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Increased systemic and adipose tissue inflammation differentiates obese women with T2DM from obese women with normal glucose tolerance

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ABSTRACT

Introduction. Obesity is strongly related to type-2 diabetes (T2DM), but there is a subset of obese individuals that remains relatively insulin sensitive and metabolically healthy. This study determined to what extent differences in metabolic health in obese women are associated with differences in adipose tissue and/or systemic inflammation.

Methods. The subject group consisted of age comparable lean (n = 12) and obese women either with T2DM (n = 28) or normal glucose tolerance (NGT; n = 26). Number of crown like structures (CLS) and adipocyte size were measured in subcutaneous and visceral adipose tissue of the obese women. Circulating cytokine and free fatty acid (FFA) levels, as well as number and activation status of peripheral leukocytes were determined.

Results. Obese T2DM subjects showed higher circulating levels of IL-6, FFA and glycerol as compared to obese NGT subjects. Obese T2DM subjects had higher absolute numbers of peripheral leukocytes which were mainly due to an increase of T helper cells. Activation status of circulating cytotoxic T (CD8+CD25+) and B (CD19+CD38+) cells was significantly increased in obese NGT subjects as compared to lean but was not different between the two obese groups. Subcutaneous adipose tissue of obese T2DM subjects contained more CLS than adipose tissue of obese NGT subjects.

Abbreviations: BMI, body mass index; CD, cluster of differentiation; CLS, crown like structures; CRP, C-reactive protein; FFA, free fatty acid; FSC, Forward scatter; HDL-C, high density lipoprotein cholesterol; HOMA, homeostasis model assessment; IFN-γ, interferon gamma; IHC, immunohistochemistry; IL, interleukin; IMDM, Iscove's modified Dulbecco's medium; IRMA, immunoradiometric assay; LDL, low density lipoprotein; NGT, normal glucose tolerance; PBMC, peripheral blood mononuclear cell; PHA, Phytohaemagglutinin; PMA, phorbol myristate acetate; RIA, radioimmunoassay; SSC, Sideward scatter; T2DM, type two diabetes mellitus; TG, triglycerides; TNF-α, tumor necrosis factor alpha.

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Conclusion. Obese T2DM subjects show higher FFA levels and adipose tissue macrophage infiltration in addition to higher levels of circulating IL-6 and numbers of CD4+ T cells than obese NGT subjects. Hence, obese T2DM subjects show a higher extent of inflammation at both the systemic and adipose tissue level.

1. Introduction

The metabolic syndrome comprises a combination of risk factors that increase the risk of developing type-2 diabetes and cardiovascular disease [1]. Obesity, in particular abdominal obesity, is one of the main risk factors of the metabolic syndrome. The majority of obese individuals (~80%) will eventually develop metabolic abnormalities associated with a reduced life expectancy. However, there is a subset of obese individuals who remain relatively insulin sensitive and metabolically healthy throughout life [2]. The reason why these individuals are unaffected is still not completely understood.

The pathological metabolic consequences of obesity are closely linked to the expanding adipose tissue that at a certain level responds with stress signals to the energy overload [3]. Adipose tissue functions as a metabolic and endocrine organ releasing fatty acids and adipokines, both of which have immune modulatory activities as reviewed in Refs. [4,5]. Obesity induces adipose tissue dysfunction with increased secretion of pro-inflammatory cytokines and chemokines. Adipose tissue acquires a chronic inflammatory state which is characterized by macrophage accumulation in crown like structures that surround stressed and dying adipocytes [6,7]. Adipose tissue inflammation may affect systemic immune responses that contribute to the initiation and progression of obesity induced metabolic and cardiovascular dysfunctions. Several studies in obese subjects have shown elevated levels of adipose tissue released pro-inflammatory cytokines – such as leptin, TNF-α and IL-6 – in contrast to a decreased level of the anti-inflammatory cytokine adiponectin [8]. Also, the levels of the acute phase protein, C-reactive protein (CRP), are higher in subjects with obesity [9], indicating that obesity is associated with (low grade) systemic inflammation.

Numerous studies have investigated the effects of obesity or type 2 diabetes on systemic inflammation [8–17], but they have not considered differences between obese individuals that develop T2DM and those that remain relatively healthy. We hypothesize that in obese individuals that have developed type 2 diabetes, the intensity of adipose tissue inflammation and/or the systemic inflammatory state may be higher as compared to obese individuals that still have normal glucose tolerance (NGT). To this end, we compared the extent of abdominal subcutaneous and visceral adipose tissue inflammation between age comparable severely obese women with T2DM and NGT. Moreover, we compared systemic inflammation between lean and obese women either with T2DM or NGT by determining number and activation- or memory status of peripheral leukocytes in addition to circulating levels of pro-inflammatory cytokines, CRP and free fatty acids (FFAs).

2. Materials and Methods

2.1. Subjects

The study group consisted of 12 lean and 54 obese women of whom 28 had type-2 diabetes. The three groups were comparable in age and the obese groups in BMI. All the obese women had been morbidly obese (mean BMI = 42.8 ± 4.7 kg/m^2) for at least five years. Subjects who reported the use of weight loss medications within 90 days prior to enrollment in the study were excluded. Body weight of all subjects had been stable for at least 3 months prior to inclusion. All subjects were non-smokers, had no signs of any infections and had no history of auto-immune diseases. The subjects were investigated in the morning after an overnight fast. Venous blood samples were taken for determination of number of leukocytes as well as determination of glucose, insulin, lipids, cholesterol and cytokines in serum. Moreover, ~50-ml of venous blood was taken for subsequent flow cytometry analysis. Around 4 weeks after the first examination a subgroup (n = 35 of whom 14 had T2DM) of the obese individuals underwent bariatric surgery (gastric bypass or banding). Within 1 h after opening the abdominal wall adipose tissue specimens were taken from the epigastric region of the abdominal wall (subcutaneous) and from the major omentum (visceral). These samples were used for determination of cell size and extent of adipose tissue inflammation.

The study was approved by the Ethics Committee of Leiden University. All subjects gave informed consent to participate in the study.

2.2. Medication

For obvious reasons we could not restrict to obese subjects not using any type of medication. All T2DM subjects were treated with oral medication only (metformin or sulfonylurea derivatives). Participants were allowed to use cholesterol lowering statins and antihypertensive medication.

The use of drugs such as statins and antihypertensive drugs was slightly higher in the T2DM subjects. At baseline, statins were used by 60% of the T2DM subjects and 25% of the NGT subjects. Of the T2DM patients, 50% used anti-hypertensives (diuretics n = 7, ACE-inhibitors n = 5, β-blockers n = 6) against 33% in the NGT patients (diuretics n = 4, ACE-inhibitors n = 3, β-blockers n = 4). The patients were using neither any medication that affects lipid or glucose metabolism nor any anti-inflammatory agents (i.e. thiazolidinediones, steroids (prednisone) or NSAIDS).

2.3. Blood measurements

Serum Glucose, Total cholesterol, High Density Lipoprotein cholesterol (HDL-C), Triglycerides (TG) and C-reactive protein