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Inflammatory characteristics of distinct abdominal adipose tissue depots relate differently to metabolic risk factors for cardiovascular disease
Distinct fat depots and vascular risk factors

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ABSTRACT

Objective: Abdominal obesity is associated with insulin resistance and metabolic syndrome. However, specific contributions of distinct adipose tissue (AT) depots to metabolic complications of obesity are still unclear. In this study, the inflammatory profile of four distinct abdominal AT-depots and the relation between AT-characteristics and obesity-induced metabolic complications was evaluated.
Methods: In 28 men undergoing abdominal aortic surgery, biopsies were collected from subcutaneous fat (SAT), and 3 visceral AT-depots: mesenteric (MAT), omental (OAT) and periaortic (PAT). The AT biopsies were characterized morphologically (adipocyte size, capillary density, CD68 + macrophages and crown-like-structures (CLS)) and the *ex vivo* adipokine secretion profile was determined by multiplex-immunoassay. The relation between depot-specific inflammatory characteristics and clinical parameters (waist circumference, insulin resistance and metabolic syndrome) was assessed by multivariable linear regression analysis.
Results: PAT contained the smallest adipocytes, highest capillary density and secreted abundant amounts of adipokines. SAT contained the lowest amount of macrophages and adipokines, while MAT and OAT displayed a similar inflammatory profile. In contrast to the other depots, MAT inflammation was most strongly related to metabolic complications of obesity, as adipocyte size and CLS were related to insulin resistance (β2.0; 95%CI1.15–2.85 and β0.24; 95%CI0.06–0.43) and MAT adipocyte size was associated with 79% higher odds of having metabolic syndrome (OR1.79; 95% CI1.13–2.89).
Conclusions: There are significant differences in the inflammatory profile of distinct abdominal fat depots, of which MAT characteristics were mostly associated with metabolic complications of obesity. These findings suggest a differential contribution of AT-depots to systemic metabolic dysfunction which precedes type 2 diabetes and vascular diseases.

1. Introduction

The worldwide prevalence of obesity has doubled over the last 20 years, and with that the risk for type 2 diabetes mellitus, metabolic syndrome and cardiovascular diseases has increased tremendously [1–3]. Obesity is the result of chronic energy excess, which challenges adipose tissue (AT) function to regulate metabolic homeostasis [4]. Obesity results in AT-derangements, characterized by adipocyte hypertrophy, altered AT-resident immune cell composition and consequently an altered adipokine and cytokine secretion profile, often referred to as AT-dysfunction [5,6]. In

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addition, AT of obese subjects has less capacity to expand the capillary network surrounding adipocytes, resulting in adipocyte hypoxia and necrosis [7]. This process is marked by presence of crown-like structures (CLS); e.g. macrophages surrounding necrotic adipocytes [5,8]. Presence of these inflammatory characteristics reflecting AT-dysfunction is considered a key mechanism leading to systemic metabolic complications such as low-grade inflammation, insulin resistance and dyslipidaemia [9,10]. These obesity-induced metabolic complications often precede the risk for type 2 diabetes and recurrent vascular events in the general population as well as in patients with established manifest vascular disease [11–13].

In the intra-abdominal region several AT-depots can be distinguished based upon anatomic location. Subcutaneous AT (SAT) is located underneath the skin and visceral AT (VAT) surrounds organs. Distinct visceral fat depots can be distinguished, of which mesenteric AT (MAT) lines the surface of the intestine, omental AT (OAT) relates to the greater omentum, and periaortic AT (PAT) surrounds the abdominal aorta [14,15]. Epidemiological studies indicate that mainly abundance of visceral fat is related to development of type 2 diabetes mellitus and cardiovascular disease, although these studies do not discriminate between distinct visceral fat depots [11,16]. SAT is considered to be the least active AT-depot, with lower macrophage infiltration and adipokine secretion compared to visceral fat [17,18]. In contrast, CLS are repeatedly shown to be present in SAT and associated with metabolic complications as well [9,19]. PAT is located just outside the adventitial layer, and capable of secreting adipokines, which can diffuse directly into the vascular wall and thereby contribute to atherogenesis [20,21]. VAT is considered a highly active secretory organ, and the direct release of VAT-derived adipokines into the portal vein might directly affect hepatic glucose and lipid metabolism [22,23]. However, less is known about the differential contribution of distinct VAT-depots to obesity-induced metabolic derangements as MAT and OAT are often conjointly termed VAT, and considered one and the same depot. However, OAT secretes more abundant amounts of adipokines compared to MAT, while in MAT lipolysis activity as well as adipocyte necrosis is more prominent compared to OAT [24,25]. Such intrinsic differences between MAT and OAT indicate that these distinct abdominal VAT-depots might contribute differently to distinct metabolic complications of obesity.

In order to unravel the mechanisms whereby AT dysfunction contributes to metabolic complications of obesity, accurate knowledge of depot-specific contribution is crucial. Accurate assessment of putative differences between distinct visceral fat depots requires analyses of distinct fat biopsies obtained from a single individual. Although differences between subcutaneous and one type of visceral fat has been broadly studied, data on the inflammatory profile of distinct abdominal AT-depots by equal comparison is scarce.

In this study, the inflammatory profile of four distinct abdominal fat depots (SAT, MAT, OAT, PAT) obtained within one individual was characterised. The primary objective was to directly compare the morphology and the *ex vivo* adipokine secretion profile of four abdominal AT-depots (SAT, MAT, OAT, PAT). The secondary objective was to relate morphological characteristics of each AT-depot to metabolic complications of obesity such as insulin resistance and metabolic syndrome.

2. Subjects and methods

2.1. Subjects

Male patients undergoing elective abdominal aortic surgery for aneurysmatic or stenotic aortic disease at the University Medical Centre Utrecht (UMCU) were eligible for participation in the study in the period from October 2010 to March 2013. Exclusion criteria were hypothyroidism (thyroid-stimulating hormone >5.0mUl^{-}1), elevated liver enzymes (aspartate aminotransferase (ASAT) or alanine aminotransferase (ALAT) > 2 times the upper limit of normal), renal failure (MDRD <30 ml/min/1.73 m^2), known malignancy in the past 2 years, use of thiazolidinediones and a history of liposuction, as these conditions are associated with altered adipokine regulation. All patients gave written informed consent and the study was approved by the Medical Ethics Committee of the UMCU.

2.2. Anthropometric and metabolic measures

During a single visit before planned abdominal aortic surgery, height and weight were collected, and the body mass index was computed as the weight in kilograms divided by the square of the height in meters. Waist circumference was measured as the circumference in centimetres halfway between the lower rib and the iliac crest. Further, blood was drawn in a fasting state for biochemical analysis including plasma glucose, insulin, glycated haemoglobin-A1c, total cholesterol (TC), triglycerides (TG), HDL-cholesterol, hsCRP, creatinin, ASAT, ALAT and gamma glutamyltransferase. TC, TG and fasting glucose were measured with a commercial enzymatic dry chemistry kit (Johnson & Johnson, New Brunswick, Quebec, Canada) and HDL-c was measured with a commercial enzymatic kit (Boehringer, Mannheim, Germany). LDL-cholesterol was calculated with the Friedewald's formula. Plasma insulin was measured with an immunometric technique in an IMMULITE 1000 analyzer (Diagnostic Products Corporation, Los Angeles, USA). The formula homeostasis model assessment parameter of insulin resistance (HOMA-IR: fasting glucose (mmol/mL) X insulin (IU/mL)/22.5) was used to assess insulin resistance, only performed in patients without antihyperglycaemic drugs [26]. Low-grade inflammation was measured by hsCRP levels, determined by immunonephelometry with a lower detection limit of the test of 0.2 mg/L (Nephelometer Analyser BN II, Dade-Behring, Marburg, Germany). The Adult Treatment Panel (ATP) III criteria were taken for the definition of metabolic syndrome [3].

2.3. Adipose tissue collection and handling

Approximately 5 g of SAT, MAT or OAT and approximately 1–2 g of PAT were obtained during surgery. SAT was obtained 2–3 cm to the right of the umbilicus, MAT was obtained in the right iliac region, in between the ileum and cecum, OAT was obtained from the right lumbar region near the hepatic flexure and PAT was obtained from the abdominal aorta at the site of the aneurysm. The AT biopsies were cut into different pieces. One part was incubated in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEMf12) supplemented with 50 IU/ml penicillin and 50 mg/ml streptomycin, for 24 h and weighed afterwards. Culture supernatant was centrifuged for 5 min at 500 g to remove cells and stored at −80 °C until further processing. Another part of AT was fixed in 10% formaldehyde until further processing. Two patients did not have enough PAT for sampling. Of one other patient the AT biopsies were not cultured. Altogether, AT supernatant of all 4 depots of 25 patients was eligible for *ex vivo* adipokine measurement. Histological examination was performed on AT of all patients.

2.4. (Immuno)histochemistry

Fixed AT samples were embedded in paraffin and 4 μm sections were processed for histological staining with hematoxylin & eosin (H&E). For quantification of macrophages (anti-CD68, mouse