocyte. It appears that resistin may be related to the occurrence of insulin resistance in the case of obese individuals with type II diabetes mellitus (33). Although its mechanism of action is not known, it has been proven in *in vitro* assays on adipocyte cell lines that resistin inhibits glucose uptake by the Glut-4 transporter in insulin dependent tissues (34). In tissue, this molecule may exhibit proliferative, antiapoptotic, pro-inflammatory, pro-angiogenic function and has appeared as an important link between obesity and inflammatory processes by regulating oxidative and nitrosative stress pathways (35). Indeed, resistin has been shown to influence pro-inflammatory properties which may be associated with its production by resident monocytes and macrophages in AT in human (36). Being resistin a key molecule in the complications of obesity development, it has been proposed as a diagnostic and prognostic marker of obesity comorbidities (35).

Omentin (or interlectin-1) is an adipokine preferentially produced by visceral AT. Omentin enhances insulin-stimulated glucose uptake in human adipocytes through Akt signalling *in vitro* and its expression in visceral AT is reduced in obesity and IR (36, 37). Serum omentin levels correlate negatively with BMI, serum insulin and the Homeostasis Model Assessment (HOMA)-IR index. Thus, omentin could be a nutritional marker reflecting body weight and IR (38).

Plasminogen activator inhibitor 1 (PAI-1) is a prothrombotic adipokine and an important inhibitor of the fibrinolytic system. It is the primary inhibitor of both tissue-type plasminogen activator (t-PA) and urokinase type plasminogen activator (u-PA). Elevated plasma PAI-1 level is a central feature of MS (39), and also, high concentration suppresses fibrinolysis an increases the risk of thrombosis. Thus, it could be considered as a strong risk factor for coronary artery disease (40).

Tumour necrosis factor (TNF-α) is a pro-inflammatory cytokine involved in many inflammatory diseases, principally produced by monocytes and macrophages. Its expression is up regulated in the AT as well as in other tissues. Subjects with obesity have elevated serum TNF-*α*, whereas during weight loss, the levels rapidly decreases (41). This cytokine acts in adipocytes, decreasing the secretion of adiponectin, leading to the development of IR and dyslipidaemia (42).

Among the other proinflammatory cytokines present in chronic inflammation, there are interleukin 6 (IL-6) and C-reactive protein. IL-6 is associated with atherogenesis promotion, dyslipidaemia, hypertension and IR through activated macrophages and lymphocytes, produced by several cells-like fibroblasts, endothelial cells, monocytes and adipocytes (43). Approximately one third of circulating IL-6 originates from AT (44). It has been demonstrated that circulating levels of IL-6 have direct effects on insulin signalling in adipocytes and hepatocytes (43): in fact, plasma levels of IL-6 are elevated in patients with obesity and involved in the development of IR and T2D (45). C-reactive protein is an inflammatory biomarker associated with the severity and number of features of MS, particularly high-sensitive CRP (hsCRP) (46), it has been proposed as an independent risk factor for CVD. High concentrations are observed in subjects with obesity and T2D (32) and it has become a target for assess the pharmacological strategies to prevent atherosclerosis and vascular events (46).

3.3. Gut microbiota as a biomarker of obesity

In recent years, new biomarkers have been integrated with the information of the anthropometric assessment and the inflammatory state of the body: the gut microbiota. Several studies showed that the dysbiosis of gut microbiota is associated with an increase in body weight and BMI, leading to chronic inflammatory states that can generate IR and dyslipidaemia among others. The use of high-throughput sequencing techniques allows describing the presence of specific species in the gut microbiota. The microbiota dysbiosis has been proposed as potential biomarker of metabolic diseases associated with obesity (47).

Gut microbiota consists of a mix of bacteria, archaea and viruses, and the majority of these microorganisms are commensals (48). There are approximately 10 to 100 trillion microorganisms in the gut microbiota (48), which contain 100- to 150-fold more genes than the human genome (49). It has been considered that *"a healthy microbiota is defined by high diversity and ability to resist change under physiological stress"* (50). Thus, dysbiosis could be caused by decrease of species diversity, fewer beneficial microorganisms and/or the presence of pathogens (50).

Decrease in gut species diversity could be considered a biomarker of an altered gut microbiota. It has been demonstrated that during obesity the diversity of the gut microbiota decreases (47). Studies comparing the gut microbiota of lean and obese adults (also comparing twins, either lean or obese) showed that the gut microbiota diversity is significantly lower in the obese subjects (51, 52). Also the ratio among

different gut bacterial strain could become an index of dysbiosis. Bacteroidetes and Firmicutes are the most abundant phyla in the gut microbiota, representing >90% of the bacteria in the gut (50). The abundance of these two phyla may be taken as a reference during a dysbiosis caused by obesity. Several studies have demonstrated that during obesity the abundance of Bacteroidetes decreases whereas the abundance of Firmicutes increases, suggesting that Bacteroidetes-Firmicutes ratio is an indicator of the decrease of microbiota diversity (51, 53). However, it has not been fully proved if this behaviour is constant across all the individuals. Recent studies have demonstrated that the presence of specific species correlates significantly with several metabolic alterations. Thus, these species have received important attention in recent years as indexes of specific metabolic abnormalities. The main bacteria species are the following:

3.3.1. *Akkermansia (A.)muciniphila*

Akkermansia (A.)muciniphila is a gram-negative bacteria capable of using mucin as an energy source (54), although it only represents 3-5% of the gut microbiota (55). The abundance of *A. muciniphila* has been inversely related with obesity and weight gain in pregnant women (56). Interestingly, a study showed that higher abundance of *A. muciniphila* in obese subject was associated with a healthier metabolic status, especially in the levels of fasting plasma glucose, plasma triglycerides and body fat distribution. When these same participants went into a caloric restriction diet, those who had the higher abundance of *A. muciniphila*, had a better improvement in glucose homoeostasis, blood lipids and body composition (57).

3.3.2. Faecalibacterium (F.) prausnitzii

Levels of *Faecalibacterium (F.) prausnitzii* strain have been negatively correlated with inflammatory markers, suggesting that this bacteria could modulate inflammation during obesity (58). This bacterium has important anti-inflammatory activities mainly because its production of butyrate in the gut; presence of butyrate at moderate levels prevent insulin insensitivity induced by a high fat diet since it increases the mitochondrial beta oxidation and prevents glucose sensitivity and adiposity (59). In obese and T2D patients, *F. prausnitzii* was less abundant and this specie expression was correlated with the reduction of low-grade inflammation (60).

3.3.3. *Prevotella (P.) copri*

Recent evidence showed that the increase in the abundance of *Prevotella (P.) copri* strain is associated with improved glucose tolerance, which has been linked with the production of succinate (61). Succinate is a precursor of propionate (62), and it can act as a glucose precursor in the gut (63), and also as a substrate for intestinal gluconeogenesis resulting in improved glucose tolerance and insulin sensitivity (61). These beneficial effects could be related with the modulation on the host metagenome by *P. copri*. Thus, beneficial effects of *P. copri* are related with the interactions between components of fibres and its own genome.

Other metabolic components, such as Short-Chain Fatty Acids (SCFA) and lipopolysaccharide (LPS), have been described to have a role in the regulation of the relation of gut microbiota with obese host. Some studies, comparing the SCFA concentration between lean and overweight or obese subjects, showed a higher faecal concentration of SCFA in overweight or obese subjects compared to the normal weight subjects (64). The fermentation of dietary fibre by the bacteria present in the gut microbiota generates SCFA (acetate, propionate and butyrate) and these SCFA provide 10% of the total dietary energy supply in humans (65). It is hypothesized that SCFA are substrate for hepatic *de novo* lipogenesis, increasing the capacity of host energy harvest from food (66). The SCFA produced by gut microbiota reach the liver via the portal system, where, acetate, propionate and butyrate are converted to co-factors (acetyl-CoA and propionyl-CoA) that guide gluconeogenesis and *de novo* lipogenesis (67, 68).

During obesity, a high fat diet increases the concentration of LPS resulting in changes in body weight, altered glycaemia and presence of inflammation (69). LPS is one of the component of the outer membrane of gram-negative bacteria. An increase of LPS concentration is called “metabolic endotoxemia”, a phenomenon associated with gut barrier dysfunction (70). During obesity, it has been observed that high levels of LPS, associated to the dysbiosis of gut microbiota, increases intestinal permeability by altering the barrier function of intestinal epithelium. This process is mediated by a decrease in the expression of genes coding for tight junction proteins (i.e., zonula occludens-1 (ZO-1) and occluding) (71).

4. BIOMARKERS OF SKELETAL MUSCLE FUNCTIONING AND PHYSICAL ACTIVITY HABITS

4.1. Biomarkers of muscle functional impairment in obesity

During the execution of physical activity, obese individuals suffer from a number of functional limitations which are mainly related to the excess of their body mass. This impairment is particularly critical since an increase in physical activity is considered an important component in the strategy for obesity treatment through a daily negative energy balance.

In fact, overall motor performance in obese people is grossly reduced during anaerobic tasks (i.e. short and intense efforts) by the imbalance between the available skeletal muscle engine and the notably increased body mass to move; during aerobic exercise (i.e. walking or running at moderate velocity) the principal limitation is provided by the greater metabolic energy required to move the heavier body, or single body segments involved in movements, which may ultimately exceed the limits of the individual's aerobic capacity (72).

Though, overweight and obesity have also a role in affecting skeletal muscle attributes, such as size, architecture and composition (in terms of fat infiltration in muscle tissue), which have been shown to significantly interfere with mechanical capabilities relative to muscle volume. Thus, these changes, detectable through imaging techniques, may provide useful markers of muscle impairment in conditions of body mass excess.

Overweight and obesity are considered to have a loading effect on whole body antigravity muscles, so that obese individuals have more muscle mass and higher absolute strength respect to normal-weight individuals (73-77). However, the increased body mass observed in obesity largely outbalance the obesity-induced increase in muscle size, and the net functional result is a decrease in muscle mass per unit of body mass (74, 75, 77), which may lead to a detectable motor impairment, especially in women who are inherently endowed with a lower amount of skeletal muscle relative to body mass.

4.1.1. Muscle quality

A more precise biomarker of skeletal muscle intrinsic functionality is considered the capability of strength (or tension) generation per unit muscle size (often referred to as "muscle quality"). This parameter has been estimated taking as reference either total body or single limb fat-free mass (an indicator, though rather crude, of skeletal muscle mass), assessed through methods evaluating body or regional composition (73, 74, 76) or the specific dimension of the investigated muscles (such as cross section area or volume) determined by imaging techniques (78, 79). Although conflicting results are reported (76), overall these studies agree in detecting a lower muscle quality in obese compared to their normal-weight counterpart, the actual muscle specific functionality of obese persons decreasing with their degree of adiposity. Muscle quality, as an adiposity-dependent marker of muscle intrinsic capabilities, has been, on turn, associated with factors linked to both muscle architecture and composition, found to be influenced by obesity.

4.1.2. Muscle architecture

Skeletal muscle architecture, defined as "the arrangement of muscle fibres within a muscle relative to the axis of force generation" (80), is an important structural property of whole muscles influencing their functional performance. Indeed, in pennate muscles (like Quadriceps femoris and other important muscles involved in human locomotion) fascicles are arranged at a certain angle (i.e. the pennation angle, PA) in respect to the axis of whole muscle tendon shortening. Therefore, the measurement of PA represents a quantitative assessment of the divergence of the vector of muscle fibre force production from the vector of whole muscle force production, being the total force transmitted to the whole muscle tendon proportional to the cosine of PA (i.e. total muscle force decreases as PA increases).

Tomlinson *et al*. (78) showed that obesity leads to an increase in skeletal muscle size and PA, with partial differences between young and old people. In addition they found a positive significant correlation between muscle size e BMI, providing further evidence to the hypothesis that the loading stimulus of increased body mass may act as a stimulus for muscle hypertrophy. Similarly, Rastelli *et al*. (79) detected a significant increase in PA and muscle cross sectional area of Quadriceps femoris of obese older women as compared to their normal-weight counterpart, and identified a negative correlation between PA and muscle quality. Thus, the increased PA observed in obese individuals seems to derive from the obesity-related increase in muscle size, in line with a general phenomenology evidenced also in strength-trained athletes (81). An example of changes induced by obesity in muscle architecture is given in Figure 1.

A common non-invasive imaging technique allegedly considered the present standard for investigating muscle architecture is B-mode ultrasound, which is relatively easy to use and permits real time measurement of different muscle architectural features, among which are fibre length and PA. However, main limitations of the B-mode ultrasound are: a) the image field of view often too small to capture the entire length of the fibre, b) the presence of experimental errors (such as muscle deformation due to probe pressure and inaccuracy in probe repositioning when measure repetitions are required), and c) the two-dimensional nature of images obtained, in face to the three-dimensional arrangement of muscle fibres. An emerging new approach to muscle architecture study overcoming the above issues is provided by Diffusion Tensor Imaging (DTI), a modality of Magnetic Resonance Imaging yielding quantitative information about water diffusion in tissues and permitting the visualisation

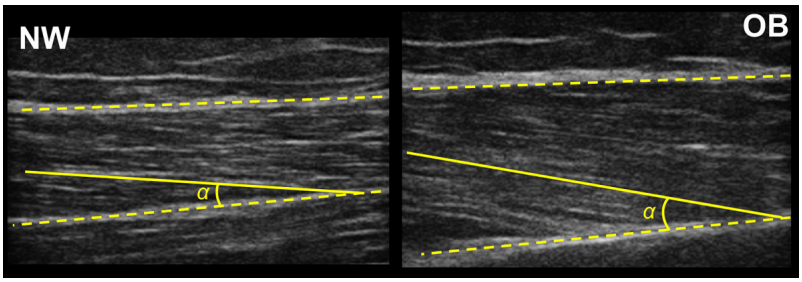

Figure 1. *Rectus femoris* architecture in a normal-weight (NW) and obese (OB) woman obtained with B-mode ultrasonography imaging. Yellow broken lines represent superficial (upper line) and deep (lower line) aponeurosis; solid yellow line represents muscle fascicle orientation; the angle of insertion of muscle fascicles on deep aponeurosis (pennation angle) is indicated as α. The image evidences the muscle architecture difference detected between OB and NW individuals (data from (79)).

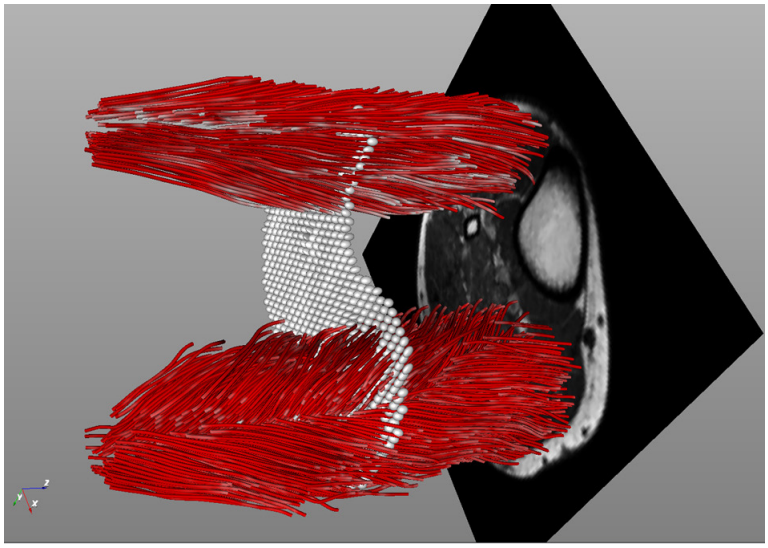

Figure 2. Example of fibre tractography of the gastrocnemius (bottom) and tibialis anterior (top) muscle in a human calf (reprinted from (82), with permission).

of fibre structure and arrangements. The technique, also referred to as tractography, widely employed in the study of brain connectivity, has been found to be very accurate for the detection of macroscopic muscle architecture (82). After 3D reconstruction of muscle structure, the tractography, permits to map the fibres trajectories between the origin and the insertion inside the muscle, as it is appreciable in Figure 2, and allows the quantification of several architectural parameters, including PA (83, 84). Although requiring expensive instrumentation and costly facilities, beside needing a relatively complex post-processing analysis of the images, DTI-based skeletal muscle fibre tracking seems to be a very promising approach, candidate to become the gold standard for the study of markers of muscle architecture.

However, some caution has to be taken when using DTI in obesity, since fat-infiltrated muscles have diffusivities that are typically lower than those of healthy muscle (85). Williams *et al*. (86) reported that the regions of interest considered in DTI analysis have to include at least 76% of muscle tissue to allow measurements accurately reflecting the properties of pure muscle. In addition, they recommend to co-register quantitative fat-water images, in order to exclude fat-infiltrated regions of muscle from the analysis.

4.1.3. Muscle composition (intramuscular fat content)

Disparate investigations have reported that overweight/obesity, and body adiposity in general, are associated with modifications in skeletal muscle composition due to a different degree of fat infiltration in muscle tissue which leads to reductions in muscle performance (77, 79, 87). Moreover, such fat infiltration within muscles has been shown to be linked to an increased risk of diabetes and poor insulin sensitivity (88, 89).

Using muscle tissue attenuation (which represents an inverse proxy assessment of lipid infiltration (87)) from computed tomography images of lower limb, recently Lafortuna *et al*. (77) evidenced a rise in muscle fat content proportional to the overall body adiposity in a sample of men and women, ranging from normal-weight to obesity. On turn, muscle fat content has been detected as a significant negative correlate of muscle quality (i.e. the capability of strength production per unit muscle section) in a very large sample of older men and women performing isokinetic knee extensions (90). Exploiting a different technological approach, Rastelli *et al*. (79) measured lower limb muscle fat content at mid-thigh by means of MRI (three-point Dixon sequence) in obese and non-obese older well-functioning women, along with isokinetic and isometric muscle strength during knee extension. Their results show a significantly negative effect of intramuscular fat content on muscle performance per unit cross-sectional area, and confirm the role of muscle composition in the modulation of muscle quality.

Physical activity is recognized as an integral part of the comprehensive obesity management and should be individually tailored to the specific characteristics of each subject (91). Biomarkers of muscular functional impairment may inform about muscle functionality and consequently they are important in the choice of exercise modalities to be undertaken. In particular, the role of the prolonged aerobic exercise (i.e. walking, jogging, swimming, bicycling) for the stimulation of lipid oxidation is well known (92), but it was also recognized the usefulness of the short duration strength exercise modality for protection of lean body mass and to preserve or increase muscle strength (93).

4.2. Biomarkers of sedentary behaviours associated to increased risk of obesity and metabolic abnormalities

4.2.1. Biological impact of sedentary behaviour

In the last 50 years, contemporary society has recorded, parallel to the increment of the number of people suffering from overweight and obesity, a dramatic increase of sedentary behaviour, defined as a prevalence of activities during waking hours, characterised by low energy expenditure (≤1.5. Metabolic Equivalent of Task, METs), performed while sitting or in reclined position (94).

Epidemiological studies evidenced that most of adults living in developed countries, spend the majority of their day sitting (up to 70% of their waking time) both for occupational reasons, and during leisure time (watching TV, playing videogames, reading books, driving, etc.) (95, 96).

Prolonged sedentary activity has been found to be highly associated to obesity, other metabolic conditions, such as type II diabetes and coronary heart disease. This knowledge has been achieved mostly through cross-sectional studies, that leave open the question on what are the baseline mechanisms originating this association. The study of sedentary activity and health is encompassed by what is provocatively called inactivity physiology, opposite to the traditional exercise physiology (97).

The fundamental reason for the inactivity physiology to be created as an independent branch of physiology is that the biological effects of sedentary activity has been evidenced to be independent from the physical activity the individual performs.

More in details, the biological mechanisms at the basis of the association between physical activity, obesity prevention and improvements in health are object of several research domains (i.e. exercise physiology) and have been widely investigated in the last 50 years (98, 99). However, from research, it emerges that physically inactive people are more exposed to disorders involving poor skeletal muscle metabolism (such as type II diabetes and obesity) compared to those meeting physical activity recommendations. Inactivity is also recognized as a major risk factor for coronary heart disease and consequently for death (100, 101). For that reason the reduction of sedentary time, in favour of physical activity at, at least, moderate intensity has been included in the international recommendations.

The underlying processes (probably involving muscle lipoprotein lipase (LPL) regulation) associating inactivity, low-intensity activity and disease are a relatively new research domain. A research study conducted in 2003 (102), aiming at the study of LPL regulation dependent on exercise intensity, tested on mice the hypothesis that physical inactivity decreases the normally high LPL activity in muscle and that restoring ambulation in previously inactive animals would raise muscle LPL activity. The main (and striking) output of the study is that inactivity caused significant decreases of intracellular LPL protein content and that, within 4 hours after inactivity, treadmill walking was able to raise LPL activity again. This study gives an important insight on the link between muscle inactivity and metabolic diseases, and possibly also on the role of moderate physical activity as protective factor against disorders involving poor lipid metabolism.

4.2.2. Sedentary behaviour as a risk factor for health independent from low physical activity

Apart from the animal model, and despite the opinion that being sedentary is unhealthy (because the opposite, being active, is healthy) is commonly

Table 1. Summary of behavioural markers linked to obesity and related metabolic abnormalities

Behaviour Marker	Definition	Guidelines/Recommendations
Overall physical activity	Minutes of moderate to vigorous physical activity	For adults, at least 30 minutes per day, five days a week, of at least moderate-intensity physical activity
Sedentary behaviour	Overall time (in minutes) spent in activities at <1.5. METs* in sitting or reclined position during waking hours	Reduction of sedentary behaviour
Uninterrupted sedentary activity	Length of bouts of continuous sedentary activity	Introduction of frequent breaks during sedentary activities

*MET (Metabolic Equivalent of Task): physiological measure expressing the energy cost of physical activities, 1 MET being the rate of energy expenditure of an average person seated at rest (reference value, per unit body mass: 1 kcal·kg−1·h−1)

accepted, the relationship among sedentary time, physical activity and health in humans appears to be a complex issue.

Overall, it emerges from the current literature that, beside the behavioural marker of a life style including the recommended amount of physical activity (represented by the time daily devoted to the performance of moderate to vigorous physical activity), the time devoted to sedentary pursues (i.e. daily sedentary time) may be considered a reliable and independent prognostic marker of the health risks deriving from physical inactivity.

Most of recent studies have focused on the effect of physical activity and sedentary time on health as separated parameters (103, 104). The picture emerging is that increased sedentary time is strongly associated to adverse cardio vascular health outcomes, while physical activity has a positive impact on health in a dose-response relationship.

Loprinzi *et al*. (105) have been the first who moved the focus on the effects of physical activity combined with sedentary behaviour on health markers. They found that the engagement in at least 150 minutes per week of moderate to vigorous physical activity is associated with more favourable levels of metabolic markers, compared to the levels of those performing less than 150 min/week, but even when the cut-off of 150min/week is not met, those who had less sedentary activity had more favourable levels of triglycerides and insulin.

Comparable results have been found in a study published in 2015 (106). Using the data coming from the Health Survey for England, Bakrania *et al*. identified four behavioural categories named: 'Busy Bees', those who meet physical activity requirements and are low in sedentary behaviour, 'Sedentary Exercisers', physically active, but spending long time sitting during their day, 'Light Movers' (not meeting physical activity prescriptions, but spending little time sitting during the day, and 'Couch Potatoes' (physically inactive and high in sedentary behaviour). Comparing the members of the different categories for health outcomes, it emerged that physically active subjects had a better health profile, even when highly sedentary, while low sedentary time independent of physical activity had a positive association with HDL-cholesterol.

In addition to this, recent studies (107) have shown that frequent breaks in sedentary time are associated with better health outcomes (such as waist circumference, BMI, triglycerides and 2-h plasma glucose), so that the amount of uninterrupted sedentary time provides a promising behavioural marker of health risks associated to sedentariness.

Table 1 provides a summary of behavioural markers relevant for prevention (or linked to an increased risk) of obesity and associated metabolic abnormalities.

4.2.3. Subjective and objective measure of sedentary behaviour

Sedentary behaviour exists as a research focus, since it has been given an univocal and agreed definition of it. According to this definition, sitting time is the proxy for the measure of sedentary behaviour. It derives that the study of sedentary behaviour can not disregard the capacity of measure the amount of time spent sitting by an individual.

Several studies in the past have used diaries, self or proxy-report questionnaires (108), centred usually on the estimation of daily screen time as a marker of overall sedentary behaviour. These tools rely on what the subject refers, therefore are called subjective measures. Subjective measure can provide an acceptable estimation of the habitual sedentary time, but, when compared to objective measures (based on accelerometers, gyroscopes, inclinometers) they show a relevant underestimation of overall sedentary time (103).

At the moment ActivPal (108, 109) is considered as the gold standard for the measurement of sitting time. It is a tri-axial accelerometer; it is fixed on the upper third of subject thigh with tape. It is able

to display the overall quantity of sitting time and the duration of bouts (a parameter that has been found to be relevant for health).

ActiGraph (108, 109) is another tool that has been employed for physical activity and sedentary time assessment, since it is able to stratify the physical activity performed by the subject, based on its intensity, on the basis of accelerometer count.

4.2.4. Role of objective measure of physical activity and sedentary behaviour in obesity care and prevention

The objective measure of physical activity and sedentary behaviour has a theranostic value in obesity treatment and prevention, since it can be used for monitoring individual level of activity, his/her compliance to the intervention and the measure of behaviour change achieved (110). Moreover the introduction to the market of low cost and miniaturized devices for activity monitor (now also embedded in low-cost smartphones), and the use of improved algorithms for movement detection, could represent a new frontier for m-health industry and global health governance, since the flow of information regarding individuals' habits and their overall health status, may be used to generate new models for health care and disease prevention, have a great impact on the understanding of the relations linking behaviours and lifestyles, as well as on the knowledge shaping health policy choices for public health (111).

5. EMERGING EPIGENOMIC BIOMARKERS WITH POTENTIAL THERAPEUTIC TARGET APPLICATION

Epigenetics could be defined as an inheritable, reversible process that affect gene expression without altering the genomic sequence. Epigenomics, the field that study the epigenetic modification, is able to clarify which is the influence of the environment (e.g. diet, smoking, exercise) and the lifestyle on the gene expression that generates a specific phenotype. Among the epigenetic processes, we consider DNA methylation status, chromatin modifications (i.e., histone acetylation and methylation) and small non-coding RNA expression. All these factors create a link between lifestyle and the risk of disease. Indeed, epigenetic processes have been proposed to be sensitive biomarkers and potential therapeutic targets for obesity management.

5.1. DNA Methylation

DNA methylation is the best studied epigenetic regulatory mechanism (112). During this process, a covalent addition or subtraction of a methyl group to a cytosine nucleotide (the 'so called' CpG islands) in a DNA sequence occurs. These regions are usually located in the promoter regions of approximately half of all genes (112). This process is regulated by specific enzymes, called DNA methyltransferases (DNMTs). DNA hypomethylation or demethylation is one of the process that contributes to carcinogenesis, causing activation of proto-oncogenes (i.e., c-Jun, c-Myc and c-Ha-Ras). Hypomethylation favours also genomic instability by mitotic recombination. On the contrary, hypermethylation of CpG islands can result in transcriptional silencing of the gene, and subsequent loss of protein expression.

As an example, a longitudinal study on the methylation status of DNA associate the BMI index with the methylation of the locus encoding HIF3A gene (113). HIF3A in one member of the family of hypoxia inducible transcription factors (HIF), which regulates cellular and vascular responses to decreased levels of oxygen. Studies in mice suggest these proteins may play key roles in metabolism, energy expenditure and obesity (114, 115). Silencing the promoter of this gene could cause alterations in the pathway that control adiposity (116). Indeed, maternal–pre-pregnancy BMI and offspring cord blood methylation in CpG islands of HIF3A are associated, suggesting that maternal BMI could severely has an effect on baby adiposity at birth (116). The silencing of two other genes, melanocortin 4 receptor (MC4R), which controls the appetite, and hepatocyte nuclear factor 4 alpha (HNF4α), which regulates lipid homeostasis, has been associated with a higher risk of metabolic abnormalities with type 2 diabetes (T2D) and cardiovascular disease later in life in children (117-119).

5.2. Chromatin modifications

Chromatin is comprised of histones and DNA. Histones are the protein components of chromatin, the structure around which DNA is wound. Histones are octamers with variable tails that extend out of the DNA/histone complex (nucleosome). There are several types of post-translational modification that can affect the histone tails, including methylation, acetylation, phosphorylation, and ubiquitination. These modifications can affect interactions between DNA and histones, leading to alterations in gene transcription, DNA repair, DNA replication, and even the organization of chromosomes (120).

In general, acetylation of the lysine residue of histones is associated with transcriptional activation, and deacetylation is linked with transcriptional repression, while methylation of histones can positively or negatively affect transcription (121).

Obesity can be defined as an excess accumulation of white adipose mass, resulting from both an increase in adipocyte cell size and the

development of new mature cells from undifferentiated precursors. A wide array of transcription factors participate in adipogenesis, the process that convert pre-adipocytes into adipocytes, such as members of the CCAAT enhancer-binding protein (C/EBP) family, GATA2 (122), the Krüppel like factor (KLF) family (123, 124), and Nr2f2, the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ), whose expression is driven by a specific promoter controlled by epigenetic regulation (125). Recently, an histone modifying enzyme called JmjC-domain-containing histone demethylase 2A, (JMJD1A, also known as JHDM2A or KDM3A) (126), has been reported to be protective against obesity. JHDM2A-/- mice develop adult onset obesity, hypertriglyceridemia, hypercholesterolemia, hyperinsulinemia, and hyperleptinemia, hallmarks of metabolic syndrome (127). JHDM2A demethylase also regulates the expression levels of metabolic genes related to energy homeostasis, such as anti-adipogenesis genes (i.e., NR2F2 and GATA2), fat storage genes (i.e., APOC1), the gene encoding for glucose transporters (i.e., SLC2A4), and genes associated with susceptibility to type 2 diabetes (ADAMTS9) (128). All these data suggest that changes in histone modification are a key component of an epigenetic network controlling adipogenesis and energy homeostasis.

5.3. Small Non-coding RNA

5.3.1. Non-circulating miRNAs

The best studied non-coding RNAs with a regulatory function are miRNAs. They are small RNA sequences (about 22 nucleotide long) encoded by non-coding RNA regions or introns of genes. miRNAs are transcribed into the nucleus as pri-miRNAs and their undergo several modification steps to became functional sequence, through the activity of Dicer/DGCR8 complex, which cleave the nuclear pri-miR into pre-miR, and Drosha RNase enzyme, which transform the pre-miR exported in the cytosol into mature miRNA (129). The activity of a single miRNA is due to its ability to recognize a specific complementary sequence on the 3' untranslated region (UTR) of target RNA. If the miRNA is fully complement to the RNA, miRNA binds to it and degrades through the action of RISC complex. If there is an imperfect match between miRNA and the target RNA, miRNA partial binding to the 3' UTR of the RNA inhibits the action of RNA transfer (120).

MiRNA expression levels could be altered by multiple factors. Alteration in processing enzymes could cause a decrease in the efficiency of miRNA maturation, decreasing the final amount of miRNAs. The failure in miRNA biogenesis has been associated with cancer (130-132). The first evidences on the role of miRNA in obesity and metabolic diseases came from studies on DGCR8-/- and Drosha-/- mouse models: knockout animals displayed an enlarged brown fat, accompanied by decreased expression of brown fat markers (133, 134). These two studies suggest that microRNAs are important for brown adipocyte differentiation and functional maintenance.

A role for miRNAs in fat cell metabolism and obesity has been described in genetic screens on Drosophila, where miR-14 deletion caused apoptosis, enlarged adipocyte lipid droplets, elevated triglycerides (TG) and diacylglycerol (135). In humans, increased fat mass associated with obesity is due to both increased adipocyte size and/or increased adipocyte number (136). Pre-adipocytes can proliferate and differentiate into mature adipocytes. Multipotent mesenchymal cells can originate lineage-committed pre-adipocytes upon hormone stimulation, and then differentiate into mature adipocytes (137). Recent evidences suggest that miRNAs are involved in the regulation of differentiation of mesenchymal cells into adipocytes (miR-24, miR-30c, miR-31, miR-143, miR-642a-3p) (138-141).

Looking for the signature of miRNA in obese tissues, the first report of miRNA-profile in human obesity identified miR-17-5p, miR-34a, miR-132, miR-145, miR-181a to be correlated with obesity (142). The cluster containing miR-143/145 is one of the best studied together with miR-130 in relation to obesity. In particular, it has been demonstrated that miR-143 is a positive regulator of ERK5 signalling (138), influencing adipocyte differentiation. Moreover, miR-143 expression is regulated by TNFα treatment, suggesting that obesity-associated inflammation may deregulate miR-143 expression affecting adipogenesis (143).

In human subcutaneous adipose tissue, overexpression of miR-519d was reported to be associated with severe obesity (144). During adipogenesis, miR-519d is stimulated in a dose-dependent manner, which suggests miR-519d may be a factor in adipocyte hypertrophy and increased adipose tissue mass in human obesity, mainly by regulating PPARα level of expression, as suggested by the observation that PPARα mRNA is highly expressed in obese subjects (144). Also in subcutaneous adipose tissue from obese subjects miR-150 was reported to be up regulated and miR-659 was reported to be down regulated (144). An overview of the miRNAs altered during adipogenesis is depicted in Figure 3.

5.3.2. Circulating miRNAs

In the past year circulating miRNAs have generated much research interest as novel diagnostic disease markers. They have been isolated in almost all bio fluids (i.e., saliva, urine, plasma, serum, blood, amnion, milk, teardrop, cerebrospinal fluid (145)). Their stability, their association with specific disease and the easy methods for their isolation and detection make

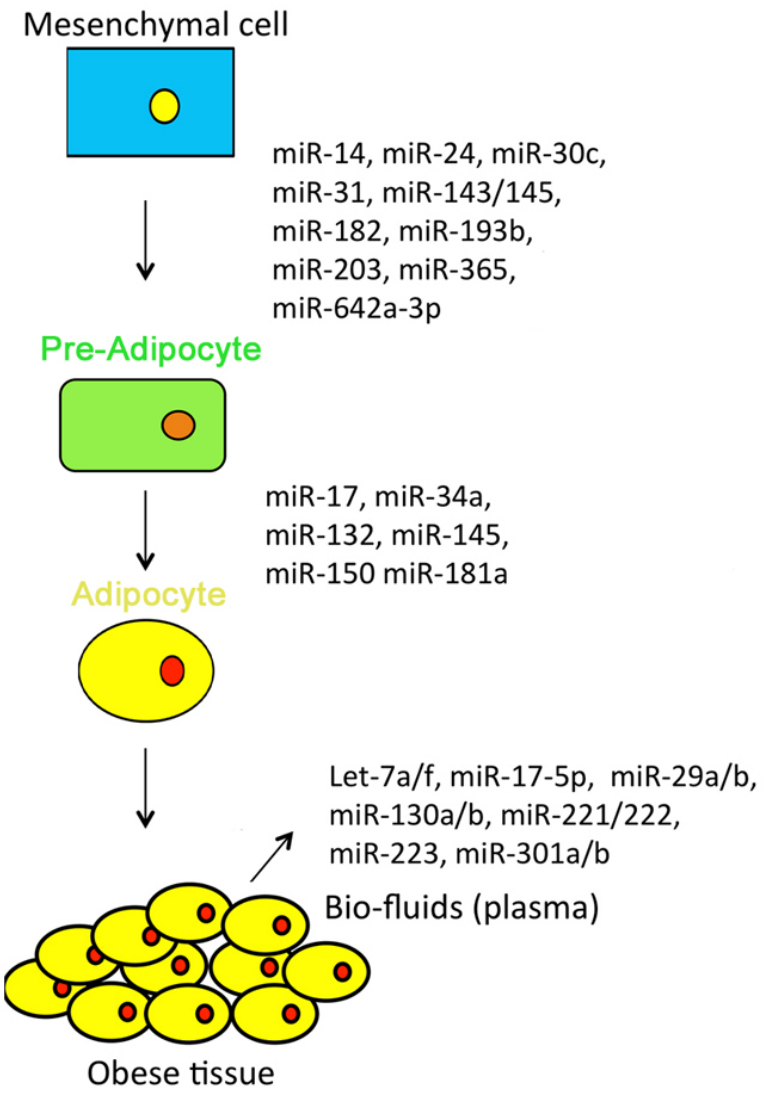

Figure 3. Representative miRNAs, involved in adipogenesis, altered in obesity. MiRNAs altered in obesity or in type 2 diabetes are represented. For each miRNA a possible influence on adipogenesis process is suggested. Secreted miRNAs, found in biofluids (i.e. blood or plasma) are also depicted.

them important diagnostic molecules with potentiality to become biomarkers. In this section we describe some circulating miRNAs that have been proposed as metabolic syndrome biomarkers.

miR17-5p, as well as miR-130 family members (miR-130a, miR-130b, miR-301a and miR-301b), have been found already in obese tissue, and confirmed also in blood of obese patients (146-148). Plasma level of Let-7a and Let-7f were lower in obese diabetic patients versus normal subjects (149). These miRNAs have been already revealed their importance by *in vitro* experiments on pre-adipocytes, where the overexpression of Let-7 reduced adipogenesis (150, 151). This group of miRNAs could modulate systemic insulin sensitivity and glucose metabolism, acting on insulin signalling/PI3K and mTOR pathways, becoming potential biomarkers of glycaemic control and metabolic disease (149). Regarding miR-221/222 cluster, they are altered in murine models of diabetes (152, 153), and also in omental adipose tissue of women with gestational diabetes (154) where they regulate adipose insulin sensitivity via repression of ERα and GLUT4 (154). *In vitro* miR-221 levels of human adipocytes were negatively correlated with tumour necrosis factor alpha (TNFα) mRNA levels and body mass index of donor (155) and decreased levels of miR-221 have been found in plasma of obese patients (145). In contrast, miR-222 is up regulated in plasma of obese subjects (147, 156) and in patients with familial hypercholesterolemia (157). The disparity of findings on circulating miR-221 and miR-222 may be owed to methodological differences or undefined variables in the considered cohorts.

Regarding miR-223, its role in obesity is linked to the fact that it regulates monocyte-macrophage differentiation and macrophage activation. MiR-223 -/- mice on a high-fat diet exhibited an increased severity of systemic insulin resistance and a marked increase in adipose tissue inflammation, compared with wild-type mice (158). In human plasma, miR-223 as well as miR-29b plasma levels were lower in T2D patients than in normal subject (159), while miR-29a urinary expression was higher in diabetic patients with nephropathy and atherosclerosis (160).

The role of miR-375 in obesity has been cleared by the study in pancreas: miR-375 is highly expressed in pancreatic β cells and is important in B cell maintenance. Mice lacking miR-375 exhibit hyperglycaemia owing to decreased insulin production, possibly via regulation of ERK1/2 signalling upstream of peroxisome proliferator-activated receptor gamma (161). In humans, miR-375 was significantly increased in the plasma of T2DM subjects vs. control groups in two studies (162, 163).

Recently, miR-503 emerged as a potential therapeutic target. Ortega *et al*. showed that miR-503 was the most down-regulated miRNA during human adipocyte differentiation (164), being able to regulate Bone Morphogenetic Proteins (BMPs)-receptor 1a (BMPR1a), a protein with an important role in adipose cell fate determination (165, 166).

Among other miRNA secreted in bio fluids there are miR-103 and miR-224, whose levels of expression in urine of diabetic patients correlate with their blood levels (167).

5.4. miRNAs as metabolic syndrome biomarkers

The features of miRNA molecules, such as their stability and their presence in bio fluids, could help in defining a new class of biomarkers for multiple disease, including type 2 diabetes and metabolic disease. With the proper development, miRNA-based biomarkers have the diagnostic potential at preclinical stage, when the metabolic problems have not manifested yet, to assess severity of the disease, to identify subjects with a predisposition to the disease, to address disease aetiology, to confirm the diagnosis based on other clinical parameters and to monitor clinical interventions.

The first step in developing a biomarker, showing a statistically relevant difference between a healthy and metabolic disease cohort, has clearly been met by multiple studies identifying numerous plasma miRNAs that are associated with metabolic disease in a range of demographics (children, women, during pregnancy). However, there are some inconsistency in the findings for specific circulating miRNAs (i.e. miR-223) that need to be cleared by the use of standardization procedure for miRNA isolation and analysis, to optimize the comparison of findings among different groups. Some miRNAs, among those discussed above, such as miR-221, miR-222, appear to be reliable candidate biomarker based on several reports. In particular, we found that miR-130b has been found by different authors in plasma of obese patients (147, 148), and miR-17-5p has been found in either tissues and plasma of obese subjects (142, 146), suggesting a possible use of these miRNAs as circulating metabolic syndrome biomarkers.

5.5. Theranostic miRNAs

To be a theranostic molecule, a miRNA should have both features of diagnostic molecules and could be used as potential target for therapy. In this section we considered those miRNAs that demonstrated to have potential diagnostic features and that have been used already in *in vivo* experiment as therapeutic tools in metabolic syndromes.

There are two main approaches to develop miRNA-based therapies: the use of oligonucleotide as miRNA mimics, to increase miRNA level of expression, or antago-miR or the so-called 'miRNA sponge', to reduce a specific miRNA sequence. Until now, the use of miRNA-based therapies has been proposed mainly for cancer treatment. Several problems associated with miRNA-based therapy are: miRNA oligonucleotides must be cell-permeable, stable and not degradable by the tissue and should specifically reach the target of interest, to avoid unwanted side-effects (168-170). Several attempts have been made in the chemistry modification of the oligonucleotides, such as modification of the sugar moiety or the conjugation with cholesterol, to increase the stability of the molecules, the binding affinity and to optimize their *in vivo* functionality (168-170). The key step in the development of a miR-based therapy is mainly the choice of the correct miRNA. A relevant miR for the development of miRNA-based therapeutics for metabolic syndrome treatment could be miR-375; its modulation profoundly affects pancreatic islet cell viability and glucose metabolism (by regulating NeuroD1, Ngn3, Pdx1 and Hnf6) and it was significantly increased in the plasma of T2DM humans (162), thus suggesting that miR-375 may be a potential target to treat diabetes (171), but nor for the obese status.

MiR-122 is important for lipid metabolism in liver. Preclinical trials in non-human primates shows encouraging pharmacokinetic properties of locked-nucleic acid (LNA) modified oligonucleotides against miR-122 (172): systematic administration of LNA anti-miRs against miR-122 resulted in a dose-dependent improvement of plasma cholesterol with no indication of hepatic toxicity, but further analysis are necessary on its use against obesity.

Another possible candidate could be miR-21, being increase in diabetic mouse model (173) and being

Table 2. miRNA having a role in metabolic disease

miRNA Type	Model	Reference
Non circulating		
miR-182 miR-193b miR-203 miR-365	Brown adipocytes differentiation	(176)
miR-14	Regulation of adipocytes metabolism in Drosophila model	(135)
miR-24 miR-30c miR-31 miR-143 miR-642a-3p	Differentiation of mesenchymal cells into adypocytes	(138-141)
miR-17-5p miR-34a miR-132 miR-145 miR-181a	Human adipose tissue	(142)
miR-519d (up) miR-150 (up) miR-659 (down)	Human adipose tissue	(144)
miR-503	Pre-adipocyte differentiation	(166)
Circulating		
miR-17-5p	Blood of obese subjects	(146)
miR-130a miR-130b miR-301a miR-301b	Plasma of obese subjects	(147)
miR-130b	Plasma of obese children	(148)
Let-7a Let-7f	Plasma of obese diabetic patients	(149)
miR-221 miR-222	Plasma of obese diabetic patients	(147, 156)
miR-223	Plasma of T2D patients	(159)
miR-29a miR-29b	Plasma and urine of T2D	(159, 160)
miR-103 miR-224	Blood and urine of T2D	(167)

involved in several compliance due to diabetes (i.e. diabetic nephropathy) (174). In a model of metabolic syndrome, LNA-antagomiR-21 has been already successfully used, demonstrating that reduction of this miRNA reduced body weight, adipocyte size, serum triglycerides, and, modestly, the cardiac collagen in hypertrophic hearts of the mouse model (175). AntagomiR-based silencing of miR-103/107 (RG-125(AZD4076) developed by Regulus Therapeutics and AstraZeneca for the treatment of non-alcoholic steatohepatitis in type 2 diabetes/pre-diabetes patients) in mice was followed by decreased liver triglyceride content and improved insulin sensitivity (http://ir.regulusrx.com/releasedetail.cfm?releaseid=905305).

Until now, miRNA-based therapies for metabolic disorders are few, most likely due to the difficulties of efficient tissue delivery and the lack of identification of miRNA-targets specific for this disease. In Table 2 is provided a summary of current miRNAs relevant in the approach to the biology of body mass excess.

6. CONCLUSIONS

The body mass excess condition entailed by overweight and obesity has a negative impact for health affecting a widespread span of biological processes and bodily function of very different nature. Thus, the biomarkers relevant for overweight and obesity management differ considerably between each other in their capability to contain information with diagnostic, prognostic and therapeutic impact, as summarised in Table 3. While biomarkers in the domain characterising epigenetics and cellular mediators of inflammation may have a full theranostic value, for all the other parameters, a remarkable gap still exists between the phenomenology represented by the biomarker and the molecular processes underlying that phenomenology. Nevertheless, although lacking a specific molecular basis, these biomarkers, such as anthropometric biomarkers, still have a powerful capacity to evaluate the on going level of body mass

Table 3. Summary of principal biomarkers linked to obesity and related metabolic abnormalities

Biological Domain	Biomarker	Description	Biological and theranostic impact
Anthropometry	Body fat content	Percent of fat in body	Body composition-based indicator of obesity. Used to guide obesity treatment, focused to weight loss and improvement of metabolic abnormalities of obesity
	Waist circumference (WC)	Waist circumference	Indicator of abdominal obesity, metabolic and cardio-vascular risk predictor. Used to guide obesity treatment, focused to weight loss and improvement of metabolic abnormalities of obesity
	Waist to hip ratio (WHR)	Ratio between waist and hip circumference	Metabolic and cardio-vascular risk predictors. Used to guide obesity treatment, focused to weight loss and improvement of metabolic abnormalities of obesity
	Waist to height ratio (WHtR)	Ratio between waist circumference and height	
Inflammatory mediators	Adiponectin, Leptin, Resistin, Plasminogen activator inhibitor 1	Released by adipose tissue.	Metabolic and cardio-vascular risk predictors. Used to guide interventions to reduce insulin resistance and improve adipocyte functionality
	Tumor necrosis factor-alpha, Interleukin 6, C-reactive protein	Released by several cell types	Associated to a chronic inflammatory state, cardio-vascular risk predictors. Used to guide interventions to reduce inflammation and cardio-vascular risk
Microbiota-host interactions	Microbiota species diversity	Ratio between different specific species	Change in defined ratios and reduction in species diversity is detected in obesity and correlates with several metabolic abnormalities. Potential impact on appropriate diet strategy
	Short-Chain Fatty Acids, Lipopolysaccharides	Outer membrane components of microbiota species	Negatively affect gut barrier permeability in obesity (increase gluconeogenesis and lipogenesis). Used in the microbiota evaluation
Muscle functioning	Muscle quality	Force per unit muscle cross-sectional area	Specific muscle performance is reduced in obesity. Used to evaluate degree of functional impairment and to guide physical activity individualised protocols.
	Muscle architecture	Arrangement of muscle fibres	Contribute to reduced specific muscle performance in obesity and correlate with metabolic risk. Used to evaluate effects of intervention and to guide physical activity protocols.
	Muscle composition	Fat infiltration in muscle tissue	
Sedentariness and physical activity	Overall physical activity	Amount of physical activity	Negative correlate of obesity risk. Monitoring of activity used for motivational and behavioural interventions to prevent and care overweight/obesity
	Sedentary behaviours	Amount of sedentary activity	Positive correlates of obesity and metabolic risk. Monitoring of sedentariness used for motivational and behavioural interventions to prevent and care overweight/obesity
	Sedentary activity bouts	Amount of continuous sedentary activity	
Epigenetics	DNA methylation	HIF3A methylation status MC4R methylation status	Higher methylation is associated to overweight/obesity and increased metabolic risk. Potential target for the development of new drugs against obesity
	Chromatin modification	JMJD2A	Protective role against obesity regulating fat storage genes, glucose transporters genes and anti-adipogenesis genes. Potential target for the development of new drugs against obesity
	miRNAs	Circulating miRNAs Non-circulating miRNAs	Overall, non-circulating miRNAs influence adipocytes differentiation and increase adipose tissue mass; circulating miRNAs act on insulin sensitivity and obesity-related inflammation. Potential targets for the development of new drugs against obesity

excess and predict related risks and outcomes, as well as providing a quantitative guide to personalised diet and physical activity interventions.

In particular, the assessment of microbiota-host interactions appears to have a considerable theranostic potential. Indeed, environmental factors and diet are important aspect of human life infuencing the gut microbiota, and, on turn, the interactions between microbiota and host metabolism have been shown to produce an impact on body weight and body composition. In addition, the gut microbiota has a role in the inflammatory and metabolic responses. Thus, the evaluation of gut microbiota can yield valuable information about the individual's overall metabolic condition, with impact in the selection of diet strategy.

Under the theranostic perspective, a very promising category of biomarkers is that including

indicators of behaviours and habits which overall contribute to the definition of lifestyle. In fact, these concur in the classification of different modalities of behaviours and have definite impact on the prediction of the risk for obesity and metabolic abnormalities development, thus possessing a both diagnostic and prognostic value. Moreover, the effects of interventions based on behaviour change approach at individual or societal level can be explored by these markers of lifestyle. We thus propose that this class of behavioural biomarkers for lifestyle assessment could be considered to have also a real theranostic value, although still not directly linked to a definite molecular underlying process.

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