**Ectopic Fat: the true culprit linking obesity and cardiovascular disease?**

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“Ectopic Fat: the true culprit linking obesity and cardiovascular disease?”

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Abstract

Obesity is a major risk factor for cardiovascular disease (CVD) and its complications. However, not all fat depots share the same characteristics. Recent studies have found ectopic fat accumulation, rather than subcutaneous fat, associated with increased cardiometabolic risk. However, ectopic fat accumulation can be seen initially as a protective mechanism against lipotoxicity and only when the adipose tissue becomes dysfunctional it is becoming responsible for metabolic alterations either systemic (through release of cytokines) or by altering specific organ functions. The purpose of this review is to summarize the current available data on the impact of excess adiposity vs. ectopic fat in the development of cardio-metabolic diseases.

Introduction

It is recognized that obesity is a major risk factor for cardiovascular disease (CVD), in particular coronary heart disease (CHD) and stroke, but also for systemic hypertension, metabolic dyslipidemia, inflammation, and thrombosis (1-4). These results prompted the American Heart Association to considered obesity as a major CVD risk factor in 1998 (5). Furthermore, obesity has been shown to be associated with the development of type 2 diabetes (T2DM), with excess adiposity being a key contributor to the development of insulin resistance through the increased release of free fatty acids (FFA) and the development of lipotoxicity (6-8). With the advance of imaging techniques it has been shown that ectopic fat can accumulate in organs such as liver, heart, muscle and pancreas impairing organ function, and also releasing factors that can increase cardiovascular risk (8, 9). Thus, it has been suggested that not general obesity per se but ectopic fat accumulation was responsible for increased cardiometabolic risk.

In the following paragraphs we have reviewed the current available data to understand the impact of excess adiposity vs. ectopic fat in the development of CVD.

Sites of adipose tissue accumulation

Adipose tissue accumulates predominantly as subcutaneous (SAT), visceral fat (VAT) and intrathoracic fat (10-12). SAT forms a fat layer under the skin, the visceral adipose tissue surrounds inner organs in the abdominal cavity while the intrathoracic fat is accumulating around the heart as epicardial or mediastinal fat (10, 11). Moreover, ectopic fat can accumulate inside organs as liver, heart, pancreas and muscle, altering their metabolic activity (9, 11, 12). Many studies have shown that it is not the total amount of fat but rather its distribution that increases the risk of CVD and that excess abdominal fat (measured as increased waist circumference, WC) is a CVD risk factor stronger than BMI (13-30).

This also explains, at least in part, why men, that are more prone to accumulate abdominal fat, are at higher risk than women to develop CVD. This observation was initially made by GB Morgagni who observed
more than 250 years ago, during an autopsy, the accumulation of fat as visceral and mediastinal adipose tissue, and described the association between visceral obesity, hypertension, hyperuricemia, atherosclerosis and obstructive sleep apnea syndrome, long before the modern recognition of the metabolic syndrome (31). Several years later, in 1947, a French physician Jean Vague, reported that his obese patients with diabetes and clinical signs of CVD had a central distribution of body fat (referred as male-type or android obesity), whereas he suggested that the typical female body fat pattern of lower fat accumulation (he introduced the term “gynoid” obesity) was rarely associated with complications and more frequently found in premenopausal women (31, 32). He concluded that the common complications of obesity, such as insulin resistance, atherogenic dyslipidemia, T2DM, and CVD, were more closely related to the distribution of body fat than to the absolute degree of fatness per se.

Since then, the advance in imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) have increased the ability to precisely and reliably quantify individual differences in body fat distribution. Using these techniques, the substantial variation in regional fat accumulation at any BMI value has been documented, showing that the ability to store fat in various adipose tissue compartments could markedly differ from one individual to another (33). Moreover, using MR spectroscopy it has been shown that fat can also accumulate in ectopic sites such as liver, pancreas and heart (Figure 1) causing lipotoxicity and derangement in organ metabolism possibly increasing cardiometabolic risk (8).

The metabolically healthy obese (MHO) vs normal weight but metabolically obese subjects (NWMO).

The adipose tissue is now recognized as an endocrine organ with an important role in the regulation of glucose metabolism, lipolysis and FFA release in the peripheral circulation and also as a source of pro- and anti-inflammatory markers. However, it is only when the adipose tissue becomes dysfunctional that obesity is associated with the development of disease.

Not all obese subjects are at risk of cardio-metabolic disease and not all lean subjects are free of risk. Two particular phenotypes are representative of this phenomenon: the metabolically healthy obese subjects (MHO) and the subjects with normal weight but metabolically obese (NWMO). Several studies reported data on metabolically ‘healthy’ obese (MHO) individuals that seem to be protected against metabolic and cardiovascular obesity comorbidities and often display absence of dyslipidemia, hypertension, T2D and lower intima media thickness of the carotid artery than the majority of metabolically ‘unhealthy’ obese patients (34-37). It has been estimated that over 30% of obese patients are metabolically healthy, have normal insulin sensitivity and do not show any criterion of metabolic syndrome, according to the IDF criteria, excluding waist circumference (34, 38). Data from the RISC study have shown that even when free from metabolic syndrome and with normal glucose tolerance, overweight/obese subjects have a less favorable cardiometabolic profile, characterized by a higher total and LDL-cholesterol value, lower HDL, increased values of high-sensitivity C-reactive protein and blood pressure, as well as thicker intima media of
common carotid segment. This suggests a potentially susceptibility to CVD (38). However, in long term prospective studies (7-15y) MHO were not found at increased risk of CVD and all-cause mortality (39, 40). It has not been clarified whether healthy obese individuals can maintain insulin sensitivity during the entire life or whether healthy obesity simply represents delayed onset of obesity related insulin resistance (41).

Several normal weight individuals are characterized by hyperinsulinemia, hyperglycemia, insulin resistance (IR), impaired glucose tolerance (IGT), hypercholesterolemia and hypertriglyceridemia, and are therefore ‘metabolically obese’ (NWMO) (42). These characteristics in lean individuals mark a departure from common human patterns in which metabolic disease is a consequence of weight gain. These phenotypes are very prevalent. The analysis of the Framingham study have shown that over 40% of men and women had increased VAT, despite an average BMI of 27 kg/m2 in women and 28 kg/m2 in men (43). Metabolic abnormalities can be observed also in non-obese subjects with ectopic fat accumulation, such as non-alcoholic fatty liver disease, characterized by hepatic steatosis (44) or in lean women with Polycystic Ovary Syndrome (PCOS) (45). Elevated risk for CVD is common in subjects NWMO (46), as well as elevated risk for hypertension, T2D and other metabolic complications and it has been recently reported that normal weight adults at the time of diagnosis of diabetes have higher cardiovascular and non-cardiovascular mortality than adults who were overweight or obese (47).

When the “adipose organ” becomes dysfunctional

The adipose tissue is a dynamic organ where new adipocytes are formed and old adipocytes change in size and metabolism. It is only when the adipose tissue becomes dysfunctional, with infiltration of chronic inflammatory cells like macrophage and lymphocytes that it releases inflammatory factors and shows alteration in lipid metabolism, contributing to the development of insulin resistance, endothelial dysfunction and in general to the increase in cardiometabolic risk.

Failure to make new adipocytes and enlarged adipocyte size is considered a marker of adipocyte dysfunction (48). Human studies of adipose tissue morphology have shown that we have on average of between 20 to 40 billion mature adipocytes but this number can be doubled or even tripled in massively obese patients depending upon their adipose tissue mass (49-51). Human adipocytes can grow up to ~20 fold in diameter and several thousand-fold in volume (52). Studies that have examined the relationship between adipose cell size and number have generally reported that large subcutaneous fat cells are associated with metabolic derangements possibly due to hypoxia whereas adipose cell number does not appear, per se, to be detrimental to cardiometabolic health in the presence of normal size adipocytes (53). In non obese subjects lower insulin sensitivity is correlated with large abdominal adipocytes, increased adipokine release and lower GLUT-4 expression, (54).

Initially, it was believed that subcutaneous fat cells themselves could contribute to the dysmetabolic state of obesity until it was found that excess visceral fat is more closely related to metabolic complications than
subcutaneous fat cell size. The reason for this had remained unclear until the hypothesis was put forward that excess visceral adiposity may rather be a marker of the relative inability of the subcutaneous adipose tissue to expand through hyperplasia in the face of a positive energy balance (29). An hypothesis recently confirmed in a work studying the effect of overfeeding (55). The study has shown that not all subjects increase visceral fat (VF) but only those with a defective gene expression in the regulation of lipid-storing genes in subcutaneous adipose tissue (SC) (55). Although ectopic fat accumulation was not measured in this study, this supports the hypothesis that in presence of lipid overflow and impairment in SC tissue to store it, this accumulates in other tissues. Under conditions of fat overflow (56) SC tissue would enlarge adipocytes, that would secrete pro-inflammatory adipokines, reduce secretion of anti-inflammatory and insulin sensitizing cytokine, e.g., adiponectin, and would become prone to apoptosis (57-59). As a consequence, such hypertrophic adipose depots may become invaded by macrophages and a vicious cycle would develop with a harmful cross-talk of deleterious secretory products between the macrophages and the enlarged fat cells (57-59).

Some of the adipokines, like TNF-α and IL-6, impair adipocyte differentiation, reduce lipid accumulation, and increase adipocyte lipolysis others interfere with insulin signalling. Hypertrophic adipocytes are not only stressed, but also display reduced ability to take up and release free fatty acids (FFAs). This induces a redirection of lipids towards peripheral tissues, including skeletal muscle, liver, pancreas, and heart, causing ectopic fat deposition (Figure 1). If in these tissues, lipid supply exceeds oxidative capacity intracellular lipid accumulation occurs, causing lipotoxicity and impairment in organ function (Figure 1).

Ectopic fat and CVD

A key unanswered question is the respective contribution of the various ectopic fat depots, including the expanded visceral adipose tissue, to cardiometabolic risk. It is well established that ectopic fat accumulation is not preferentially located in one organ but it is often found simultaneously in several organs, among which liver, heart, muscle and pancreas (12, 60, 61). Ectopic fat in muscle and pancreas are possibly related to peripheral insulin resistance and impaired beta cell function that are risk factors for the development of T2DM. However, there are no evidences that ectopic fat could be directly implicated in the increase CVD risk. On the other hand ectopic fat in heart and liver seems to have a direct role in CVD risk as well as the fat accumulated around these organs, i.e., visceral and epicardial fat. Below we are reviewing the current knowledge.

Epicardial and Intra-Myocardial fat and CVD

Around 80% of the heart is surrounded by epicardial fat which accounts 20% of total heart weight (11, 62, 63). If epicardial fat is contributing to increase cardiometabolic risk is still controversial (64). Several cross-sectional studies have suggested a positive relationship between increased epicardial fat volume and
coronary artery disease (65-68), myocardial infarction (69), systemic inflammation (70), reduced cardiac energy metabolism (71), insulin resistance in obese nondiabetic patients (72) and in Chinese T2DM subjects (73, 74). In the studies where epicardial fat was found associated with CVD also mediastinal fat was associated with CVD (64), and the increase in epicardial fat is mirrored by the increase in mediastinal as well as total fat (11, 63).

As demonstrated in animal models, epicardial fat might protect the myocardium from high circulating fatty acid levels providing fatty acids as a direct energy source (11, 75, 76). Moreover, epicardial fat might have a mechanic function by surrounding coronary arteries and reducing artery shear stress (11). Epicardial fat compartment could act as a metabolically active organ by secreting cytokines (70), so it is possible that only when in subjects where it has become dysfunctional it is associated with cardiometabolic risk, as in patients with established disease (11). However, imaging studies can only measure the size of fat accumulation and not the metabolic status.

Different is the ectopic fact accumulated inside cardiomyocytes as intramyocardial triglyceride, IM-TG (64). Patients with IGT and T2DM have increased myocardial TG content compared to obese and lean controls (77-79) showing that insulin resistance can favour intramyocardial fat accumulation. High rate conversion of fatty acids in myocardial TG is associated with intermediates (e.g.,ceramide) release which is associated with cardiac dysfunction in animal models (80, 81). In T2DM patients, increased TG myocardial content was associated with impaired left ventricular diastolic function (81).

**Hepatic fat and CVD**

Subjects with non alcoholic fatty liver disease (NAFLD) have an increased cardiometabolic risk (82-84). The real prevalence of CV events in patients with NAFLD is still not known and probably underestimated. NAFLD is often not diagnosed since in the great majority of NAFLD subjects hepatic enzymes are within normal ranges and ultrasound technique is unable to detect NAFLD when fat infiltration is below 30% or (85, 86). In general the cardio-metabolic risk is increased in subjects with NAFLD, even if they do not have the metabolic syndrome and they are at low risk for CVD (84). If hepatic fat plays a direct role in the development of CVD is however still controversial.

Some factors that can explain the increased CVD risk in subjects with NAFLD are the increased lipolysis and VLDL secretion (44, 87), the atherogenic lipoprotein profile (19), but also hyperglycemia due to hepatic overproduction of glucose. Fat accumulation in the liver and oxidative stress induce the secretion of inflammatory markers such as fibrinogen and C reactive Protein (CRP), IL-6, IGF-1, TNF-α, Fetuin-A, (44, 88, 89). All these metabolic abnormalities, common in subjects with NAFLD, have been shown to directly or indirectly promote atherosclerosis as confirmed by studies that showed increased IMT and coronary atherosclerosis (83, 84, 88, 90, 91). NAFLD has been found also associated with endothelial dysfunction and coronary artery disease (84, 90, 92-94).
Visceral fat and CVD

An excess of intra-abdominal or VF has been reported to be associated with a constellation of metabolic abnormalities including insulin resistance, hypertension, hyperinsulinemia, glucose intolerance, type 2 diabetes mellitus, atherogenic high triglyceride. Moreover, subjects with VF have increased serum concentration of small dense low-density lipoprotein, low high-density lipoprotein, dyslipidemia, inflammation, altered cytokine profile, impaired fibrinolysis, and increased risk of thrombosis, and endothelial dysfunction (29, 95-104). In patients with type 2 diabetes mellitus, plasma lipoprotein levels (105) and inflammatory markers (106) are strictly correlated with visceral adiposity that was independent from metabolic control. In addition VF accumulation has been correlated with hepatic and cardiac fat and similarly predicted metabolic and cardiovascular alterations (63, 68, 89, 104, 107). Recent large cohort studies such as the Framingham Heart Study and the Jackson Heart Study that have extensively used CT, have shown that excess visceral adiposity (along with other markers of excess ectopic fat deposition such as increased cardiac, hepatic and intrathoracic fat) is significantly correlated with various cardiometabolic abnormalities in a manner that is independent of the amount of subcutaneous fat (68, 107-112). However, these results should be interpreted with caution since none of these studies was designed to answer this question and correlations do not prove causation. Moreover, none of the above described abnormalities (e.g., insulin resistance, hyperlipidemia, increased risk of thrombosis, endothelial dysfunction) can be directly related to excess visceral fat. On the other hand VF could secrete adipokines that can impair the metabolic signalling, at least in part contribute to hyperlipidemia through hepatic lipid overflow and explaining its association with increased CVD risk (60).

Ectopic fat reduction: a therapeutical target?

Reducing CVD risk by weight loss and/or increasing energy expenditure is still a matter of discussion. Despite the variety of weight loss methods, it remains unclear whether relative rates of change in ectopic fat and/or VAT can be specifically manipulated and in a long term. A recent study with

Lifestyle intervention: caloric restriction

Caloric restriction, as low (LCD) or very low caloric diet (VLCD, < 800 kcal/die), is effective in decreasing both total and ectopic fat and in improving cardiometabolic profile (113). All ectopic fat depots have been shown to be decreased with weight loss. In particular hepatic and visceral fat have been shown to decrease more in the early weeks of diet and percent weight loss was inversely associated to the relative ratio of percent changes in VF vs SC fat (113). Weight loss was also associated with decrease in hepatic fat and both changes in visceral and hepatic fat correlated with the improvement in peripheral and hepatic insulin resistance (114). However, subjects with high VAT and LF have a lower chance of profiting from lifestyle
intervention and may require intensified lifestyle prevention strategies or even pharmacological approaches to improve insulin sensitivity (115). Diet-induced weight loss was associated with a significant decrease in epicardial fat thickness measured over the right ventricle wall by echocardiography (11, 12, 116). Moreover, the change in diastolic function was also positively correlated with the change in epicardial fat thickness (116). The effects of diet on pericardial fat and other TG stores in obese T2DM patients treated for 16-weeks with VLCD followed by 14 months regular diet were evaluated by MR imaging and spectroscopy. Loss of visceral and epicardial fat were more pronounced as compared to subcutaneous fat during intervention study, but VF was in part regained during 14 months follow-up period. Surprisingly, epicardial fat was the only fat compartment that did not expand during the additional 14 months follow-up (117). Weight loss was also associated to a decrease in intramyocardial TG. Obese patients with T2DM following for 16-week a VLCD (450 kcal/day) had a significant decrease in BMI (from 35.6 ± 1.2 to 27.5 ± 1.3 kg/m²) associated with a significant decrease in myocardial TG content (from 0.88 ± 0.12% to 0.64 ± 0.14%) and an improvement in left ventricular diastolic function (118).

Several studies have shown that weight loss decreases hepatic TG content in obese and T2DM subjects (62, 115, 119, 120). Studies measuring both hepatic TG content and hepatic insulin sensitivity with a hyperinsulinaemic euglycaemic clamp in obese NGT and T2DM patients, found an improvement in hepatic insulin sensitivity that was associated with the decrease in intrahepatic lipids (115, 120).

**Lifestyle intervention: physical exercise**

The effects of exercise per se on ectopic fat volume and distribution are still not well established. After a 6-week aerobic exercise program (60–85% of VO2max for a minimum of 20 min at least three times per week), despite no significant effects on body weight and hepatic TG content as measured by 1H-MRS were found, both peripheral and hepatic insulin sensitivity (measured by the hyperinsulinaemic euglycaemic clamp) improved (121). On the contrary, in obese NGT subjects, a one-month aerobic exercise training (3 times/week, 3–45/min at max 70%VO2max) decreased hepatic TG concentration by 21% (121). In general if exercise was not accompanied by weight loss there was little decrease in liver fat but no improvement in liver metabolism (122).

In a 3 months exercise program in obese NGT middle-aged Japanese men, BMI decreased by 4.3 ± 3.0% (circa −1 kg/m²) and VO2max increased by 20% leading to a change in visceral adipose tissue (−15%) that was significantly correlated with the change in epicardial adipose tissue (−8.6%) (123). This findings were confirmed in a trial in which 32 obese postmenopausal women were randomized to diet-only or diet combined with moderate or intensive exercise for 20 weeks. The three groups had similar reduction in bodyweight (-15%) and pericardial fat (-17%) that were not affected by the type of intervention [100].
Bariatric surgery

Bariatric surgery (BS) has been demonstrated to induce important weight loss in obese patients and to reduce cardiovascular mortality and cardiovascular events by 50% in patients with severe obesity (124, 125). BS is associated with decrease in all fat depots and a drastic reduction in biological cardiovascular risk markers, such as inflammatory and insulin resistance parameters (126, 127). Moreover, already after 6 months it is possible to observe an improvement in cardiac function, particularly diastolic function. In general all fat depots decreased but the reduction in epicardial fat volume (EFV) was independent of the reduction in body mass index and VF, while no change was observed in intramyocardial fat (128). Similarly BS decreased also hepatic fat often improving liver histology and resolving NASH (129). In general after bariatric surgery improves the entire cardiometabolic profile with decrease in serum transaminases, γ-glutamyltransferase, glucose, insulin and triglycerides.

Conclusions

Ectopic fat may be assimilated to an endocrine organ able to secrete a number of active substances. Data support its role as a relevant link between risk and clinical conditions such as metabolic syndrome, diabetes, hypertension and cardiovascular diseases. Only when the adipose tissue becomes dysfunctional and mitochondrial oxidation is impaired, reactive oxygen species (ROS) are produced, cytokines released and ectopic fat becomes responsible for impairment in organ metabolism and implicated in the development of CVD. However, this relationship remains to be fully elucidated in properly designed clinical trials. Imaging techniques show an accurate diagnostic yield, but their clinical use is not standardized and clinical algorithms do not envision fat assessment for risk stratification. Ectopic fat may become the target for therapeutic interventions, aimed at its reduction to counteract its dysfunctional activities. It is conceivable that ectopic fat becomes the diagnostic target in metabolic disorders shifting the focus from quantity (obesity) to quality (dysfunctional fat).

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Conflict of Interest

The authors declare no conflict of interest for this work.
**FIGURE LEGEND**

Figure 1.

Adipocyte enlargement is more prone to macrophage infiltration leading to adipokine release, inflammation, impaired lipolysis and lipogenesis.

This results in adipocyte insulin resistance with fatty acid overflow, inflammatory processes and eventually, adipocyte necrosis. These events send “signals” (release of adipokines, hormones, other unknown factors) that locally induce inflammation, recruit macrophages, and increase ectopic fat accumulation leading to lipotoxicity-induced metabolic dysfunction that results in reduced mitochondrial activity in all tissues, excessive HGP and VLDL production, reduced hepatic insulin clearance and beta cell function, endothelial dysfunction, atherosclerosis, and plaque formation.

**Abbreviations:**

FFA (Free Fatty Acid), HGP (Hepatic Glucose Production), VLDL (Very Low Density Lipoprotein), NAFLD (Non-alcoholic fatty liver disease), VO$_2$ max (maximal oxygen consumption)

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