



# Adipose tissue as an endocrine organ: implications of its distribution on free fatty acid metabolism

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## KEYWORDS

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Lipolysis

Free fatty acids (FFAs) are the main circulating lipid fuel in the post-absorptive state. Elevated plasma FFA concentrations are associated with increased endogenous glucose production rates and insulin resistance in skeletal muscle, whereas long-term exposure of pancreatic  $\beta$ -cells to elevated FFA impairs insulin secretion, with a consequently increased risk of developing type 2 diabetes. A range of adverse changes occur in the vascular endothelium in response to elevation of plasma FFA concentrations that are similar to key early steps in atherogenesis. These include reduced endothelium-dependent vasodilatation and increased expression of adhesion molecules. We have found that the majority of systemic plasma FFAs originate from upper-body subcutaneous fat, with lesser contributions from leg fat and intra-abdominal adipocytes, even in persons with abdominal obesity. However, systemic FFA concentrations correlate positively with visceral fat, so that FFA release from visceral depots appears to serve as a marker of systemic insulin resistance. In addition, the secretion of a range of bioactive substances from intra-abdominal adipocytes (adipokines) is disturbed, with potentially deleterious consequences for metabolism and overall cardiometabolic risk. Interventions that reduce circulating FFA, either due to or independently of weight loss, are likely to improve insulin sensitivity,  $\beta$ -cell function, and cardiovascular risk. Weight loss in overweight or obese patients would have the additional benefit of reducing intra-abdominal adiposity and tending to correct the pathologically altered adipokine secretion associated with abdominal obesity.

## Introduction

Obese individuals are often insulin resistant, which is associated with an increased risk of adverse cardiovascular outcomes.<sup>1</sup> Research during the previous decade has linked upper-body (abdominal) obesity in particular with insulin resistance and increased cardiovascular risk.<sup>2–6</sup> Moreover, prospective observational studies have shown that abdominal obesity, measured by high waist circumference or increased waist-hip ratio, provides additional prognostic information relating to the development of cardiovascular risk factors, the metabolic syndrome, or type 2 diabetes, compared with

increased body mass index (BMI) alone.<sup>7–9</sup> Accordingly, the presence of abdominal obesity is now a pre-requisite for a diagnosis of the metabolic syndrome, according to the latest set of diagnostic criteria from the International Diabetes Federation.<sup>10</sup>

Many studies have sought to define mechanistic links between abdominal obesity, especially with increased visceral fat, and the development of cardiovascular disease. *In vitro* studies of adipocytes from different body regions have shown that intra-abdominal adipocytes are more lipolytically active than subcutaneous adipocytes. Because free fatty acids (FFAs), the product of adipose tissue lipolysis, are elevated in abdominal obesity and artificial elevations of FFA can induce insulin resistance in non-obese persons, it is natural to link enlarged intra-abdominal fat stores with FFA-induced

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insulin resistance. In addition, with obesity, there is altered secretion of a range of biologically active substances, including of the so-called adipokines: leptin, adiponectin, plasminogen activator inhibitor-1, interleukin (IL)-6, and tumour necrosis factor alpha.<sup>11,12</sup> These metabolic disturbances have the potential to mediate insulin resistance, inflammation, endothelial dysfunction, and atherothrombosis in the setting of obesity.

It remains uncertain whether abdominal obesity promotes generalized insulin resistance directly<sup>13</sup> or serves as a marker for this cardiovascular risk factor.<sup>14</sup> Nevertheless, disturbances to FFA metabolism occur during the development of abdominal obesity, which can adversely alter insulin sensitivity in some circumstances. The purpose of this review is to evaluate the contribution of disturbances in FFA metabolism to elevated cardiometabolic risk in the setting of abdominal obesity.

## Abdominal obesity and FFA metabolism

### Normal metabolism

Almost all fat stored in the body is derived from dietary triglycerides, and the vast majority of triglycerides is stored in adipocytes. Adipose tissue lipolysis releases FFA into the circulation to provide fuel for use by other tissues. Although heart, liver, and muscle are major users of FFA, only neural tissue, erythrocytes, and the renal medulla seem completely dependent on glucose; all other tissues can use FFA for fuel.<sup>15</sup> FFAs derived from adipose tissue lipolysis provide the principal source of lipid fuel in post-absorptive state.

The availability of FFA is strongly regulated by hormonal factors that modulate the activity of hormone-sensitive lipase.<sup>15</sup> Insulin is perhaps the most powerful regulator of lipolysis. When compared with the insulin-deficient state, overnight post-absorptive insulin concentrations have already restrained lipolysis by ~50% and the insulinaemic response to a typical meal can reduce the rate of lipolysis by another 80–90% in lean and healthy individuals. Catecholamines, epinephrine and norepinephrine, are potent stimulators of lipolysis, whereas growth hormone is a somewhat less potent lipolytic. During prolonged exercise, the combination of elevated catecholamines and growth hormone together with falling insulin concentrations can allow lipolysis to increase by as much as 300–400%. Thus, FFA concentrations and availability can vary over a range of two orders of magnitude. This makes FFA one of the most flexibly regulated substrates in the body.

### Disturbances of FFA metabolism in the setting of obesity

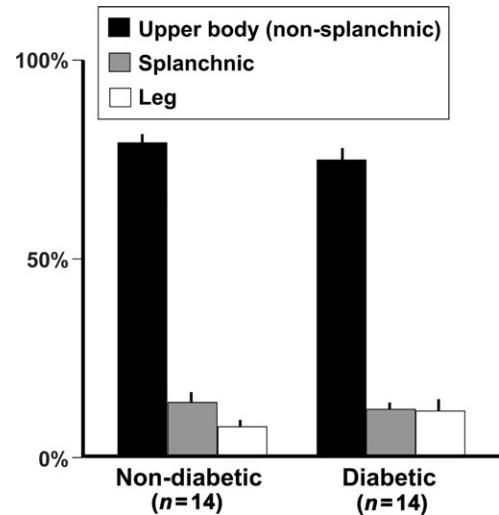
Obesity, and especially abdominal obesity, is associated with increased post-absorptive and post-prandial FFA concentrations. Post-absorptive FFA concentrations in abdominal obesity are variably (~20–30%) greater than that are found in lean, healthy persons and in

peripherally obese persons. The most striking difference between abdominally obese persons and peripherally obese or non-obese is the degree of FFA suppression following meal ingestion. Despite much higher post-prandial insulin concentrations, FFA concentrations and FFA release are about three-fold greater in abdominally obese adults than in non-obese or peripherally obese adults.<sup>16,17</sup> Given the results of *in vitro* studies of human omental adipocytes mentioned earlier, we have tested the hypothesis that increased visceral adipose tissue lipolysis is responsible for the elevated post-absorptive and post-prandial FFA release/FFA concentrations seen in upper-body obesity. In order to accomplish this, we combined the measures of FFA turnover using isotope dilution techniques with regional (splanchnic and leg) blood flow measurements and sampling of blood from the arterial circulation, the hepatic vein, and the femoral vein. We also measured regional lean and fat masses. This allowed us to partition systemic FFA release into that coming from leg (lower-body fat), the splanchnic bed (visceral fat), and upper-body subcutaneous fat (total systemic release minus leg and splanchnic release).

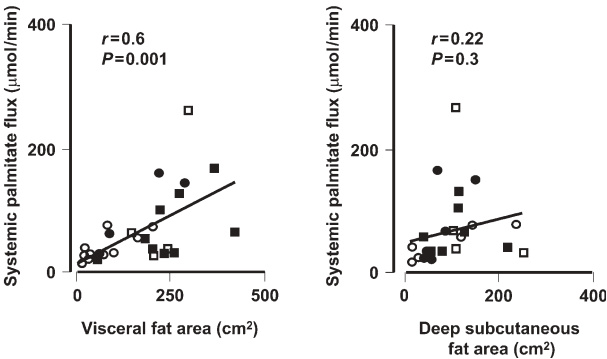
The initial study of upper-body obese, lower-body obese, and non-obese women indicated that intra-abdominal fat was also not a major source of circulating FFA in the post-absorptive state.<sup>18</sup> Splanchnic FFA release, which is the net contribution of visceral adipose tissue lipolysis (after hepatic uptake),<sup>19</sup> accounted for between approximately one-third and one-fifth of the upper-body FFA release in each group, with no significant differences between them. Because we did not measure visceral fat with imaging techniques in that study, however, and because we had no data on the post-prandial or insulin-suppressed condition, further studies were undertaken.

The subsequent studies disclosed similar findings. We found that upper-body/viscerally obese women had three-fold greater post-prandial FFA concentrations and FFA release compared with lower-body obese women and that the entire excess of FFA release came from upper-body subcutaneous adipose tissue.<sup>17</sup> To select a group with even greater amounts of visceral fat, we studied persons with type 2 diabetes and 14 control subjects matched for age, BMI, and proportion of body fat.<sup>20</sup> In the presence of hyper-insulinaemia and hyperglycaemia, a large and similar majority of FFA release occurred from upper-body subcutaneous fat in both the diabetic and non-diabetic subjects, even though FFA release was significantly greater in the type 2 diabetic population (*Figure 1*). The contribution of FFA from the splanchnic tissues was very small (~15%) for each group (*Figure 1*). When data from diabetic and non-diabetic subjects were combined, systemic FFA flux and visceral fat area correlated significantly (*Figure 2*). This is consistent with the previous report, but indicated that association is not causation. Visceral fat was not the source of greater FFA release despite being correlated with increased FFA release.

Although only a minority of systemic FFAs originate from the intra-abdominal depot, excess intra-abdominal



**Figure 1** FFA release from different anatomical regions. Columns show regional fractional release of palmitate during an infusion of insulin in subjects with type 2 diabetes and matched controls. Reproduced with permission from Basu *et al.*<sup>20</sup>



**Figure 2** Relationship between intra-abdominal adiposity, subcutaneous adiposity, and FFA flux: data from subjects with and without type 2 diabetes. Open circles, non-diabetic women; filled circles, diabetic women; open squares, non-diabetic men; filled squares, diabetic men. Measurements were made during an infusion of insulin as part of a euglycaemic hyper-insulinaemic clamp. Reproduced with permission from Basu *et al.*<sup>20</sup>

adiposity is clearly associated with increased FFA flux and with other metabolic complications of obesity. What might be the role of excess visceral fat in omental and mesenteric adipose tissue lipolysis if not in providing the excess systemic FFA seen in upper-body/visceral obesity? We conducted a large study, involving 68 lean and obese men and women with measures of regional FFA uptake and release as described earlier to address this issue.<sup>12</sup> Furthermore, we were able to use hepatic vein blood samples to estimate the relative contribution of visceral adipose tissue lipolysis to hepatic FFA delivery.<sup>19</sup> The results confirmed that in the post-absorptive state, the vast majority of systemic FFAs originate from subcutaneous adipose tissue in lean and obese men and women. We did find, however, that the proportion of hepatic FFA delivery that comes from visceral adipose tissue lipolysis increases as a function of visceral fat and that this is more striking in women than in men.

**Table 1** Overview of the deleterious effects of elevation of plasma FFA in key body systems involved in the regulation of metabolism and cardiometabolic risk in the setting of insulin resistance

System	Changes during FFA elevation
Liver	↑ Gluconeogenesis ↑ Hepatic glucose production in diabetes
Skeletal muscle	↑ Insulin resistance ↓ Insulin-mediated glucose disposal
Pancreas	↑ Insulin release (short-term) ↓ β-cell function (long-term)
Vasculature	↓ Endothelium-related vasodilatation ↓ Endothelial activation (pro-atherogenic)

See text for details and supporting references.

Thus, in visceral obesity, the excess systemic FFAs come from upper-body subcutaneous fat but visceral fat contributes to the exposure of the liver to even higher FFA concentrations in the portal vein.

Pathophysiological consequences of disturbed FFA metabolism

Chronically increased plasma FFA has deleterious long-term consequences for metabolism and cardiometabolic risk. Table 1 gives an overview of these adverse changes, and a summary of the most important pathophysiological effects of FFA in the liver, skeletal muscle, the pancreas, and the vasculature are described subsequently in more detail.

Liver

Increased hepatic FFA delivery induces increased gluconeogenesis in the liver.<sup>21</sup> This has been proposed to occur via several mechanisms relating to increased availability of biochemical substrates, such as ATP (energy source for gluconeogenesis and inhibitor of glycolysis at the level of phosphofructokinase), acetyl-CoA (which activates pyruvate carboxylase), NADH (involved in the conversion of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate), and citrate (also inhibits glycolysis at the level of phosphofructokinase).<sup>22</sup> This does not necessarily result in increased hepatic glucose production, because of compensatory increases in insulin release, which reduces the rate of glycogenolysis.<sup>22,23</sup> The increased rates of gluconeogenesis and hepatic glucose production may result from a failure of this 'hepatic autoregulation'. For example, a study in 44 patients with type 2 diabetes and 29 matched controls demonstrated increased gluconeogenic flux, without a compensatory decrease in the rate of glycogenolysis.<sup>24</sup> The rate of gluconeogenesis was significantly related to total body fat and intra-abdominal adiposity and to plasma FFA and glucose in a multivariate analysis adjusted for age, gender, and ethnicity. Poorly controlled diabetes, obesity *per se*, and

intra-abdominal adiposity were identified as important driving forces for the increased gluconeogenic flux observed in the diabetic group.

An increase in hepatic FFA flux has been proposed as a primary driving force for systemic insulin resistance. The 'single gateway' hypothesis of insulin action suggested by Bergman in 1999 proposed that insulin stimulates glucose uptake by muscle directly and reduces hepatic glucose through a reduction in portal FFA delivery to the liver. The reduction in hepatic FFA delivery is in turn supposed to arise from insulin-mediated suppression of lipolysis in visceral adipocytes.<sup>25</sup> Conversely, increased hepatic glucose production from excess FFA from insulin-resistant intra-abdominal adipocytes could, in theory, drive the development of insulin resistance. This could be especially important in women, in whom the increase in hepatic FFA delivery per unit of increased visceral fat area is greater than in men.<sup>12</sup> The importance of visceral lipolysis to the overall exposure of the liver to FFA as been little studied, but currently available evidence does not support the concept of isolated hepatic insulin resistance as a driver of systemic insulin resistance.<sup>14</sup> The studies described earlier have shown that visceral FFA production accounts for a minority of circulating FFA. Moreover, evidence from studies in humans suggests that increases in subcutaneous fat usually accompany increased intra-abdominal adiposity, which would tend to preserve the predominant role of subcutaneous fat as the primary source of hepatic exposure to FFA.

Finally, increased hepatic FFA delivery may underlie the development of the atherogenic lipid profile characterized by elevated triglycerides, low HDL-cholesterol, and a shift in the LDL subclass profile towards smaller, denser lipoprotein particles that is often encountered in subjects with insulin-resistant states such as the metabolic syndrome or type 2 diabetes.<sup>26</sup> According to the 'portal hypothesis', increased hepatic delivery of FFA promotes the formation of triglyceride-rich VLDL, which accounts for the observation of hypertriglyceridaemia. Lipolysis of this excess triglyceride content reduces the size and buoyancy of the lipoproteins, leading ultimately to the formation of small, dense LDL. Meanwhile, the elevated triglyceride content of the ApoB-containing lipoproteins drives an increased rate of transfer of triglycerides in exchange for cholesteryl ester via cholesteryl ester transfer protein. Lipolysis of these triglycerides in HDL reduces the size and buoyancy of these lipoproteins, in a manner similar to that described earlier for LDL. Finally, small, dense HDL is more rapidly cleared from the circulation, resulting in low circulating levels of HDL-cholesterol.<sup>27,28</sup>

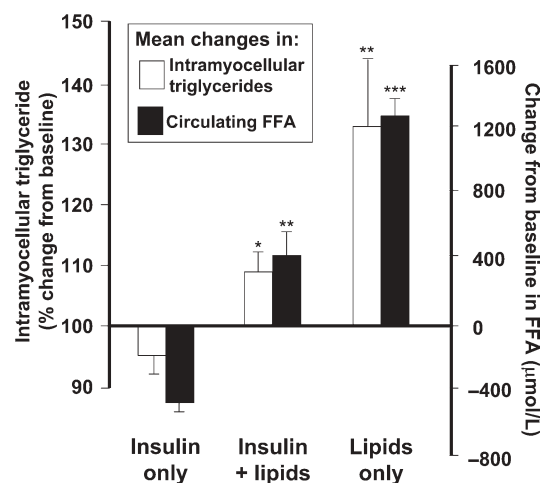
## Muscle

Skeletal muscle accounts for the largest destination for insulin-stimulated glucose disposal and may therefore be considered to be a principal determinant of systemic insulin resistance. Intracellular lipid content in skeletal muscle has been shown to correlate with insulin sensitivity measured during a euglycaemic-hyper-insulinaemic glucose clamp in a study in non-obese, non-diabetic

individuals.<sup>29</sup> A further study of 20 healthy individuals showed that subjects with higher intramyocellular lipid content had decreased insulin receptor autophosphorylation and decreased signalling via IRS-1 and PI-3-kinase, suggesting that the efficiency of the post-insulin receptor tyrosine kinase cascade was impaired in subjects with higher intramyocellular lipid.<sup>30</sup>

A number of other studies have demonstrated that accumulation of intramyocellular triglycerides accompanies the development of insulin resistance in pre-diabetic and/or obese adolescents,<sup>31,32</sup> type 1 or type 2 diabetic patients,<sup>33,34</sup> women with prior gestational diabetes,<sup>35</sup> and offspring of type 2 diabetes patients.<sup>33,36</sup> Dietary intervention has been shown to deplete intramyocellular lipid stores in a selective manner in severely obese subjects (mean BMI 49 kg/m<sup>2</sup>), resulting in improved insulin sensitivity.<sup>37</sup> It should be noted that a significant association between intramyocellular lipid and insulin resistance does not occur in all populations, however; for example, no such correlation has been found in South Asians.<sup>38,39</sup>

In man, acute infusion of FFA induces insulin resistance characterized by defects in decreased glycogen synthesis and, to a lesser extent, decreased glucose oxidation, within several hours.<sup>40</sup> There is strong evidence from healthy human subjects that plasma FFAs are an important determinant of intramyocellular lipid content.<sup>41</sup> Three evaluations, each of 4-h duration, were undertaken: a euglycaemic-hyper-insulinaemic clamp (i) without and (ii) with simultaneous infusion of lipids and (iii) an infusion of lipids alone. Changes in plasma FFA closely paralleled changes in intramyocellular lipid content under these three conditions (Figure 3). Overall, a highly significant correlation was observed between intramyocellular lipid content and plasma FFA ( $r = 0.74$ ,  $P < 0.0003$ ). Extramyocellular lipid



**Figure 3** Relationship between changes in plasma FFAs and changes in intramyocellular lipid content in healthy volunteers during a euglycaemic-hyper-insulinaemic glucose clamp with and without infusion of lipids or during infusion of lipids without insulin. \* $P < 0.05$ ; \*\* $P < 0.02$ ; \*\*\* $P < 0.0001$  vs. baseline. Bars represent SEM. Copyright© 2001 American Diabetes Association from Diabetes 2001;50:1612-1617. Modified with permission from the American Diabetes Association.



content did not change between the three experimental situations.

The closely paralleled time courses of changes in FFA and lipid accumulation in skeletal muscle in this study lend support to the hypothesis that these phenomena are interrelated at a mechanistic level and are an important component of the pathophysiology of insulin resistance in this tissue. Recent evaluations have implicated a relative inability of muscle to switch between glucose metabolism and fat oxidation in the pathogenesis of insulin resistance.<sup>42</sup>

## Pancreas

Long-term exposure to FFA is toxic to the  $\beta$ -cells of the pancreas. We have known for decades that acute exposure of the pancreas to FFA increases insulin secretion.<sup>43</sup> More recently, it has been shown that chronic exposure of pancreatic tissues to elevated FFA causes an impairment of insulin secretion *in vitro*<sup>44</sup> and *in vivo*.<sup>45</sup> The development of hyperglycaemia in the Zucker rat is preceded by marked increases in FFA levels and an apparently associated accumulation of triglycerides to a level 10-fold higher than normal within the pancreatic tissues.<sup>45,46</sup> The secretory defect following prolonged exposure to elevated FFA levels is characterized by a loss of glucose sensing by the  $\beta$ -cells.<sup>45,47</sup>

The mechanism of FFA-induced impairment of  $\beta$ -cell function is under intense study. Increased apoptosis through pathway involving caspase and ceramide has been proposed as a mechanism underlying these effects.<sup>48</sup> The pancreata of patients with poorly controlled type 2 diabetes are exposed to long-term hyperglycaemia as well as elevated levels of FFA, in many cases. Hyperglycaemia and elevated FFA may act synergistically in causing damage to the  $\beta$ -cell, as hyperglycaemia inhibits fat oxidation, a potential means of detoxifying lipids accumulating within the  $\beta$ -cell.<sup>49</sup>

FFA-induced impairment of  $\beta$ -cell function is likely to be of clinical importance. Type 2 diabetes is usually diagnosed when progressive  $\beta$ -cell dysfunction renders the pancreas unable to maintain the level of hyperinsulinaemia required to control glycaemia in the face of a relatively constant level of insulin resistance. The deleterious effects of FFA on pancreatic function may therefore provide sufficient stimulus to push many patients from a state of glucose intolerance maintained by hyperinsulinaemia to clinical type 2 diabetes mellitus.

## Vasculature

Endothelial dysfunction is an early event in atherogenesis. Initially, the ability of the endothelium to generate vasodilator substances, such as nitric oxide or prostacyclin, is impaired, with a consequent shift towards vasoconstriction and hypertension. In addition, the damaged endothelium expresses adhesion molecules which facilitate the entry of inflammatory cells into the arterial intima. These go on to differentiate into macrophages and not only take up lipids to become foam cells but

also secrete numerous bioactive substances that attract more inflammatory cells and promote deleterious arterial remodelling.

Elevations in FFA levels promote endothelial dysfunction, and studies in humans have demonstrated impaired responses to endothelium-dependent vasodilators in subjects subjected to lipid infusions.<sup>50,51</sup> One study in 16 lean and 12 obese women explored this phenomenon by increasing FFA levels acutely in the lean group with an intralipid infusion (obese women received saline) and by reducing FFA levels in the obese group by the administration of acipimox, an inhibitor of lipolysis.<sup>51</sup> Reducing FFA levels improved the recruitment of capillaries and endothelium-dependent vasodilator response to acetylcholine, whereas increasing FFA levels impaired these parameters. Experimental studies in cultured cells have implicated a number of mechanisms consistent with these findings, including reduced synthesis of nitric oxide or prostacyclin,<sup>52–54</sup> induction of IL-8 synthesis,<sup>55</sup> endothelial apoptosis,<sup>56</sup> reduced proliferation of endothelial cells,<sup>56,57</sup> increased expression of endothelial adhesion molecules,<sup>58,59</sup> generation of oxidative free radicals,<sup>58</sup> and triglyceride accumulation within endothelial cells.<sup>60</sup>

## Discussion

Adipose tissue is biologically highly active and undertakes a range of metabolic and endocrine functions. FFAs released from adipocytes provide the body's main source of fuel in the post-absorptive state. Although the majority of circulating FFAs arise from outside the intra-abdominal region, FFAs from this source serve as a marker for insulin resistance and associated increases in cardiometabolic risk.

Although the liver's function of glucose production and VLDL synthesis and secretion may be driven to a significant extent by visceral adipose tissue lipolysis, the effects of FFA on muscle, pancreatic  $\beta$ -cells, and the vasculature must be primarily related to upper-body subcutaneous fat. In addition, adipocytes secrete a range of bioactive substances ('adipokines') which have the potential to influence metabolism and cardiometabolic function directly. The extent of the contributions of individual adipokines is unclear, although increased secretion of pro-inflammatory mediators and decreased secretion of adiponectin from intra-abdominal adipocytes have been strongly associated with an increased risk of adverse cardiovascular outcomes.<sup>61</sup>

The evidence discussed earlier shows clearly that most circulating FFA arise from upper-body subcutaneous adipose tissue. Interventions that reduce body weight in overweight or obese subjects are known to effectively reduce circulating FFA concentrations, especially when plasma insulin concentrations are increased.<sup>62</sup> Such interventions will also improve intra-abdominal adiposity, with the potential for a further improvement in insulin resistance and tendency to normalize FFA and secretion of adipokines.<sup>63</sup> However, given the clear mechanistic links between excess FFA production and adverse metabolic changes discussed earlier, it is reasonable to

suppose that improving FFA metabolism alone may improve the overall cardiometabolic risk profile, even where clinically significant weight loss is not achieved.

## Conclusions

Increased FFA production is associated with multiple adverse cardiometabolic changes, especially in the setting of abdominal obesity and insulin resistance, including promotion of insulin resistance, dyslipidaemia, and impaired  $\beta$ -cell function. These adverse changes are consistent with increased risk of adverse cardiovascular outcomes and an increased risk of developing type 2 diabetes. Therapies that normalize FFA secretion may improve cardiometabolic risk by mechanisms related to and/or independent of weight loss.

**Conflict of interest:** none declared.

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