

Adipose Tissue Dysfunction: Clinical Relevance and Diagnostic Possibilities

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Key words

- adipose tissue
- dysfunction
- diagnosis
- metabolic syndrome

Abstract

Adipose tissue dysfunction is defined as an imbalance between pro- and anti-inflammatory adipokines, causing insulin resistance, systemic low-grade inflammation, hypercoagulability, and elevated blood pressure. These can lead to cardiovascular disease and diabetes mellitus type 2. Although quantity of adipose tissue is an important determinant of adipose tissue dysfunction, it can be diagnosed in both obese and lean individuals. This implies that not only quantity of adipose

tissue should be used as a measure for adipose tissue dysfunction. Instead, focus should be on measuring quality of adipose tissue, which can be done with diagnostic modalities ranging from anthropometric measurements to tissue biopsies and advanced imaging techniques. In daily clinical practice, high quantity of visceral adipose tissue (reflected in high waist circumference or adipose tissue imaging), insulin resistance, or presence of the metabolic syndrome are easy and low-cost diagnostic modalities to evaluate presence or absence of adipose tissue dysfunction.

Abbreviations

BMI:	Body mass index
DM2:	Diabetes mellitus type 2
ATD:	Adipose tissue dysfunction
FFA:	Free fatty acids
TNF- α :	Tumor necrosis factor alpha
IL-6:	Interleukin 6
PAI-1:	Plasminogen activator inhibitor 1
WC:	Waist circumference
IL-8:	Interleukin 8
IL-18:	Interleukin 18
VAT:	Visceral adipose tissue
CT:	Computed tomography
MRI:	Magnetic resonance imaging
SAT:	Subcutaneous adipose tissue
RBP-4:	Retinol binding protein 4
TG:	Triglycerides
PUFA:	Poly unsaturated fatty acids
TUFA:	Total unsaturated fatty acids
HOMA-IR:	Homeostatic model of assessment-insulin resistance
SFRP-5:	Secreted frizzled related protein 5
HGF:	Hepatic growth factor
IP-10:	Interferon gamma induced protein 10
MCP-1:	Monocyte chemo attractant protein 1
ELISA:	Enzyme linked immunosorbent assay

1. Introduction

Worldwide, an estimated 1 billion people are overweight (defined as a BMI > 25 kg/m²). Another 500 million are obese (BMI > 30 kg/m²) [1]. A BMI above 25 kg/m² is associated with a 30% increase in overall mortality, a 40% increase in vascular mortality, and a 120% increase in mortality due to complications of diabetes as compared to a BMI \leq 25 kg/m². It is estimated that 1 in every 7 cases of cardiovascular disease is attributable to overweight and 8 in every 10 cases of incident type 2 diabetes mellitus (DM2) [2,3]. Moreover, a high BMI is associated with an increased incidence of multiple cancer types [4,5]. On the other hand, BMI is an imperfect measure to estimate the contribution of adiposity to future disease risk and mortality [6], since not the quantity of adipose tissue itself is the causal factor in the occurrence of cardiovascular diseases, DM2, and cancer, but the metabolic consequences of adiposity as a result of adipose tissue dysfunction (ATD). Insulin resistance, hypertriglyceridemia, low HDL-cholesterol, hypertension, hypercoagulability, and low-grade inflammation are metabolic risk factors related to ATD [7–9]. Obese patients who are metabolically healthy do not exhibit unfavorable metabolic changes [10], are not insulin resistant [11], and have a low risk of

received 01.08.2015

accepted 11.02.2016

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DOI <http://dx.doi.org/10.1055/s-0042-103243>
Horm Metab Res 2016;
48: 213–225
© Georg Thieme Verlag KG
Stuttgart · New York
ISSN 0018-5043

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developing cardiovascular diseases [9], indicating that not only adipose tissue quantity matters, but also adipose tissue function. This concept of ATD is signified by patients who are metabolically obese despite a normal weight [12, 13]. These patients have an increased risk for DM2 and cardiovascular disease. The diagnosis or identification of ATD may therefore be of clinical relevance serving as a tool for stratifying risk for cardiovascular diseases, DM2, and even cancer, and may guide preventive treatment with both medication and lifestyle interventions [14]. ATD may even serve as a direct treatment target [15]. This would be in contrast to a more general approach with the focus on adipose tissue quantity reflected by overweight and obesity as measured with BMI.

In this review, we evaluate current evidence of different options for diagnosing ATD, ranging from anthropometric measurements to tissue biopsies and advanced imaging techniques. In the absence of the possibility of a direct diagnosis of ATD, we use consequences of ATD as surrogate indication of the presence of ATD.

2. Adipose Tissue Dysfunction

When total energy intake exceeds energy expenditure, this excess energy is stored in adipose tissue leading to enlargement of adipocytes. As a consequence, hypertrophic adipocytes produce chemotactic adipokines, which attract macrophages to adipose tissue [16, 17]. Inflamed adipose tissue is able to produce large amounts of free fatty acids (FFA) and pro-inflammatory adipokines, such as tumor necrosis factor alpha (TNF- α), leptin, chemerin, and interleukin-6 (IL-6), whereas the production of the protective adipokine adiponectin is reduced [18–20]. ATD refers to the imbalanced production and release of pro- and anti-inflammatory adipokines. The systemic metabolic consequences of ATD include systemic low-grade inflammation, hypercoagulability, elevated blood pressure, dyslipidemia, and insulin resistance. Insulin resistance occurs as a result of interference with the intracellular insulin signaling cascade by TNF- α and FFA in various target organs [18]. Part of these systemic metabolic consequences are clustered in the metabolic syndrome, which is defined as the presence of ≥ 3 of the following items: waist circumference > 102 cm (men) or > 88 cm (women), triglycerides ≥ 1.7 mmol/l, HDL-c < 1.03 mmol/l (men) or < 1.29 mmol/l (women) or use of lipid-lowering medication, systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg or use of blood pressure-lowering medication, fasting plasma glucose ≥ 5.6 mmol/l or use of glucose-lowering medication [21, 22].

Although obesity is the most important driver of ATD development, not all, but approximately 80% of obese individuals become insulin resistant [10]. Moreover, 10–40% of nonobese individuals develop insulin resistance [12, 13, 23], indicating that other factors are also involved in the development of ATD. Both exogenous factors such as physical inactivity [24, 25] and the dietary intake of saturated fat [26, 27], as well as endogenous susceptibility such as a low birth-weight [28, 29], genetic predisposition [30, 31], and an overactive sympathetic nervous system [32, 33] may all contribute to the development of ATD.

Dysfunctional adipose tissue contributes to the development of diabetes mellitus by causing insulin resistance [34] and through cytotoxic effects of pro-inflammatory adipokines and free fatty acids on pancreatic beta cells, leading to diminished insulin pro-

duction (● Fig. 1) [35]. There are various pathophysiological mechanisms linking ATD to atherosclerotic vascular diseases, including systemic low-grade inflammation by production of IL-6 by adipose tissue, a procoagulant state as a result of plasminogen activator inhibitor-1 (PAI-1) production, direct effects of adipokines on the endothelium, activation of the renin-angiotensin-aldosterone system by adipose tissue production of angiotensinogen, and activation of the sympathetic nervous system possibly due to high levels of insulin, leptin, and angiotensin II centrally exerting a sympatho-excitatory response [36–38]. Diagnosing ATD may identify patients at high risk for the development of diabetes mellitus and vascular diseases and may guide preventive measures in an early stage. Potential diagnostic tools for identifying patients with ATD are outlined below. The diagnostic value will be evaluated in the context of pathophysiological characteristics (morphologic changes in adipose tissue and plasma adipokine concentrations), as well as to clinical outcome of ATD (metabolic syndrome, DM2, and cardiovascular diseases).

3. Anthropometric Measurements

There is a strong relation between the quantity of adipose tissue and ATD [39–41]. Adipose tissue quantity, as measured with either BMI or waist circumference (WC) is related to plasma concentrations of adipokines, to morphologic characteristics of adipose tissue, and to the development of the metabolic syndrome, DM2, and vascular diseases [39–41]. Pro-inflammatory adipokines (IL-6, IL-8, IL-18, TNF- α , PAI1, and leptin) are known to be positively correlated with both BMI and WC [42–45], whereas the anti-inflammatory adiponectin is negatively correlated with BMI and WC [42–46]. In subcutaneous adipose tissue biopsies, IL-6 and IL-8 expression are associated with waist circumference [47]. Moreover, both BMI and WC are associated with the amount of macrophages in both subcutaneous and omental adipose tissue [48], and with adipocyte size [48].

The relation between adipose tissue quantity, measured with BMI and WC, and metabolic disturbances is illustrated by the fact that only 5% of normal weight individuals (BMI < 25 kg/m²) fulfill the criteria for the metabolic syndrome [11, 39, 49, 50], compared to 20% in subjects with a BMI 25–30 kg/m² and 50% in obese (BMI > 30 kg/m²) individuals [11, 39, 49, 50]. Accordingly, the prevalence of insulin resistance increases when BMI is higher, ranging from 6% in normal weight subjects (BMI < 25 kg/m²) to 60–80% when BMI is > 35 kg/m² [11, 39, 51, 52].

Waist circumference reflects visceral adipose tissue (VAT) rather than general adiposity [53–55] and has a stronger relation with the metabolic syndrome and insulin resistance than BMI [39–41]. Within strata of BMI, a high WC (i.e., > 88 cm in women and > 102 cm in men) doubles the risk of metabolic syndrome compared to persons in the same BMI category with a normal WC [39]. Moreover, the risk for cardiovascular morbidity and mortality is better reflected by WC than by BMI [40]. This is in line with the observation that ATD is most prominently related to the quantity of VAT [40, 41, 56].

Clinical recommendation

Based on these facts we recommend the use of waist circumference rather than BMI in the evaluation of possible ATD.

4. Imaging of Adipose Tissue

As described in Section 3, ATD is strongly related to the quantity of adipose tissue and especially to the quantity of VAT [40,41,55]. Therefore, precise quantitative measurement of (visceral) adipose tissue is important in the diagnosis of ATD. Several imaging modalities are capable of measuring adipose tissue depots in different anatomical locations. Ultrasonography, computed tomography (CT) as well magnetic resonance imaging (MRI) have all been used for this purpose [57–59]. In addition, MRI is well-suited to probe adipose tissue (dys)function using MR spectroscopy [60].

Quantitative measurement of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) with imaging modalities

Ultrasound measurements of adipose tissue are obtained by measuring the distance between the skin and the linea alba in cm (SAT) and the distance between the peritoneum and the lumbar vertebrae in cm for VAT [61,62]. As such these measurements are a proxy for the total amount of adipose tissue. CT or MRI measurements of adipose tissue are considered the reference standard. Adipose tissue can be measured in a cross-sectional fashion, for example, at the level of the L4-L5 vertebrae, where the amount in cm² is computed, or as the total volume of adipose tissue in the abdomen using planimetric software [59,61–63]. In most studies, quantification relies on manual segmentation, but semi- and fully-automated methods are under

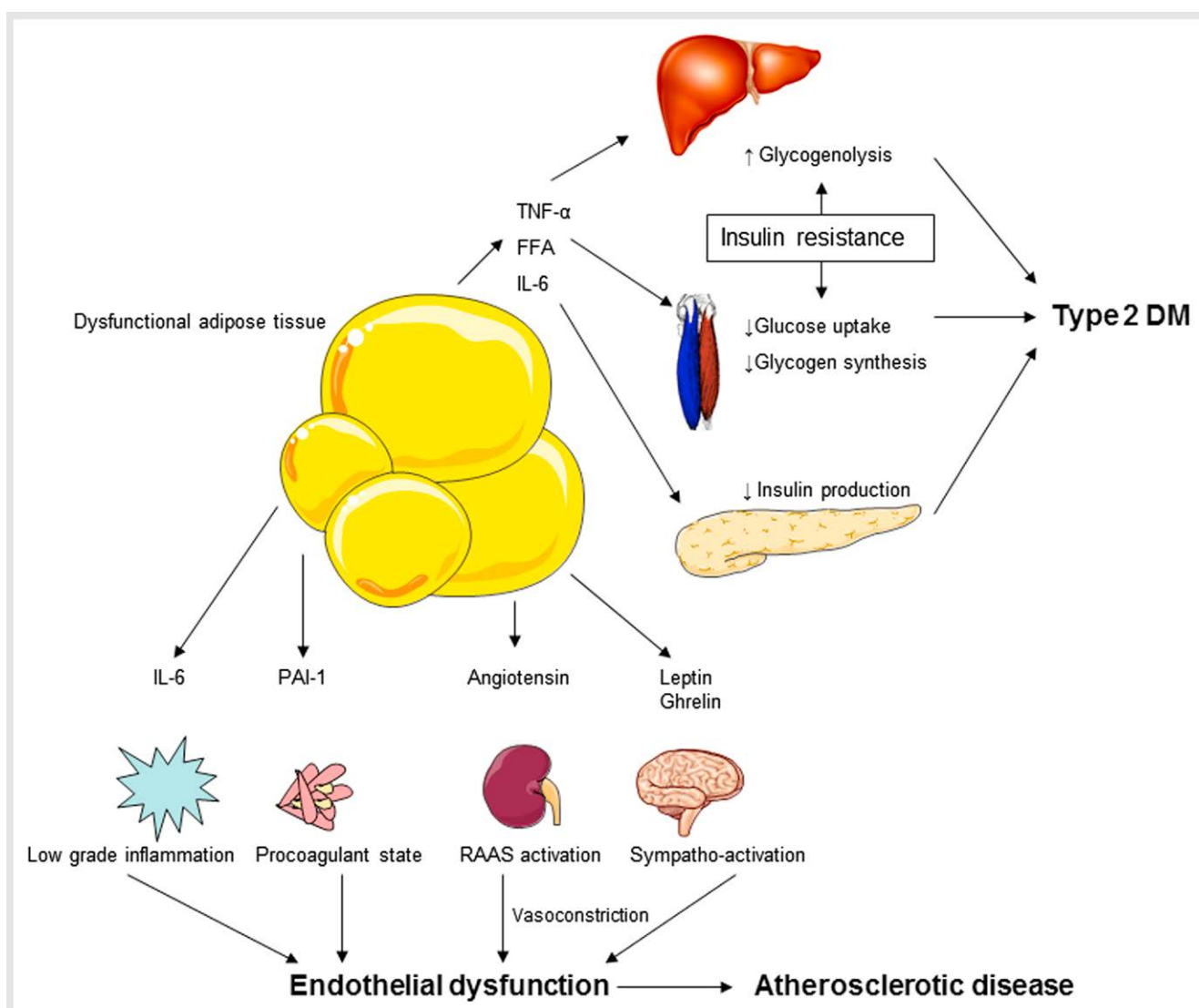


Fig. 1 Adipose tissue dysfunction leads to diabetes mellitus and atherosclerotic disease. Elevated levels of free fatty acids (FFA), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) interfere with the insulin signaling cascade of the liver and muscles (insulin resistance). As a consequence, there is less glucose uptake and glycogen synthesis in the muscle and enhanced glycogenolysis in the liver, causing a raise in serum glucose levels (impaired glucose tolerance). The production of insulin itself is diminished due to toxic effects of IL-6 and FFA on the pancreatic beta-cells. The lack of insulin combined with an impaired glucose tolerance leads to the development of type 2 diabetes mellitus. Atherosclerotic

disease starts with the release of pro-inflammatory (IL-6), procoagulant [plasminogen activator inhibitor 1 (PAI1)], and hormonal (angiotensin, leptin, ghrelin) factors causing, respectively, systemic low grade inflammation, a procoagulant state and activation of the renin-angiotensin-aldosterone-system (RAAS) and sympathetic nervous system. The RAAS and sympathetic nervous system activation cause vasoconstriction, in combination with the inflammation and prothrombotic state leading to endothelial dysfunction and eventually atherosclerotic disease. (Color figure available online only)

development [64,65]. Ultrasonographic measurements of adipose tissue are highly correlated to CT or MRI measurements of adipose tissue, with Pearson correlation coefficients of 0.64–0.81 [61,62].

Perhaps the most interesting and promising family of techniques to quantify the amount of adipose tissue in different body regions are based on multi-echo 3-dimensional chemical-shift-encoding water-fat imaging, also known as 'Dixon' methods. These methods encode both spatial position and chemical shift during the acquisition and subsequently estimate the contributions of water and fat to the measured signal in each voxel [66]. Using these methods, adipose tissue and water can be automatically separated per scanned voxel, allowing direct imaged based water and adipose tissue quantitation [67].

Examples of ultrasound, CT, MRI and Dixon adipose tissue measurements are provided in **Fig. 2,3**.

The quantity of VAT, as measured with ultrasound, CT, or MRI, is positively related to plasma concentrations of the pro-inflammatory adipokines IL-6, TNF- α , leptin, and retinol binding protein 4 (RBP-4) [68–72]. For SAT, this relation is considerably attenuated or nonexistent [68–72]. Adiponectin levels are negatively correlated to both VAT and SAT [69,72–75], although the correlation with VAT is consistently stronger than the correlation with SAT [69,72,74].

Volumetric CT, MRI, and ultrasound measurements of VAT are consistently correlated more strongly to cardiometabolic risk factors (hypertension, impaired fasting glucose) and the development of the metabolic syndrome, DM2 and (subclinical) atherosclerotic disease compared to SAT [56,76–78]. About 20% of men and 10% of women have a high amount of VAT (>90th percentile healthy referent sample) at CT-scanning, despite having a normal waist circumference [79]. These persons have a 20% higher risk of developing the metabolic syndrome compared to subjects with an elevated waist circumference, but with a low amount of VAT on CT-scan [79,80].

Functional imaging of adipose tissue with ¹H magnetic resonance spectroscopy

A promising MR imaging based technique is proton magnetic resonance spectroscopy (¹H MRS). With ¹H MR-spectroscopy it is possible to quantify localized lipid content in relation to the amount of water. Chemical characteristics of water and fat and their reaction to magnetic forces in the MRI-scanner are used to obtain a visual spectrum of metabolites [81]. ¹H MR-spectroscopy has been used to quantify free fatty acids, such as triglycerides (TG), poly-unsaturated fatty acids (PUFA), total unsaturated fatty acids (TUFA), and saturated fatty acids (SFA), in the myocardium, liver, breast, muscle, bone marrow, and SAT [82–86]. In general, a reduction in saturated fatty acid ingestion reduces the risk of a cardiovascular event, with 17%, probably via reduction of LDL-cholesterol levels. There is no effect of reducing saturated fatty acids on cardiovascular or all-cause mortality. Patients with DM2 have more unsaturated fatty acids in the liver compared to patients without DM2, influencing insulin resistance as reflected in higher glucose and HOMA-IR (Homeostatic Model Assessment – Insulin Resistance) levels [86]. Exercise reduces intrahepatic triglyceride content up to 50%, especially in males [87], even in the absence of changes in total body fat or VAT [88]. Low hepatic triglyceride content is related to a lower risk of developing nonalcoholic fatty liver disease, a condition frequently seen in obese subjects [87].

Hepatic triglyceride content increases from 2.0 to 4.3% in healthy men after a 3-day high fat high energy diet, consisting of 800 ml whipped cream added to a normal diet of about 2100 kcal/day [85]. Contrary, a 3-day low fat low energy diet, consisting of less than 500 kcal/day reduced intrahepatic triglyceride content by 4% in patients with DM2 [89]. These observations stress the short-term and flexible reactions of different (nonadipose) tissues to diet and exercise interventions as measured with ¹H MRS.

Only limited data on lipid composition and reactions to interventions concerning (abdominal) adipose tissue are available. Quantification of triglyceride content of SAT with ¹H MRS at 1.5 Tesla revealed that there was no correlation with serum lipid concentrations [82,90]. The amount of unsaturated fatty acids in abdominal adipose tissue correlated negatively with the amount of SAT and positively with the amount of VAT [90]. PUFA/TUFA and PUFA/TG ratios, as measured with MR spectroscopy, are higher in persons with the metabolic syndrome, especially in the omental adipose tissue depot, compared to subjects without the metabolic syndrome [60]. MR-spectroscopy is a noninvasive technique and a direct way of measuring metabolic characteristics of abdominal adipose tissue, which makes this technique a promising diagnostic tool for the identification of ATD.

Clinical recommendation

Although both CT and MRI measurements of adipose tissue provide detailed information of VAT and SAT, and (in the case of MRS) are feasible in research, implementing these modalities in clinical practice might be difficult due to both costs and logistics. Ultrasound measurements might be a useful alternative.

5. Insulin Resistance and Metabolic Syndrome

Insulin resistance is a condition with decreased sensitivity or responsiveness to the metabolic actions of insulin, caused by interference of the intracellular insulin signaling cascade by TNF- α and FFA [18]. Insulin resistance causes reduced capacity of adipocytes to store FFAs, causing lipid accumulation in muscles, pancreas, and liver contributing to insulin resistance. As a result, glucose clearance is diminished and glucose production enhanced in the liver, leading to a hyperinsulinemic state [91]. Insulin resistance or sensitivity can be assessed using several mathematical rules, such as the HOMA-IR or revised Quicki methods. The revised Quicki method correlates better with the reference standard [92] (euglycemic clamp) but necessitates more laboratory values (such as nonesterified fatty acids) than the HOMA-IR (which uses insulin and glucose levels). However, both methods have been used in clinical research regarding adipokines.

Insulin resistance related to adipokines and morphology of adipose tissue

Adiponectin is consistently negatively correlated to insulin resistance [93–97] whereas leptin is consistently positively correlated [96–99]. Together, adiponectin and leptin levels explained 38% of HOMA-IR variance in a group of elderly individuals [97]. Resistin [99] and TNF- α [100] were also found to be positively correlated to HOMA-IR. For IL-6 no relation was seen with insulin resistance in nonobese diabetic patients [96], but in patients with a BMI >27 kg/m² a relation between IL-6 and insulin resistance exists [101,102], suggesting a role for the quantity of adipose tissue. In insulin resistant mice, larger adipocytes and more mac-

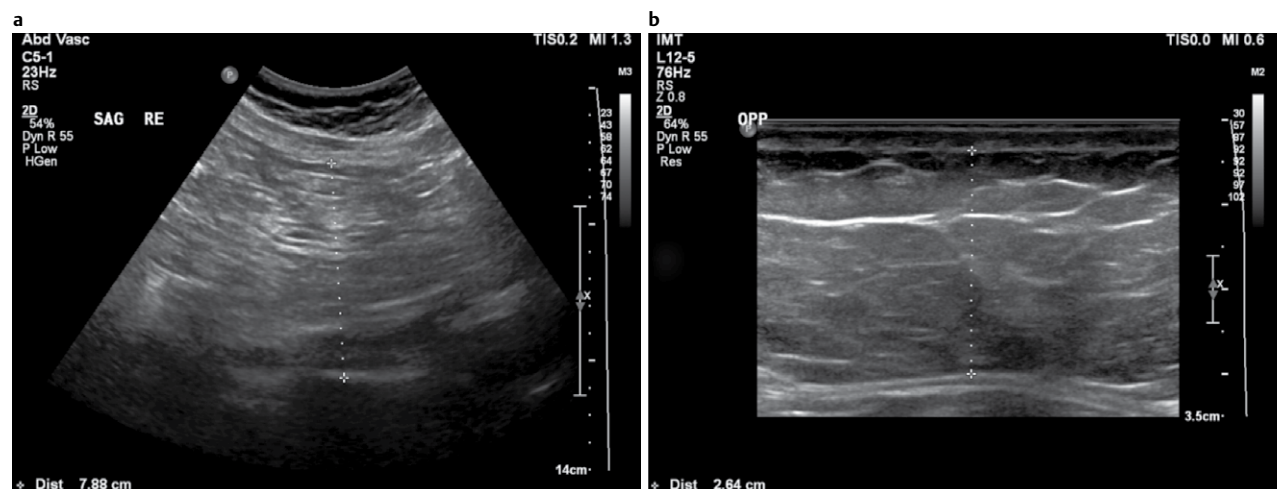


Fig. 2 Ultrasonography is well suited for distinguishing subcutaneous and intra-abdominal adipose tissue. Although intra-abdominal adipose tissue is not visualized directly with ultrasonography, the anteroposterior distance between the peritoneum behind the rectus musculature and the vertebral column (panel a) can serve as reasonable proxy measure for the amount of adipose tissue. Measurements are performed with a 5 MHz transducer. Subcutaneous adipose tissue is measured by measuring anteroposterior distance between the skin and the linea alba between the rectus abdominis muscle with a 12.5 MHz transducer (panel b).

rophage infiltration were seen than in mice without insulin resistance [103]. In patients undergoing bariatric surgery or cholecystectomy, the presence of foam cells (macrophages loaded with lipids) in VAT was positively correlated to insulin concentrations, whereas there was no correlation between foam cells in the SAT and insulin concentrations [104]. Therefore, measuring insulin resistance is a diagnostic tool for identifying ATD.

Insulin resistance related to metabolic syndrome, DM2, and cardiovascular disease

Yearly, 5–10% of people with insulin resistance develop overt diabetes [105] and the presence of insulin resistance and the metabolic syndrome are highly correlated [106], with about 60% of patients with insulin resistance also fulfilling the criteria for metabolic syndrome [107,108]. In a large meta-analysis it was shown that there is a 46% increased risk of coronary heart disease per 1 standard deviation increase of HOMA-IR [109], the risk for all-cause mortality is 64% increased in patients with a HOMA-IR >2.8 as compared to patients with a HOMA-IR <1.4 [110].

Metabolic syndrome related to adipokines and morphology of adipose tissue

Since almost all characteristics of the metabolic syndrome can be regarded as systemic metabolic consequences of ATD, it is not surprising that there are strong associations of the metabolic syndrome with elevated plasma levels of pro-inflammatory adipokines (leptin, TNF- α , IL-6) and lower adiponectin levels [45,94,98,111,112]. Moreover, in SAT biopsies of subjects with metabolic syndrome the macrophage content is higher compared to subjects without the metabolic syndrome, illustrating the relation between morphologic changes in adipose tissue, ATD, and clinical features [113].

Metabolic syndrome related to DM2 and cardiovascular diseases

Since characteristics of the metabolic syndrome are systemic consequences of ATD, consequently, associations between metabolic syndrome and DM2 and cardiovascular diseases originate in the presence of ATD. There is a significant relationship between the metabolic syndrome and the occurrence of incident coronary heart disease, with a 60–200% higher risk for subjects with compared to subjects without the metabolic syndrome [114,115]. Also, both cardiovascular (80% higher risk) and overall (40% higher risk) mortality are higher in subjects with the metabolic syndrome compared to subjects without the metabolic syndrome [115,116]. The presence of the metabolic syndrome constitutes an increased risk of 137% of developing type 2 diabetes, independent of glucose levels [117].

Clinical recommendation

Determining a HOMA level and the presence or absence of metabolic syndrome is recommended.

6. Plasma Concentrations of Adipokines as a Surrogate of ATD

Adipokines are produced by adipose tissue and secreted into the systemic circulation and can be measured in peripheral venous blood samples. The plasma concentrations of various adipokines vary widely between patients and patient groups and can be influenced as a result of interventions such as weight loss and medication [118–125]. Elevated levels of pro-inflammatory adipokines and decreased levels of anti-inflammatory adipokines are key features of ATD.

Pro-inflammatory adipokines

The adipokine leptin is the product of the *obese*-gene and is known for its inhibitory effect on the sense of appetite [126,127]. The production of leptin by adipose tissue is stimulated by pro-

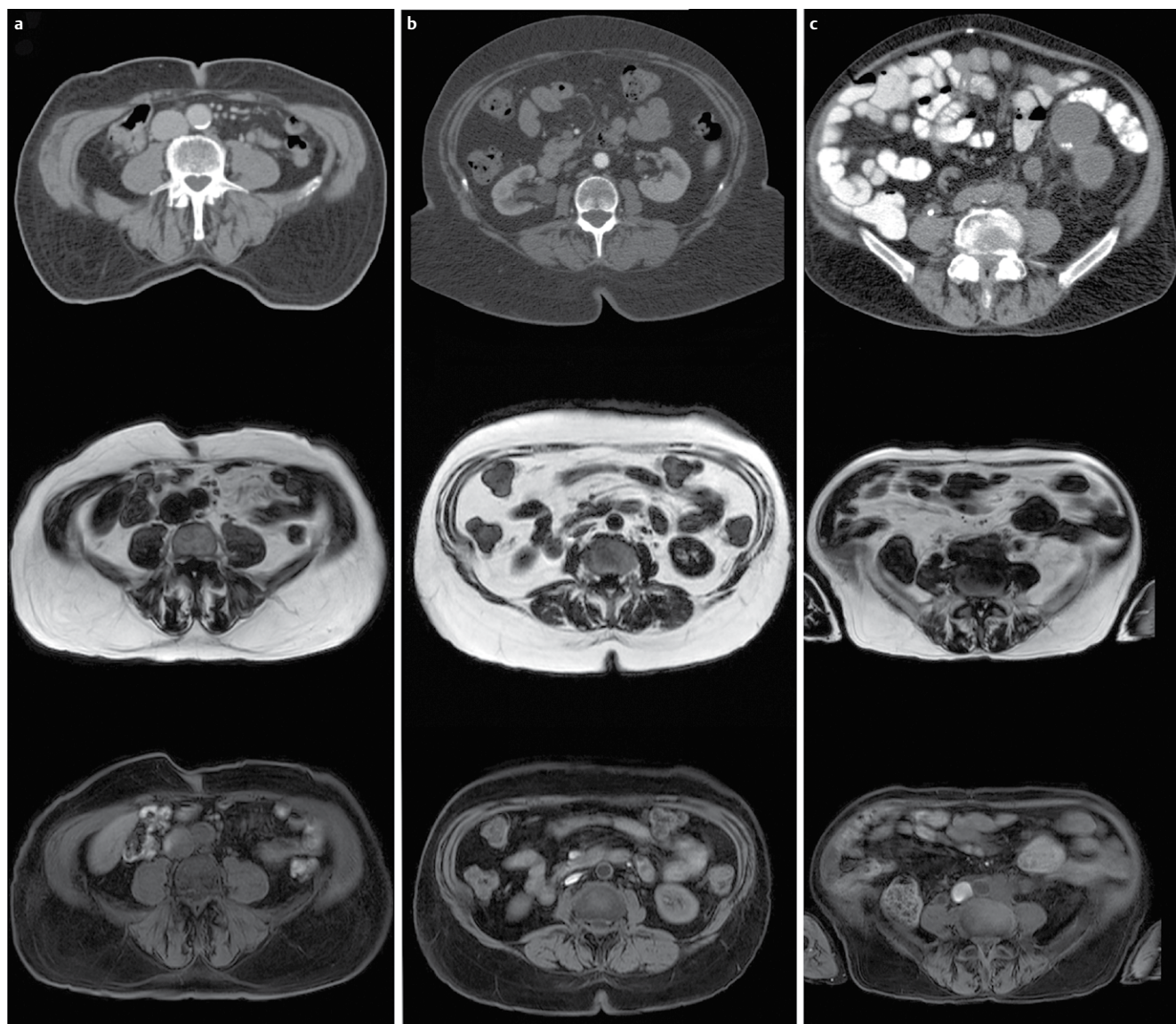


Fig. 3 Both CT (top row) and MRI (middle and bottom rows) are highly reliable modalities to map the spatial distribution of abdominal adipose tissue. The left column **a** shows a subject with relatively little intra-abdominal adipose tissue and relatively abundant subcutaneous adipose tissue. In the middle column a subject with abundant intra-abdominal as well as subcutaneous adipose tissue is shown, whereas the subject in column **c** has little subcutaneous adipose tissue, but abundant intra-abdominal adipose tissue. MRI images were obtained using the Dixon technique (bottom row), which allows for separation of the signals from water and adipose tissue and reconstruction of separate images showing both components separately, greatly aiding quantification.

inflammatory cytokines such as TNF- α and by lipopolysaccharide [16]. At a certain point the brain may become desensitized for the inhibitory effects on the food intake, a state called leptin resistance, creating a vicious circle of overeating, gaining weight, and developing insulin resistance [128, 129].

Retinol-binding protein-4 (RBP-4) is an adipokine involved in the transport of retinol (vitamin A) throughout the body [130]. It is secreted by hepatocytes, adipocytes, and macrophages [131, 132] and is important in regulating glucose homeostasis. Expression of RBP-4 is inversely related to the cellular expression of glucose transporter type 4 [133]. High plasma concentrations of RBP-4 are related to decreased insulin sensitivity and to features of the metabolic syndrome [134, 135].

Produced by monocytes, macrophages, and adipocytes, TNF- α is a pro-inflammatory cytokine that plays an important role in the development of insulin resistance by inducing apoptosis of adipocytes [91] and by interfering with the intracellular insulin signaling pathway downstream from the insulin receptor [133].

Anti-inflammatory adipokines

Adiponectin was discovered in 1996 [136] and is the most intensely studied adipokine. Adiponectin has anti-inflammatory and anti-atherogenic properties, and is positively correlated with insulin sensitivity [137]. Infusion of adiponectin in rats increases insulin sensitivity [138]. There is a strong relation between adiponectin plasma levels and the amount of VAT [139], whether this is an independent effect regardless the size of other adipose tissue depots is subject of debate [74, 139–141]. Recently, secreted frizzled related protein 5 (Sfrp5) is identified as a novel adipokine with anti-inflammatory characteristics, being an antagonist of the inflammatory protein WNT5a, preventing WNT5a from binding to its receptor. In Sfrp5 deficient mice, a high calorie diet induced severe glucose intolerance and an accumulation of macrophages in their adipose tissue, both diminished after administration of Sfrp5 [142]. Sfrp5 is down-regulated in obese individuals, causing high levels of WNT5a, possibly leading to inflammation and insulin resistance [133].

No studies have been performed yet towards the association between Sfrp5 and cardiovascular diseases or DM2.

Adipokine concentrations related to morphology of adipose tissue

In nonobese individuals, adipose tissue mainly consists of adipocytes, small amounts of pre-adipocytes, lymphocytes, macrophages, fibroblasts, and vascular cells [143]. Two phenotypes of macrophages are abundant in adipose tissue; the M1 macrophages produce pro-inflammatory cytokines [16,144] (IL-6, TNF- α) and stimulate adipocytes to secrete pro-inflammatory adipokines (leptin, resistin, RBP-4) [133]. M2 macrophages downregulate the synthesis of pro-inflammatory adipokines by adipocytes and upregulate secretion of anti-inflammatory adipokines (SFRP-5, adiponectin) [16,133]. In obese individuals, enlarged adipocytes produce chemotactic cytokines that mainly attract M1 macrophages causing an imbalance in pro- and anti-inflammatory adipokines, a condition referred to as ATD [17,145].

Plasma levels of adipokines relate to morphologic characteristics of both SAT and VAT [146–148]. Plasma progranulin [148], adiponectin [147], HGF [147], IP-10 [147], and MCP-1 [146] are strongly correlated with the number of infiltrated macrophages and adipocyte size in adipose tissue biopsies. These observa-

tions show that plasma levels of adipokines adequately reflect the inflammatory (and dysfunctional) state of the adipose tissue and can therefore be used in diagnosing ATD. • **Table 1** shows an overview of frequently studied adipokines and their characteristics.

Adipokine concentrations related to metabolic syndrome, DM2, and cardiovascular diseases

Elevated concentrations of plasma adipokines are associated with the development of the metabolic syndrome. High levels of (pro-inflammatory) leptin, RBP-4, PAI-1, and visfatin and a low level of the protective adiponectin are seen in patients with insulin resistance, metabolic syndrome, and DM2 [45,94,98,111,134,149–162]. Interestingly, for these adipokines, associations with metabolic syndrome hold even after adjusting for BMI [45,111,154,158,159,162].

For atherosclerotic disease, the association of serum adipokine levels and the development of disease is less distinct. Although a 44% risk reduction for myocardial infarction was observed in patients with the highest levels of adiponectin [114], later studies and meta-analyses [163–167] showed no relation between levels of adiponectin, leptin, adipisin, resistin, and PAI-1 and the development of atherosclerotic disease (after adjustment for risk factors). The association between ATD and atherosclerotic

Table 1 Overview of frequently studied adipokines and their characteristics.

Adipocytokine	Characteristics				
	Anti-inflammatory	Pro-inflam-matory	Metabolic function	Chemotaxis	Other
Adiponectin	+	–	↑	–	
Adipsin	+	–	↑	–	
Apelin	–	–	↓	–	
Adipolin	+	–	↑	–	
Chemerin	–				
C-reactive protein	–	+	–	–	
Ghrelin	–	–	↓	–	
Granulocyte colony stimulating factor	–	–	–	+	
Hepatic Growth Factor (HGF)	–	–	–	–	Angiogenesis
Interleukin 1 beta (IL1- β)	–	+	–	–	
Interleukin 6 (IL-6)	+ (via inhibition IL1)	+	–	–	
Interleukin 8 (IL-8)	–	+	–	–	
Interleukin 17beta (IL-17 β)	–	+	–	–	
Interleukin 21 (IL-21)	–	+	–	–	
Interferon gamma induced protein 10 (IP10)	–	+	–	+	
Leptin	+	+	↓ ↑		
Lipocalin					
Monocyte chemoattractant protein 1 (MCP1)	+	–	–	+	
Macrophage migration inhibitory factor (MIF)	–	+	–	+	
Nerve growth factor (NGF)	–	–	–	–	Angiogenesis
Omentin	+	–	↑	–	
Plasminogen activator inhibitor 1 (PAI-1)	–	–	–	–	Prothrombotic agent
Retinol binding protein 4 (RBP-4)	–	+	↓	–	
Resistin	–	+	↓	–	
Serpin	–	+	–	–	Prothrombotic agent
Serum amyloid A protein 1 (SAA1)	–	+	–	–	
Secreted frizzled related Protein (SFRP5)	+	–	–	–	
Tumor necrosis factor alpha (TNF- α)	–	+	–	?	
Thrombopoietin (TPO)	–	–	–	–	Prothrombotic agent
Transformation growth factor beta (TGF- β)	+	–	–	–	Pro-apoptotic
Visfatin	–	–	↑	–	
Vaspin	–	–	↑	–	
Vascular cell adhesion molecule 1	–	+	–	?	

+ : Characteristic present; – : Characteristic absent; ↑ : Enhances metabolic function/insulin sensitivity; ↓ : Attenuates metabolic function/insulin sensitivity; ? : Not entirely known

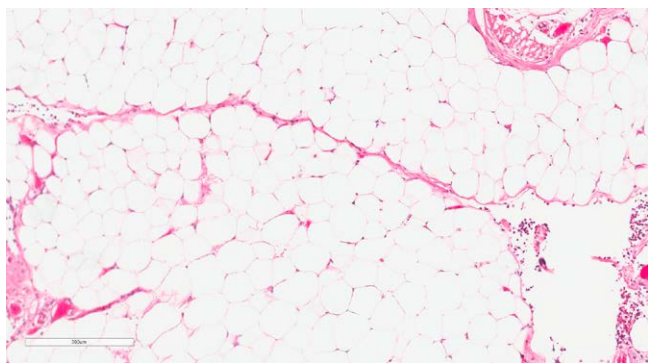


Fig. 4 Visceral adipose tissue biopsy. (Color figure available online only)

disease might be mediated via risk factors of the metabolic syndrome, by development of insulin resistance and inflammation or by direct effects of adipokines on the vessel wall [168].

Reliability of adipokine measurement in plasma

Plasma adipokine levels are fairly stable over time within individuals and a random peripheral blood sample therefore is a reliable representation of the mean level [169]. Adipokines can be measured by enzyme linked immunosorbent assay (ELISA) [170], and with a multiplex immuno-assay [171]. A fairly good correlation is seen between measurements with multiplex assay and ELISA, next to little cross-reactivity between the antibodies of the different adipokines [171]. This makes multiplex immuno-assay a suitable technique for adipokine profiling in patients or cohorts as, in contrast to ELISA, multiple adipokines can be measured in a single measurement.

Clinical recommendation

Measurement of adipokines is not routinely available in most laboratories and there are no reference values yet for adipokine plasma concentrations, making interpretation of adipokine levels on an individual level difficult and therefore these measurements are not yet useful in daily clinical practice.

7. Visceral Adiposity Index

The visceral adiposity index (VAI) was developed to estimate visceral adiposity dysfunction. It is a sex-specific index based on WC, BMI, triglycerides, and HDL-cholesterol [172]. This index is correlated to all factors of the metabolic syndrome and also to the occurrence of cardiovascular events. The association with the metabolic syndrome is not so surprising since 3 factors of the metabolic syndrome are also used in the VAI. The association with cardiovascular events, however, is interesting, since other surrogates for ATD do not show this association. Moreover, VAI is associated with many adipokines, and showed better correlations than WC or BMI. Specific measurements of VAT or SAT were not shown in this study [173]. However, if triglycerides are >3.15 mmol/l or if WC is large, the VAI is unreliable [174]. Moreover, the VAI is developed and validated in a Caucasian cohort and it is uncertain how VAI would perform in other populations.

Clinical recommendation

The VAI could be a reliable method for determining ATD, taking limitations into account.

8. Adipose Tissue Biopsies to Measure ATD

Adipose tissue biopsies are potentially the most direct way to evaluate ATD although this is a morphological evaluation and not a functional evaluation. The clinical usefulness of adipose tissue biopsies might be limited, especially with regard to VAT biopsies, as they can only be obtained during abdominal surgery. A needle biopsy of SAT, however, could be performed more easily and could be used in clinical practice. Great advantage of taking biopsies from SAT or VAT is that cellular structures of adipocytes, macrophage infiltration, and ex vivo production of adipokines can be investigated. A key feature of ATD is infiltration of macrophages in adipose tissue [17] and polarization of these macrophages predominantly to the M1-phenotype [175]. Elevated ex vivo production of pro-inflammatory adipokines by adipose tissue biopsies and diminished production of anti-inflammatory adipokines [18–20] reflect a state of ATD. In adipose tissue biopsies from subjects with either insulin resistance, metabolic syndrome, or DM2, all features of ATD are seen [113, 141, 176–180]. There is enhanced macrophage infiltration [113, 179], higher expression of pro-inflammatory adipokines [141, 178], and lower expression of adiponectin [73, 176, 177, 180] as compared to biopsies of overweight, yet metabolically healthy controls. In pericoronary adipose tissue biopsies obtained during cardiac surgery, macrophage infiltration and polarization towards the pro-inflammatory M1-type are more pronounced in patients with coronary atherosclerosis than in those without [181, 182] (► Fig. 4). Also, there is a negative association between adiponectin concentrations and macrophage infiltration in adipose tissue in patients undergoing abdominal aortic surgery [147].

After weight loss, either due to a (very) low calorie diet or bariatric surgery, significant improvements in metabolic parameters, such as insulin sensitivity, are seen [183–185]. These effects are measurable directly after the intervention and linger when the weight loss is sustained. Improvements in characteristics of ATD in adipose tissue biopsies develop simultaneously with the metabolic improvements. Reduction of both macrophage infiltration, adipocyte size and inflammatory adipokine concentrations are seen after weight loss due to bariatric surgery and very low calorie diets [186–188]. These effects were seen both shortly (5 days) and 1–3 months after the weight loss intervention, when participants had approximately lost 15% of their body weight [186–188]. Although VAT is generally believed to be more pathogenic, morphologic changes were seen in both subcutaneous and visceral biopsies [186–188]. However, no direct comparison between morphologic changes in different depots has been studied.

Clinical recommendation

In daily clinical practice, adipose tissue biopsies merely for diagnostic purposes will not be performed, and therefore studying adipose tissue biopsies will remain primarily a research area.

9. Conclusion

Adipose tissue dysfunction is an imbalance in the production of pro- and anti-inflammatory adipokines leading to insulin resistance, endothelial dysfunction, and eventually to DM2 and vascular diseases. Thus, diagnosing ATD is of clinical relevance and may even be considered a future treatment target. ATD can be diagnosed in both lean and obese individuals. Adipose tissue

biopsy is considered to be the reference standard for the diagnosis of ATD, as most features of ATD can be directly assessed. Other means are measurement of adipokine plasma levels in peripheral blood samples, although this is not implementable at an individual level due to large intra-individual variations and lack of standardization of the measurements.

Currently, we consider waist circumference, insulin resistance, and the presence of the metabolic syndrome to be the main options to be used in daily clinical practice for estimating ATD. Clearly, it would be a great advantage when more direct diagnostic tools could be used. Of the diagnostic options mentioned, measuring plasma adipokines in blood is, to our opinion, most promising, since this is relatively noninvasive and cheap compared to other options such as imaging and biopsies (especially abdominal adipose tissue biopsies). Possibly, a panel of several pro- and anti-inflammatory adipokines could be compiled, giving clinicians an 'adipokine-score' indicative of the level of ATD.

Conflict of Interest

The authors declare no conflicts of interest.

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