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Title: Insulin-sensitive obesity in humans - a "favorable fat" phenotype?

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Abstract: In most humans, obesity and insulin resistance coexist. However, a unique group of obese individuals, who exhibit better insulin sensitivity than expected for their adiposity, has been the focus of recent research interest. We critically examine cross-sectional and lifestyle intervention studies in obese humans classified as "insulin-sensitive" vs. "insulin-resistant" and review the few longitudinal studies comparing rates of cardiovascular disease, type 2 diabetes and all-cause mortality in these groups of individuals. We suggest that reduced deposition of fat, particularly of bioactive lipid intermediates, in muscle and liver is potentially protective. We propose that dynamic interventional studies in insulin-sensitive obese humans may increase understanding of the metabolic factors that play a role in obesity-associated insulin resistance in humans.

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GARVAN INSTITUTE
Breakthrough Medical Research

22nd November 2011

To: Dr Iphigenia Tzameli
Editor-in-Chief
Trends in Endocrinology & Metabolism

Re: TEM-D-11-00123

Dear Dr Tzameli,

Attached, please find a revised version of the manuscript 'Insulin-sensitive obesity in humans – a "favorable fat" phenotype?'

Yours sincerely,

Dorit Samocha-Bonet

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To: Dr Iphigenia Tzameli
Editor-in-Chief
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Re: TEM-D-11-00123

Dear Dr Tzameli,

Thank you for inviting us to resubmit a revised version of the above manuscript. We are grateful for the comments of the reviewers and editor and believe they have helped us improve this work.

The manuscript has been revised significantly. The major areas that were changed relate to (i) interpretation of longitudinal data, (ii) discussion of the contribution of lower visceral adiposity to the protective phenotype and (iii) discussion of the ectopic deposition of lipids in liver and skeletal muscle.

We have also accepted the editorial comments, included a glossary (which assisted in reducing the word count of the manuscript) and deleted complex columns from table 2.

Our specific answers to the reviewers' and editor's comments are as follow.

REVIEWER #1

We agree with the reviewer's general comment regarding the focus of the review and have centred our discussions on obese insulin-sensitive and metabolically-healthy obese, rather than obesity and insulin resistance in general. Accordingly, we have omitted references to publications that are less relevant to the obese insulin-sensitive phenomenon (e.g. overfeeding in lean-to-overweight cohorts).

Longitudinal studies

We agree with the reviewer that the data from different longitudinal studies are more consistent than was reflected in the manuscript. We have re-evaluated the data and thoroughly revised the section where we discuss the findings from longitudinal studies (pages 4-5 lines 30-67). Furthermore, an additional study that supports the notion that obese insulin-sensitive are protected from cardiovascular mortality was published recently and is now included in table 1b and the text. Further, we agree that longer follow-up will not necessarily improve the ability of longitudinal studies to determine whether the Ob_{sens} or MHO phenotypes are

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protective and have stressed that multiple assessments over longer follow-ups are better suited to address this question (page 5 lines 65-67 and in the 'concluding remarks', page 15).

We have accepted the correction to the complex sentence to read: 'Comparison of Ob_{sens} and Ob_{res} humans may help determine metabolic factors that are more closely associated with obesity *vs.* those associated with, and perhaps contributing to, insulin resistance' (page 5 lines 70-72).

We have changed 'quantifiable methodologies' to 'objective measures' (page 6 line 91) and deleted the fat-frequency questionnaires from the list.

We have deleted the discussion regarding the intra-variation of the CT technique and omitted the previously referenced paper from our group. We realize that the paragraph regarding the association between visceral adiposity and insulin resistance was unclear as written and have changed it (pages 7-8, lines 115-129). Overall, we believe that the 'abdominal fat distribution' section has improved considerably in the revised version of the manuscript.

We believe that Ob_{sens} are likely to benefit from weight loss interventions to prevent other, non-metabolic, aspects of obesity and have explained this in the revised manuscript (page 14, lines 287-289).

We have explained the rationale of studying obese individuals classified based on insulin sensitivity, rather that metabolic health (pages 14-15, lines 297-301).

Prevalence of Ob_{sens}/Obese and Ob_{sens}/whole cohort as well as MHO/Obese and MHO/whole cohort are given in tables 1a and 1b. We have spelled this out and hope it is clearer now.

The criteria used to define the Ob_{sens} and MHO in the longitudinal studies are now given at the footnote of tables 1a and 1b.

We have deleted columns 3 and 4 from table 2.

We have stressed that the early onset of obesity was not significant in Ob_{sens} in Brochu *et al.*, 2001 (P=0.09, page 7, lines 110-112) and deleted the paragraph regarding early onset obesity as a possible protective factor, as we felt that the data gathered thus far do not justify such a discussion.

REVIEWER #2

1. We agree with the reviewer that categorizing obese individuals to insulin-sensitive and insulin-resistant according to variable markers of insulin resistance and lack of consensus regarding cut-off values introduces complexity to the comparison between relative risk (RR) in different longitudinal studies (page 4, lines 40-42).
2. We have stressed that in most of the studies that found similar aerobic fitness in Ob_{sens} and Ob_{res}, BMI was not optimally matched, even when the P value was non-significant (page 6, lines 96-98).
3. We agree that the liver fat content section lacked a discussion of other mechanisms that may explain fatty liver in insulin resistance and have revised this paragraph (pages 10-12, lines 195-228).

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4. We agree that the 'ectopic lipid accumulation' section did not reflect the current controversy and complexity in the literature regarding the involvement of the bioactive lipid intermediates diacylglycerol (DAG) and ceramide in insulin resistance. Indeed, ceramide content in skeletal muscle does not always correlate with insulin resistance. We have now balanced the discussion (pages 12-13 lines 230-250) and added the skeletal muscle DAG to the figure.

EDITORIAL COMMENTS

Glossary

We have accepted the glossary suggestion. Due to the glossary word count constraints, we have limited ourselves to terms that are pertinent (and not defined elsewhere) in the text. Please advise if you prefer more terms to be included.

Tables

We have rearranged tables 1a and 1b, so that the references are given in the far right column. We agree with the editor that the 3rd column is complex and may not be appreciated by the readers and deleted it. With regards to the 4th column, we made sure that the description of the cohort and the quality of the BMI matching appear in the text and deleted this column as well. Also, we made sure that the tables are cited in the text.

Boxes

We have kept the 2 boxes, as suggested, and made sure that they do not exceed 400 words. We made sure that the boxes are cited in the appropriate places.

Figure and Figure legend

We have added a Figure legend to describe the Figure.

Length

The article word count is 3,700, Glossary- 384, Box 1- 381 and Box 2-392.

Clarity/Accessibility

We have accepted the changes suggested.

Yours sincerely,

Dorit Samocha-Bonet

Insulin-sensitive obesity in humans – a "favorable fat" phenotype?

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Abstract

In most humans, obesity and insulin resistance coexist. However, a unique group of obese individuals, who exhibit better insulin sensitivity than expected for their adiposity, has been the focus of recent research interest. We critically examine cross-sectional and lifestyle intervention studies in obese humans classified as "insulin-sensitive" *vs.* "insulin-resistant" and review the few longitudinal studies comparing rates of cardiovascular disease, type 2 diabetes and all-cause mortality in these groups of individuals. We suggest that reduced deposition of fat, particularly of bioactive lipid intermediates, in muscle and liver is potentially protective. We propose that dynamic interventional studies in insulin-sensitive obese humans may increase understanding of the metabolic factors that play a role in obesity-associated insulin resistance in humans.

Definition of "metabolically-healthy" and "insulin-sensitive" obesity

Obesity is associated with cardiovascular disease (CVD) and type 2-diabetes (T2D) and may explain, at least in part, the global rise in their prevalence. Recent studies have demonstrated that some obese humans exhibit a better metabolic profile than expected for their adiposity; indeed, this "metabolically-healthy/benign obesity" phenomenon has become the focus of study of several groups [1-3]. The definition of the phenomenon is largely based on body mass index (BMI) $\geq$30 $kg \cdot m^{-2}$ in the absence of some (or all) features of the "metabolic syndrome" (MS) [2], a cluster of CVD and T2D risk factors (Box 1). Thus, it is not surprising that there is disagreement regarding the prevalence of metabolically-healthy obese (MHO), as reviewed recently [2, 4].

Insulin resistance (glossary) is a core feature of the MS and may link its individual components [5]. Insulin resistance and abdominal adiposity are closely associated, but some obese humans exhibit comparable insulin sensitivity to that of normal-weight individuals [6]. In most studies, insulin-sensitive obese humans (Ob_{sens}) have been identified by being in the top proportion of insulin sensitivity of the population studied, based on surrogate markers such as the homeostasis model assessment [HOMA-IR] or insulin sensitivity index (ISI, glossary and Box 2). In fewer studies, typically with smaller cohorts, the gold-standard hyperinsulinemic-euglycemic clamp (clamp; glossary and Box 2) was used to measure *in vivo* insulin sensitivity.

The identification of MHO (based on the MS) is clinically interesting [2, 4], but we propose that it may be more important to study Ob_{sens} humans (i.e. individuals identified as insulin sensitive obese), as this has the potential to uncover novel targets for prevention of insulin resistance in humans. Here, we focus on Ob_{sens} humans and use the term Ob_{res} to describe their insulin-resistant obese peers. We use the terms MHO and metabolically-abnormal obese (MAO) in the context of studies that have classified obese individuals based on the MS. We

review longitudinal studies that have assessed the risk of T2D, CVD and all-cause mortality in these (supposedly protected) individuals. Next, we explore the recent literature regarding the possible factors that may contribute to the protective phenotype. Finally, we provide future directions for the study of this interesting group.

Are Ob_{sens} and MHO protected from the development of T2D, CVD and mortality?

To evaluate if Ob_{sens} and MHO are protected from the consequences of insulin resistance, we have examined longitudinal studies that defined obese individuals as Ob_{sens} and/or MHO at baseline and documented their medical status 7 – 30 years later. Two longitudinal studies have focused on T2D [7, 8] (Table 1a) and five on CVD [8-11] and all-cause mortality [9, 11, 12] (Table 1b). MHO humans were defined as having ≤1 or ≤2 components of the MS criteria (footnote to Tables 1a and 1b) and Ob_{sens} humans were defined as HOMA-IR< 75^{th} percentile of the distribution in participants without diabetes [7-9] or HOMA-IR <2.5 [11, 12]. The interpretation of longitudinal studies using HOMA-IR to stratify subjects may be limited by their use of a surrogate measure of insulin sensitivity, which is modestly correlated with direct measures of whole body insulin sensitivity using the insulin clamp. Moreover, all insulin sensitivity measures are highly variable and there is no consensus regarding cut-off values, which complicates the comparison between studies. As expected, the prevalence of Ob_{sens} was different to that of MHO (Tables 1a and 1b). The risk of T2D, CVD and all-cause mortality in Ob_{sens} or MHO and Ob_{res} or MAO relative to normal-weight insulin sensitive or metabolically-healthy individuals was reported (relative risk, RR, footnote to Tables 1a and 1b).

In all studies, Ob_{res} and MAO had significantly increased incidence of T2D, cardiovascular and all-cause mortality compared with their normal-weight insulin-sensitive and

metabolically-healthy peers (Table 1a and 1b). Ob_{sens} and MHO were not completely protected from T2D, but the magnitude of the risk of T2D in Ob_{sens} was markedly lower compared with Ob_{res} in both the Framingham Offspring Study (FOS) and the Uppsala Longitudinal Study of Adult Men (ULSAM; Table 1a). Interestingly, the difference in RR between MHO and MAO individuals was less marked in the ULSAM cohort (Table 1a).

MHO and/or Ob_{sens} were protected from cardiovascular mortality in all 4 studies in which cardiovascular mortality was an endpoint (Table 1b), consistent with reports of decreased carotid artery intima media thickness in Ob_{sens} [6, 13]. In contrast perhaps, in the ULSAM cohort, RR of cardiovascular events was significantly increased in both MHO and Ob_{sens} (Table 1b). Increased risk was also reported in all-cause mortality in Ob_{sens} and MHO in the ULSAM and the third National Health and Nutrition Examination Survey (NHANES) cohorts, but not in the Cremona cohort (Table 1b).

In summary, Ob_{sens} and MHO appear to be protected from increased CVD mortality, but may not be similarly protected from other causes of mortality. Studies with multiple assessments over longer follow-up periods are necessary to support or refute the protective Ob_{sens} (or MHO) phenotype.

Potential protective factors in Ob_{sens}

Comparison of Ob_{sens} and Ob_{res} humans may help determine metabolic factors that are more closely associated with obesity *vs.* those associated with, and perhaps contributing to, insulin resistance. To enable a valid comparison, the groups must be matched for age, BMI (preferably also fat mass) and gender. While age, BMI and fat mass are obvious confounders, sexual dimorphism in body size, fat distribution and insulin sensitivity is also well established

[14]. Moreover, gender differences in potential mediators of insulin resistance were reported in humans including adipocyte size, adipokines, pro-inflammatory cytokines, lipid species including phosphatidylcholine and sphingomyelin [14-18] and skeletal muscle lipids [19]. We review cross-sectional studies that assessed potential contributors to insulin sensitivity in obesity (Table 2). When comparing findings from different studies, one should be aware of potential complexities introduced by (i) classification based on different markers of insulin sensitivity (Box 2), (ii) clamp studies that used different insulin infusion rates (Box 2) and (iii) omission of intermediate insulin-sensitive groups in some studies.

Lifestyle factors and energy balance

An important question is whether the Ob_{sens} phenotype is associated with healthier lifestyle, including physical activity, aerobic fitness and eating habits. Physical activity and dietary intake are often over- and under- reported, respectively, in clinical studies. Using these subjective methods, physical activity was not different in Ob_{sens} and Ob_{res} premenopausal women [20]. In a mixed cohort of males and females, lower intake of saturated fat was reported, but BMI was non-significantly lower in Ob_{sens} [21] (Table 2). Large cohort studies that use objective measures, such as steps counting by pedometers and weighed food records are necessary to maximise the validity of such studies. Results regarding aerobic fitness (measured by VO_2 max) in Ob_{sens} were inconsistent, with the majority of studies reporting similar findings in Ob_{sens} and Ob_{res}. One study reported higher aerobic fitness in postmenopausal Ob_{sens} women only when classified by clamp, but not by HOMA-IR or ISI [22]. Notably, in most of the studies that did not find a difference in aerobic fitness, Ob_{sens} had lower BMI (even if significant differences were not reported), which may have confounded the interpretation of the findings.

When total energy expenditure and resting metabolic rate were evaluated by the gold-standard methodologies of doubly-labelled water and indirect calorimetry, respectively, in postmenopausal women, they were not different between Ob_{sens} and Ob_{res} (Table 2).

Metabolic flexibility (glossary) is an intrinsic property of skeletal muscle [23] and have been reported to be impaired in healthy lean individuals with a family history of T2D, possibly facilitating weight gain, obesity and insulin resistance [24, 25]. Weiss *et al* reported greater metabolic flexibility in gender and pubertal status matched Ob_{sens} children and adolescents [26]. During fasting or exercise, this may translate to channelling lipids to oxidation, rather than storage in skeletal muscle in Ob_{sens}, which may explain their preserved insulin sensitivity (Fig 1). Interestingly, Ob_{sens} were reported to have a similar family history of T2D [21] and in postmenopausal Ob_{sens} women, a tendency towards early onset of obesity (P=0.09) was reported [27] (Table 2).

Abdominal fat distribution and adipocyte size

Visceral adiposity, and in particular upper abdominal visceral adiposity, is correlated with cardiometabolic risk factors in humans [28, 29]. Several hypotheses regarding the role visceral fat may play in metabolic disease were suggested, including (i) secretion of pro-inflammatory molecules capable of inducing insulin resistance in other organs; and (ii) high rates of lipolysis in the visceral (rather than subcutaneous) fat depot, that increase the delivery of free fatty acids to the liver to induce hepatic insulin resistance [30]. Cross-sectional studies have examined the anatomical differences in abdominal fat depots between Ob_{sens} and Ob_{res} (Table 2). Most studies evaluated abdominal adipose tissue distribution by a single-slice computerized tomography (CT, glossary) and reported decreased visceral adiposity in Ob_{sens} (Table 2), but in some of these studies, Ob_{sens} tended to have lower BMI,

which may have driven this finding [20, 27, 31]. Two studies that closely matched the groups for BMI and used magnetic resonance imaging (MRI, glossary) found a decreased visceral adiposity in Ob_{sens} children and adolescents [26], but not in postmenopausal women [6]. In summary, the majority of the studies suggest that the Ob_{sens} phenotype may be characterized by lower visceral adiposity.

Failure of adipocyte proliferation and differentiation results in adipocyte hypertrophy and insulin-resistant fat cells and adipocyte size has been reported to correlate positively with ectopic deposition of fat in the liver in overweight humans [32]. Adipocyte size distribution was evaluated in visceral and subcutaneous abdominal surgery samples in morbidly-obese individuals [33, 34] and in subcutaneous periumbilical biopsy samples in overweight-to-obese individuals [35]. Smaller adipocytes were reported in omental adipose tissue in morbidly-Ob_{sens} [33] and MHO [34]. In subcutaneous samples, McLaughlin *et al* have reported a similar average adipocyte size in overweight-to-obese insulin-sensitive and insulin-resistant humans, but the ratio of small-to-large adipocytes was surprisingly lower in the insulin-sensitive group. Together with 2-3-fold higher expression of genes encoding markers of adipocyte differentiation, these findings suggest a normal *vs.* impaired adipocyte differentiation in Ob_{sens} and Ob_{res}, respectively [35]. A better adipocyte differentiation capacity in Ob_{sens} may translate into fat storage away from muscle and liver.

Circulating molecules secreted from adipose tissue

Adipose tissue is composed not only of adipocytes, but also pre-adipocytes, fibroblasts, endothelial and immune cells. Adipocytes and immune cells secrete bioactive mediators, known as adipokines and cytokines, that modulate energy and glucose homeostasis, lipid metabolism, inflammation and atherosclerosis [36].

Findings regarding circulating adipokines in Ob_{sens} and Ob_{res} are inconsistent. Adiponectin is an insulin-sensitizer in muscle and liver, and unlike other adipokines, its concentration in plasma is inversely correlated with adiposity, in particular visceral adiposity [17, 37]. Several studies, where visceral adiposity was lower [26, 33] or the ratio of females-to-males higher [38], reported higher adiponectin concentration in Ob_{sens} compared with Ob_{res}. When gender ratio and visceral adiposity were similar between the groups, no difference in adiponectin concentration was found [6]. Leptin has a central anorectic effect and a stimulatory effect on thermogenesis, lipid oxidation and insulin sensitivity in peripheral tissues, all of which are abrogated in obese animal models and humans who exhibit leptin resistance. Circulating leptin concentration positively correlates with fat mass and decrease with weight loss in humans [37], and studies evaluating circulating leptin concentration in obesity did not find a significant difference between Ob_{sens} and Ob_{res} (Table 2), suggesting that circulating leptin is merely a reflection of fat mass. Retinol binding protein (RBP)-4 plasma concentration correlates positively with BMI, is elevated in insulin resistance [36, 39] and decreases in those that improve insulin sensitivity with physical activity [39]. Interestingly, BMI-matched morbidly Ob_{sens} had lower circulating RBP-4 [33] (Table 2).

Chemokines secreted from adipocytes and immune cells residing in the adipose tissue, attract monocytes and T lymphocytes, which further exacerbate the pro-inflammatory state associated with obesity [40, 41]. Cross-sectional studies in humans have reported increased macrophage infiltration in abdominal adipose tissue of obese and pre-diabetic patients relative to lean healthy individuals [42, 43]. Visceral macrophage content was higher than subcutaneous macrophage content [43], and macrophage count was reduced after bariatric surgery, in morbidly-obese individuals [44, 45]. The most studied chemokine in obesity and T2D is monocyte chemoattractant protein (MCP)-1. Expressed more in visceral than

subcutaneous adipose tissue, MCP-1 attracts monocytes and T lymphocytes. Circulating MCP-1 concentration is higher in obese and T2D patients and bariatric surgery-induced weight loss decreases its concentration in morbidly-obese patients [40]. In a cohort of morbidly-obese males and females, serum MCP-1 was not lower in Ob_{sens}, however macrophage count and mRNA expression of CD68 (a macrophage marker) in visceral adipose tissue samples were lower in Ob_{sens} [33] (Table 2). Chemerin, a cytokine that is structurally-distinct from the chemokine family, is also secreted from the adipose tissue to attract macrophages and dendritic cells, and higher circulatory concentrations have been reported in obese humans [40]. Interestingly, chemerin concentration is lower in Ob_{sens} and may account for the decreased macrophage count in visceral samples in Ob_{sens}, in that study [33]. Pro-inflammatory pathways capable of inhibiting insulin action were described not only in adipose tissue, but also in obese liver and pancreas [41]. It may be hypothesized that Ob_{sens} will have lower concentrations of pro-inflammatory cytokines in the circulation, reflecting a lower degree of inflammation. This is the case for C-reactive protein (CRP), a molecule secreted from hepatocytes, but not for interleukin (IL)-6, secreted from both adipose tissue and liver (Table 2). In summary, circulating concentrations of some, but not all, adipokines, cytokines and chemokines are differentially expressed in Ob_{sens} and Ob_{res} (Fig 1) and may play a role in the Ob_{sens} phenotype.

Ectopic lipid accumulation

Intramyocellular and intrahepatic triacylglycerol (TAG) accumulation is strongly associated with skeletal muscle and hepatic insulin resistance. Increased fatty acid uptake coupled with decreased rate of fatty acid utilization might contribute to accumulation of ectopic fat in muscle and liver, in high fat fed and obese rodent models [46]. Fatty liver is common in obesity and is associated not only with hepatic, but also with muscle insulin resistance [47].

Moreover, in those with higher liver fat, both hepatic and muscle insulin sensitivity is impaired, despite matched visceral adiposity [48], suggesting that liver, rather than visceral, fat is a dominant factor associated with insulin resistance in humans. A major contributor to fatty liver in insulin-resistant states is enhanced *de novo* synthesis of fatty acids (lipogenesis). Insulin activates the transcription factor sterol regulatory element-binding protein-1c (SREBP-1c) which activates genes required for triglyceride synthesis in the liver. In the insulin-sensitive liver, *de novo* lipogenesis is activated by insulin and while expected to be less active in insulin resistance, the SREBP-1c pathway remains activated and *de novo* lipogenesis is stimulated, rather than down-regulated, contributing to hepatic steatosis [49].

Studies of short-term over- and under- nutrition in humans suggest that the liver is the first organ to absorb and release fat, respectively. Specifically, 3-days high fat feeding [50] and 2-days calorie restriction [51] were reported to significantly increase and reverse liver fat deposition, respectively. An almost maximal liver fat clearance and hepatic insulin sensitivity were achieved within 2-days of calorie restriction (and ~-2 kg), but muscle insulin resistance reversal lagged and required 11-weeks and ~7% weight loss [51]. In Ob_{sens}, intrahepatic lipid content is consistently reported to be lower, when measured by magnetic resonance spectroscopy (MRS, glossary) or by the surrogate hepatic enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase and γ-glutamyltransferase [GGT]) (Table 2), but hepatic insulin resistance was not reported in these studies. TAG in insulin-sensitive tissues is regarded as metabolically-inert but is a surrogate of other bioactive lipid intermediates such as diacylglycerol (DAG) and sphingolipids (including sphingomyelin and ceramide) that impair insulin action in muscle and liver [52, 53]. Although not a standard procedure in studies in humans, liver biopsies from obese individuals with hepatic steatosis revealed a ~10-fold TAG and ~2.5-fold DAG content

compared with age and BMI matched healthy individuals [54]. Interestingly, a connection between hepatic DAG content in cytoplasmic lipid droplets and insulin resistance (by HOMA-IR) was reported in severely obese individuals [55]. The role that hepatic DAG may play in insulin resistance requires further studies.

Increased skeletal muscle TAG content is reported in obesity, T2D, insulin-resistant offspring of T2D patients and elderly individuals [56]; in sedentary humans, skeletal muscle insulin sensitivity correlates inversely with muscle TAG content [57]. However, high-fat overfeeding or calorie restriction coupled with deterioration or improvement in insulin sensitivity, respectively, are not always accompanied by changes in TAG content in skeletal muscle in humans [32, 58]. Moreover, cross-sectional studies in endurance-trained, obese, impaired glucose tolerance (IGT, glossary) and T2D patients reported significantly higher insulin sensitivity in the endurance-trained individuals, but higher [59] or similar [60] skeletal muscle TAG content. Unlike findings in liver, findings in skeletal muscle in Ob_{sens} are inconsistent - some studies using MRS or Oil red O staining of vastus lateralis biopsy sections report less TAG, whereas studies using less established methodologies (CT and DXA, Table 2) report similar TAG levels. The role that skeletal muscle DAG and ceramide play in the development of insulin resistance in humans is currently under intense scrutiny. Some studies have reported elevated DAG [19] and ceramide [19, 61, 62] in skeletal muscle in obese diabetic, obese non-diabetic and insulin-resistant lean individuals compared with insulin-sensitive lean individuals, but this was not confirmed by others [60, 63]. Moreover, skeletal muscle insulin resistance does not always correlate with ceramide content [60] and may depend on the muscle fibre type distribution [60, 64]. Similarly, total skeletal muscle DAG content does not distinguish the subcellular localization of DAG and thus its biological

activity. Only one study to date examined skeletal muscle DAG and ceramide species in Ob_{sens} and Ob_{res} (Table 2) and reported lower ceramide species in the former [64].

Pancreatic fat content may also be evaluated by MRS and cross-sectional data in humans demonstrated a gradual increase in pancreatic fat from normal to IGT to combined IGT/IFG individuals (glossary) [65]. Calorie restriction and weight loss decreased pancreatic fat and restored first-phase insulin secretion in obese T2D patients [66]. Pancreatic fat is likely to be associated with impaired insulin secretion and has not been reported in Ob_{sens}.

In summary, decreased liver fat content and bioactive lipid species in skeletal muscle are possible contributors to insulin sensitivity in obesity (Fig 1). Further lipidomic analyses of muscle and plasma (considered a reflection of liver content [67, 68]) are necessary to establish an association between specific lipid intermediates and insulin sensitivity/resistance in obesity. Clearly, cross-sectional studies are limited to associative findings, but interventions known to modify insulin sensitivity have the potential to highlight those metabolic factors that change with insulin sensitivity and complement the data gathered from cross-sectional studies.

Differential effects of lifestyle intervention in Ob_{sens} and Ob_{res}?

Several groups studied the effect of calorie restriction and exercise training on insulin sensitivity in Ob_{sens}. Karelis *et al* reported that diet-induced weight loss resulted in a 26% improvement in insulin sensitivity (by clamp) in Ob_{res} and a 13% *decrease* in insulin sensitivity in Ob_{sens}. However, analysis of potential metabolic players in the response was not reported [69]. The deterioration in insulin sensitivity in Ob_{sens} reported in that study was not reproduced in other studies. Specifically, two diet-induced weight loss studies reported that

insulin sensitivity improved in Ob_{res}, but not in Ob_{sens}. It should be noted that insulin sensitivity was high in Ob_{sens} at baseline (mean HOMA-IR 1.2 [70] and 1.8 [71]), hence a further improvement may not have been detectable. Similarly, liver fat, tibialis muscle TAG content [70] and CRP [71] decreased only in Ob_{res}, but were very low at baseline in Ob_{sens}. On the other hand, Janiszewski *et al* stratified men and women separately to Ob_{sens} and Ob_{res} according to clamp and found an improvement in insulin sensitivity in the top insulin sensitivity tertile in women and a tendency for improvement in men with weight loss [72]. When weight loss was achieved by bariatric surgery in morbidly-obese participants, insulin sensitivity (by ISI) improved in Ob_{sens} and Ob_{res} in parallel with decreases in liver enzymes, fasting plasma insulin and TAG in both [73], suggesting that these factors may play a role in the improved insulin sensitivity. We propose that weight loss studies could be complemented by short-term overfeeding interventions to study the effect on insulin sensitivity in relation to potential contributing metabolic factors, as has been reported in non-obese healthy individuals with and without a family history of T2D [74]. Notably, we agree it is appropriate to suggest that enough evidence exists for lifestyle intervention in Ob_{sens} to prevent obesity-associated complications other than metabolic disturbances [75].

Concluding remarks and future directions

We propose that the major protective factors in obese humans with preserved insulin sensitivity are lower content of bioactive lipid intermediates in liver and muscle, likely through greater capacities for lipid utilization, rather than storage, in these organs with increased capacity for storing fat in adipose tissue (Fig 1). Certain adipose tissue-derived molecules may also provide protection, in particular higher adiponectin and lower RBP-4. We suggest that the definition of this pivotal obese group should be based on insulin sensitivity, rather than metabolic health parameters, because (i) cross-sectional and

299 interventional studies in obesity will provide data regarding possible contributors to insulin
300 resistance in obesity and (ii) the incidence of CVD in longitudinal studies will not be
301 confounded by the selection of cohorts based on pre-existing cardiovascular risk factors.
302 Ideally, the Ob_{sens} group should be defined as having similar insulin sensitivity to a lean
303 reference group and the obese groups should be gender, age and fat mass-matched.
304 Furthermore, to increase the validity of cross-sectional data, insulin sensitivity should be
305 evaluated by low- and high-dose insulin clamp studies with tracers, in order to distinguish
306 muscle from liver sensitivity (Box 2). State-of-the-art measures of ectopic fat deposition in
307 muscle, liver and pancreas, and detailed mass spectrometric lipidomic analyses of muscle and
308 plasma, are necessary. A dynamic approach should be taken to complement cross-sectional
309 data, including calorie restriction (with or without exercise) and short-term nutritional excess.
310 Changes in the potential metabolic players should be assessed in relation to changes in
311 muscle and liver insulin sensitivity. Finally, longitudinal data with cardiovascular and T2D
312 endpoints with multiple assessments over long follow up are necessary to support or refute
313 the long-term protective effect of insulin sensitivity in obesity.
314

Glossary

Computerized tomography (CT) is used to evaluate abdominal adipose tissue distribution and liver fat in clinical studies.

Dual-energy X-ray absorptiometry (DXA) is used to evaluate total body fat mass and fat-free mass in metabolic studies.

Endogenous glucose production (EGP) is predominantly hepatic in the post-absorptive state and can be measured in clinical studies by the tracer dilution method (Box 2).

Glucose infusion rate (GIR) is the rate of glucose infusion necessary to maintain euglycemia during the hyperinsulinemic clamp (typically during the last 30 min) and is used to measure insulin sensitivity in clinical studies (Box 2).

Homeostasis model assessment of insulin resistance (HOMA-IR) is a marker of insulin resistance and is based on fasting plasma glucose and insulin, as given in the following equation. Fasting glucose [$mmol \cdot L^{-1}$]*fasting insulin [$mU \cdot L^{-1}$]/22.5. Increased HOMA-IR corresponds with increased insulin resistance.

Hyperinsulinemic-euglycemic clamp (clamp) is the gold-standard methodology to measure insulin resistance in clinical studies (Box 2).

Impaired fasting glucose (IFG) is defined as fasting plasma glucose between 5.6 and 6.9 mmol/L.

Impaired glucose tolerance (IGT) is defined as fasting plasma glucose <7 mmol/L and 2-h plasma glucose ≥7.8 and <11.0 mmol/L after consuming 75 g glucose.

Insulin resistance is the inability of insulin secreted from pancreatic β-cells to orchestrate an appropriate metabolic response, particularly in muscle, liver and adipose tissue.

Insulin sensitivity index (ISI or Matsuda index) is a marker of insulin resistance based on plasma glucose and insulin concentrations at fasting and during OGTT, as given in the following equation. 10,000/√([fasting glucose*fasting insulin]*[mean glucose*mean insulin during OGTT]). Decreased ISI corresponds with increased insulin resistance.

Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are used to measure abdominal adipose tissue distribution and liver and muscle triglycerides, respectively in clinical studies.

Metabolic flexibility is the ability of skeletal muscle to adapt rapidly to fuel availability and can be measured by the increase in respiratory quotient (RQ) from fasting to the clamp hyperinsulinemic state, reflecting the switch from fat to carbohydrate oxidation.

Normal glucose tolerance (NGT) is defined as fasting plasma glucose <5.6 mmol/L and 2-h plasma glucose <7.8 mmol/L after consuming 75 g glucose.

Oral glucose tolerance test (OGTT) is used to diagnose IGT and T2D, whereby glucose is given orally (typically 75 g) and blood is drawn at fasting and after 2-h for measurement of plasma glucose.

353 **Box 1**: The "metabolic syndrome" – definition and limitations

354 The definition of the metabolic syndrome is based on clustering of several metabolic
355 abnormalities. Different sets of criteria were proposed since 1998 by different health
356 organizations. All versions included central obesity by waist circumference, dyslipidemia and
357 hypertension. The main difference was in mandatory components required in some, but not
358 all, sets of criteria. The first formal definition was proposed by the World Health
359 Organization (WHO) and included, in addition to the 3 common criteria, evidence of insulin
360 resistance (by IGT or IFG or T2D, glossary). In 2001, the National Cholesterol Education
361 Program Adult Treatment Panel III (ATP III) introduced another set of criteria, requiring 3
362 out of 5 of abdominal obesity, hypertriglyceridemia, reduced HDL, hypertension and fasting
363 hyperglycemia, for diagnosis. Insulin resistance per se was not required. In 2005, the
364 International Diabetes Federation (IDF) and the American Heart Association/National Heart,
365 Lung and Blood Institute (AHA/NHLBI) attempted to reconcile different clinical definitions.
366 The IDF suggested waist circumference as a mandate plus 2 of the criteria suggested by the
367 ATP III for diagnosis. AHA/NHLBI criteria were similar to that of IDF but abdominal
368 obesity was not a mandate. Notably, there was a disagreement regarding the definition of
369 abdominal obesity by waist circumference threshold with the IDF requiring a narrower waist
370 circumference that would equate to BMI ~25 $kg \cdot m^{-2}$ and the AHA/NHLBI requiring a larger
371 waist circumference threshold (BMI ~30 $kg \cdot m^{-2}$) [5]. Recently, in an attempt to settle the
372 disagreements and confusion, a unifying definition has been proposed by the IDF, NHLBI,
373 AHA, WHO, International Atherosclerosis Society and International Society for the Study of
374 Obesity and includes 3 of the following (1) elevated waist circumference (specific thresholds
375 based on population/country), (2) elevated serum triglyceride (≥1.7 mmol/L) or medication,
376 (3) reduced HDL (<1.0 and <1.3 mmol/L in males and females, respectively) or medication,
377 (4) elevated blood pressure (systolic ≥130, diastolic ≥85 mm Hg) or antihypertensive

treatment, (5) elevated fasting blood glucose (≥5.6 mmol/L) or medication [5]. The clinical usefulness of the metabolic syndrome has been often questioned with several longitudinal studies reporting that the syndrome does not predict CVD or progression any better than the sum of its components [76]. In defining the MHO, some (or all) criteria (except abdominal obesity) need to be absent, which resulted in conflicting prevalence rates and clinical findings [4].

Box 2. Methodologies used to assess insulin sensitivity in the study of Ob_{sens} humans.

In the post absorptive state, the rate of EGP (glossary), which is primarily hepatic (R_a), equals that of glucose utilized by the body (primarily by muscle, R_d). When exogenous insulin is administered, R_d increases and R_a decreases so that $R_d > R_a$, resulting in a decline in blood glucose concentration which can be countered by exogenous glucose infusion. The GIR (glossary) at the steady state of the hyperinsulinemic-euglycemic clamp (typically during the last 30 min) is used as a measure of the sensitivity to the insulin infusion. GIR normalized to fat-free mass (M value) can be compared between different individuals. R_a can be measured directly by using the tracer dilution method, where deuterated glucose is infused at a constant rate. After equilibrium (2-3 h), blood samples are collected at regular intervals and R_a is calculated to reflect basal EGP. When combined with low-dose insulin clamp, R_a suppression, which corresponds with hepatic insulin sensitivity, can also be evaluated. There are no reports of R_a suppression in Ob_{sens}. An insulin infusion rate that achieves a steady state insulin concentration of $\geq$100 $mU \cdot L^{-1}$ is considered sufficient to suppress R_a completely (R_a = 0) and in those protocols, GIR is used to measure R_d (muscle insulin sensitivity) [77]. When an insulin infusion rate of 40 $mU \cdot min \cdot m^{-2}$ body surface area was used, variability in steady state insulin concentration between individuals was large, even in healthy lean subjects; and in IGT (glossary) and T2D patients, this rate was insufficient to suppress EGP [77]. The

clamp is considered the gold-standard measurement of insulin sensitivity *in vivo*. However, due to its laborious nature, studies with larger cohorts typically use surrogate measures such as the HOMA-IR (glossary) and the ISI (glossary). HOMA-IR is based on the assumption that rising glucose concentrations lead to a compensatory increase in insulin concentration. Because fasting glucose concentrations reflect basal hepatic glucose production, HOMA-IR correlates better with R_a compared with R_d [78]. In particular, poor to no correlations were reported in individuals with IFG (glossary) and combined IFG and IGT [78], states commonly associated with obesity. The ISI was suggested as an effective marker reflecting both the response of the body to insulin sensitivity and insulin secretion [79]. The correlations reported between ISI and M-value were inconsistent and range from strong in NGT (glossary), IGT and T2D patients [79] to weak non-significant correlations in combined IGT/IFG patients [78].

Table 1a: Relative risk of type 2 diabetes in Ob_{sens}, Ob_{res}, MHO and MAO in longitudinal studies

Cohort	N	Gender (M %)	Average age at baseline (years)	Average follow up (years)	Prevalence Ob_{sens}/Obese (Ob_{sens} /whole cohort) (%)	Prevalence MHO/Obese (MHO /whole cohort) (%)	Ob_{sens} RR (*vs.* normal-weight)	MHO RR (*vs.* normal-weight)	Ob_{res} RR (*vs.* normal-weight)	MAO RR (*vs.* normal-weight)	
Framingham Offspring Study	2902	45	53	7	44 (9.3)	37 (8)	3.3 (*P* <0.01)	2.2 (NS)	10.7 (*P* <0.0001)	10.3 (*P* <0.0001)	[8]
Uppsala Longitudinal Study of Adult Men	[a] 1375 1675	100	49.7	20	30 (1.5)	32 (1.7)	11.2 (*P* <0.001)	11.7 (*P* <0.001)	17.1 (*P* <0.001)	10.1 (*P* <0.001)	[7]

[a] Cohort classified based on HOMA-IR n=1375 and based on ≤2 components of MS criteria (Box 1) n=1675

Statistical model was adjusted for age, sex, family history of T2D and IGT [8], age, smoking status and physical activity [7]

Ob_{sens} was defined as HOMA-IR< 75^{th} percentile and MHO as ≤2 components of MS criteria (Box 1) [7, 8]

Data presented for Ob_{sens} and Ob_{res} (grey shading) and MHO and MAO (no shading)

Table 1b: Relative risk of cardiovascular events, cardiovascular mortality and all-cause mortality in Ob_{sens}, Ob_{res}, MHO and MAO in longitudinal studies

Cohort	N	Gender (M %)	Average age at baseline (years)	Follow up (mean or median years)	Prevalence Ob_{sens}/Obese (Ob_{sens} /whole cohort) (%)	Prevalence MHO/Obese (MHO/whole cohort) (%)	Ob_{sens} CVD RR (*vs.* normal-weight)	Ob_{sens} All-cause mortality RR (*vs.* normal-weight)	Ob_{res} CVD RR (*vs.* normal-weight)	Ob_{res} All-cause mortality RR (*vs.* normal-weight)	MHO CVD RR (*vs.* normal-weight)	MHO All-cause mortality RR (*vs.* normal-weight)	MAO CVD RR (*vs.* normal-weight)	MAO All-cause mortality RR (*vs.* normal-weight)	
Framingham Offspring Study	2902	45	53	11	44 (9)	37 (8)	1.4 (NS)	Not reported	2.1 (*P* <0.001)	Not reported	1.5 (NS)	Not reported	2.1 (*P* <0.001)	Not reported	[8]
Uppsala Longitudinal Study of Adult Men	1758	100	50	30	25 (1)	31 (2)	Mortality 1.8 (NS) Events 1.9 (*P* <0.05)	2.0 (*P* <0.01)	Mortality 2.9 (*P* <0.001) Events 2.6 (*P* <0.001)	2.2 (*P* <0.001)	Mortality 1.2 (NS) Events 1.95 (*P* <0.05)	1.7 (*P*<0.05)	Mortality 3.2 (P<0.001) Events 2.6 (*P* <0.001)	2.4 (P<0.001)	[9]
Cremona Study	2011	44	58	15	11 (2)	Not reported	0.7 (NS)	1 (NS)	1.6 (*P*<0.05)	1.4 (*P*<0.05)	Not reported	Not reported	Not reported	Not reported	[11]
Third National Health and Nutrition Examination Survey	[a]4602 6011	50 [80]	38 [80]	9	30 (-)	38 (-)	Not reported	2.6 (*P* <0.05)	Not reported	3.1 (*P* <0.05)	Not reported	2.8 (*P* <0.05)	Not reported	2.7 (*P* <0.05)	[12]
Quebec Cardiovascular Study	1824	100	56	13	Not reported	25 (3)	Not reported	Not reported	Not reported	Not reported	1.5 (NS)	Not reported	1.8 (*P*<0.05)	Not reported	[10]

[a] Cohort classified based on HOMA-IR n=4602 and based on ≤1 component of MS criteria (Box 1) n=6011

MHO was defined as ≤2 [8, 9, 12] or ≤ 1 [12] of the MS criteria (Box 1) or ≤2 of (1) plasma TAG ≥ 1.7 mmol/L, (2) HDL ≤1.0 mmol/L, (3) % small-LDL (diameter <255A) ≥54.5, (4) apolipoprotein B ≥1.36 g/L, (5) fasting insulin ≥12 mU/L and (6) C-reactive protein ≥3.0 mg/L [10].
Ob_{sens} was defined as HOMA-IR< 75^{th} percentile [8, 9] or <2.5 [11, 12]
Statistical model was adjusted for age, sex, LDL-cholesterol and smoking [8], age, smoking status and LDL-cholesterol [9], age, sex [11, 12], income, smoking status, ethnicity and alcohol consumption [12] and age, smoking and medication use at baseline [10]
CVD was defined as fatal and non-fatal myocardial infarction, new-onset angina, stroke, heart failure [8-10], transient ischemic attack or intermittent claudication [8].
Data presented for Ob_{sens} and Ob_{res} (grey shading) and MHO and MAO (no shading)

Table 2: Possible protective factors in obese insulin-sensitive compared with obese insulin-resistant humans

Protective factor	Yes/No
Higher aerobic fitness (peak oxygen consumption, VO_2)	No [21, 27, 31, 64] Yes [22]
Increased physical activity	No (by physical activity questionnaire) [20]
Increased energy expenditure (EE)	No [22, 27, 31] Components of EE measured: total EE (by doubly-labelled water) and resting metabolic rate (by indirect calorimetry) [27, 31]
Lower energy intake	No [21]
Lower dietary fat intake	Yes [21] (in particular saturated fat)
Greater metabolic flexibility	Yes [26]
Less prevalence of family history of type 2 diabetes	No [21]
Earlier onset obesity	Tendency (48% Ob_{sens} *vs.* 29% Ob_{res} answered 'yes' to the question: 'were you overweight or obese between 13 and 19 years of age?', P=0.09) [27]
Lower visceral adiposity	Yes by CT [20, 22, 27, 31, 33, 81] and MRI [26] No by MRI [6]
Smaller adipocytes	Yes [33] (in both subcutaneous and visceral samples) No [35] (subcutaneous adipose tissue)
Higher plasma adiponectin	Yes [26, 33, 38] No [6]
Lower plasma leptin	No [21, 26, 33, 38]
Lower plasma pro-inflammatory cytokines and chemokines	Yes- CRP [31, 33], chemerin, RBP-4 [33], α-1 anti-trypsin [31] No- orosomucoid, haptoglobin [31], IL6 [26]
Less macrophage infiltration into adipose tissue	Yes [33] (in visceral, but not subcutaneous fat)
Lower liver fat content	Yes by MRS [6, 33] and liver enzymes [33, 81, 82]
Lower skeletal muscle intramyocellular lipids/leg fat	Yes by MRS [6, 26] and Oil red O staining of vastus lateralis biopsy sections [64] No by CT [27, 31] and DXA [20]
Lower skeletal muscle deleterious lipid species	Yes [64] (total ceramide and ceramide C14:0, C16:0 and C18:0, not DAG)

Figure legend: Putative protective factors in obese insulin-sensitive humans. Increased capacity for storing fat in the adipose tissue coupled with greater metabolic flexibility in skeletal muscle and decreased *de novo* lipogenesis in liver, result in decreased deposition of lipids, including bioactive species, in these organs. Also, lower visceral adiposity with increased circulating adiponectin, decreased pro-inflammatory cytokines and macrophage infiltration into the adipose tissue.

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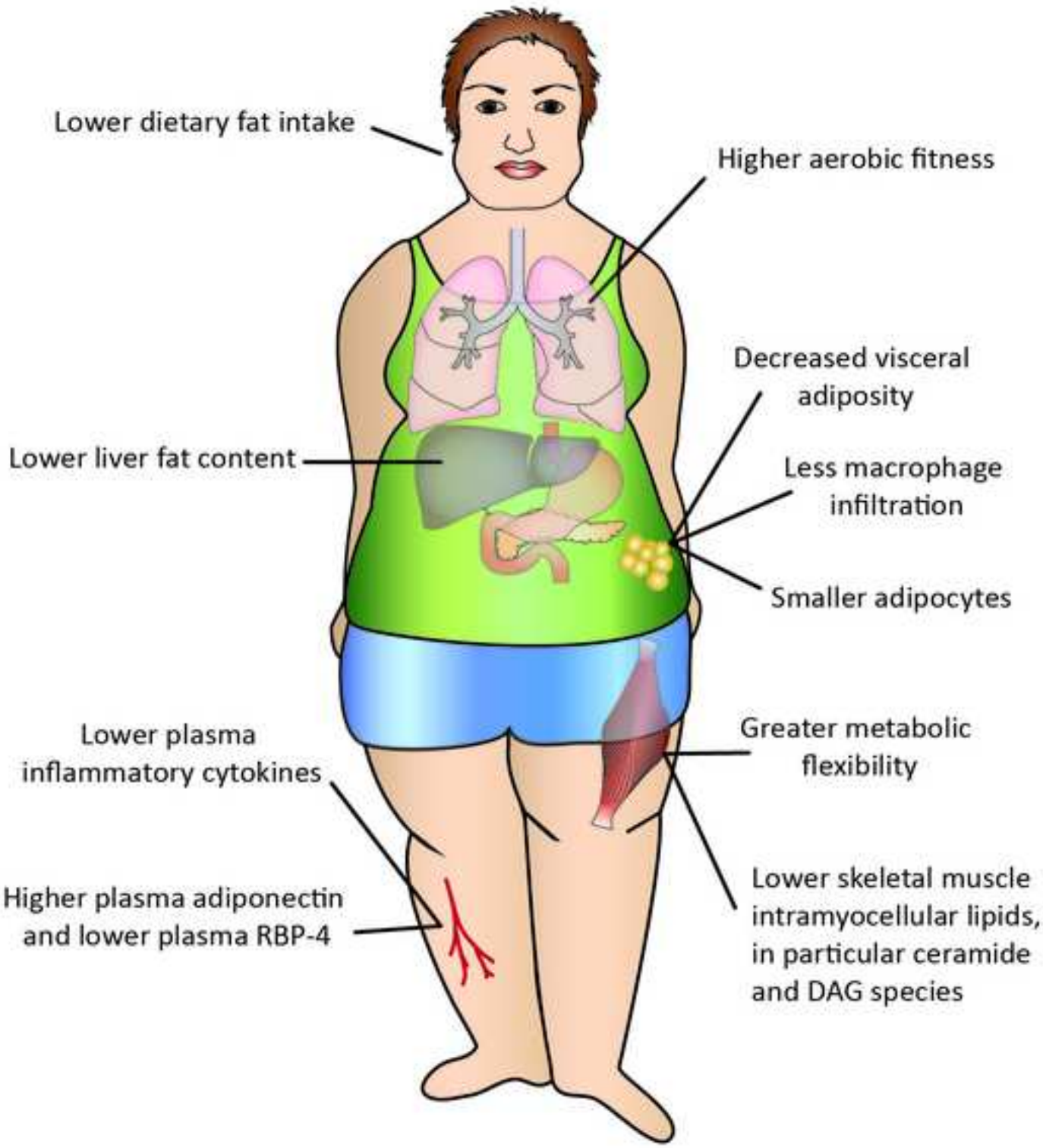

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