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Acute hyperlipidemia initiates proinflammatory and proatherogenic changes in circulation and adipose tissue in obese women

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ABSTRACT

Background: Obesity represents a high risk factor for the development of atherosclerosis and is associated with a low-grade inflammation and activation of immune cells.
Aims: The aim of our study was to investigate the effect of a short-term lipid infusion on immune cells in blood and subcutaneous abdominal adipose tissue (SAAT) in obese women.
Methods: Seven-hour intravenous lipid/control infusions were performed in two groups of women (n = 15, n = 10, respectively). Before and at the end of the infusion, SAAT and blood samples were obtained and relative content and phenotype of immune cells were analyzed using flow cytometry. Analysis of immune cell markers, inflammation and angiogenesis markers was performed in SAAT by RT-PCR and in plasma by immunoassays.
Results: Relative content of CD45+/14+ and CD45+/14+/16+ populations of monocytes was reduced in circulation by 21% ($p = 0.004$) and by 46% ($p = 0.0002$), respectively, in response to hyperlipidemia, which suggested the increased adhesion of these cells to endothelium. In line with this, the levels of sICAM and sVCAM in plasma were increased by 9.4% ($p = 0.016$), 11.8% ($p = 0.008$), respectively. In SAAT, the relative content of M2 monocyte/macrophages subpopulation CD45+/14+/206+/16+ decreased by 27% ($p = 0.012$) and subpopulations CD14+/CD206− and CD14/+TLR4+ cells increased ($p = 0.026$; $p = 0.049$, respectively). Intralipid infusion promoted an increase of mRNA levels in SAAT: RORC (marker of proinflammatory Th17 lymphocytes) by 43% ($p = 0.048$), MCP-1 (78%, $p = 0.028$) and VEGF (68.5%, $p = 0.0001$).
Conclusions: Acute hyperlipidemia induces a proinflammatory and proatherogenic response associated with altered relative content of immune cells in blood and SAAT in obese women.

1. Introduction

Obesity represents a high risk factor for the development of cardiovascular diseases and atherosclerosis. The common feature of these complications is a low-grade inflammation and activation of immune cells. It was proposed that one of the triggers of these proinflammatory processes are circulating free fatty acids (FFA) and triglycerides (TG) [1,2]. Lipids, namely saturated FFA, were found to activate classical inflammatory responses in immune cells and to regulate secretion of proinflammatory cytokines in several types of cells [3,4]. The ability of lipids to activate proinflammatory responses was documented also in vivo in humans upon the postprandial increase of lipid metabolites: the consumption of high-fat meal was accompanied with an increase of proinflammatory cytokine plasma levels [5,6] and increased circulating leukocyte counts [7,8]. Importantly, the postprandial increase of circulating lipids (TG, FFA) as well as the signs of systemic postprandial inflammatory response were more pronounced in obese [9,10].

Effects of FFA on cells can be mediated through binding to the receptors/sensors, such as toll like receptor 4 (TLR4) and fatty acid translocase (CD36), that control inflammatory signaling pathways

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[2,11]. Indeed, in humans, increased circulating levels of FFA were associated with increased expression of CD36 on monocytes, which led to lipid accumulation in these cells [12]. Such a lipid overload caused monocytes to form foam cells that are implicated in the development of atherosclerosis [13]. Atherosclerosis is now, similarly as obesity, widely accepted as a low-grade chronic inflammatory disease. It is initiated by the dysfunction of the vascular endothelium and followed by the activation and recruitment of circulating leukocytes to sites of endothelial damage [14,15].

However, the effects of lipids on immune processes within the adipose tissue (AT) are known only partially. Saturated FFA may induce an increased expression of proinflammatory cytokines in adipocytes similarly as was shown in immune cells [16]. In vivo in rats, the postprandial increase of mRNA expression of IL-6 and NF-kB activation in AT was observed after high-fat meal [17]. Moreover, FFA appeared as an important driver of macrophage accumulation in AT in mice [18]. In humans, it was documented that postprandial triglyceridemia increased levels of soluble cell adhesion molecules (sICAM, sVCAM), which may regulate the infiltration of monocytes to the endothelium [19,20] and potentially recruitment to AT [17,18].

Based on these studies, we hypothesize that acute elevation of systemic lipid levels may modify proinflammatory characteristics of AT, and so worsen the AT dysfunction and contribute to proatherogenic changes in metabolically healthy obese women. Thus, the aim of the current study was to investigate whether the acute experimentally-induced hyperlipidemia modifies the relative content and phenotype of immune cells, and other immunity-related features in subcutaneous adipose tissue (SAAT) and in circulation in obese women.

2. Materials and methods

2.1. Subjects

Seventeen obese healthy premenopausal women were sequentially recruited by referral from obesity consultations at the *University Hospital Královské Vinohrady* and by local obesity-management organizations (STOB). Subjects were randomly divided into two groups: the intervention group with Intralipid infusion (n = 15, age 43 ± 7 year, BMI 31.4 ± 2.7 kg/m^2) and the control group with infusion of glycerol (n = 10, age 44 ± 6 years, BMI 31.6 ± 3.4 kg/m^2). Exclusion criteria were weight changes of more than 3 kg within the 3 months before the study, hypertension, impaired fasting glucose, diabetes, hyperlipidemia, drug-treated obesity, smoking, drug or alcohol abuse, irregular menstrual cycle, pregnancy or participation in other studies. All individuals in the two groups of subjects showed the “metabolically healthy obese” phenotype [21,22], i.e. they did not meet the criteria of metabolic syndrome as defined by International Diabetic Federation. The two groups were homogenous from this point of view. All the subjects were non-smokers, sedentary, did not take any medications and did not suffer from any disease except for obesity. All subjects were fully informed about the aim and the protocol of the study and signed an informed consent approved by the Ethical committee of the Third Faculty of Medicine (Charles University in Prague, Czech Republic).

2.2. Experimental protocol

The subjects entered the laboratory at 7.00 a.m. after an overnight fast. A complete clinical investigation was performed, anthropometric parameters were measured and body composition was determined with multifrequency bioimpedance (Bodystat QuadScan 4000; Bodystat Ltd., Isle of Man, British Isles). Subsequently, the subjects were placed in a semi-recumbent position and a catheter was placed in the antecubital vein. To increase plasma FFA and TG concentration intravenous infusion of lipid emulsion (Intralipid 20%) was applied. Intralipid 20% (Fresenius Kabi, Bad Hamburg, Germany) consists of soya-bean oil (20%) stabilized with egg yolk phospholipids (1.2%) and glycerol (2.5%). The fatty acid composition was as follows: palmitic acid 11.3%, stearic acid 4.9%, oleic acid 29.7%, linoleic acid 46.0% and linolenic acid 8.1%. The infusion of Intralipid 20% was administered through cannula at a rate 60 ml/h for one hour and then it was continued at constant rate 90 ml/h for following six hours.

In the control group, saline infusion with 2.5% glycerol was administered at the same rate for seven hours.

Before the start of infusions and every 60 min during infusions venous blood was collected into 50 μl of an anticoagulant and antioxidant cocktail (Immunotech SA, Marseille, France) and immediately centrifuged (1300 rpm, 4 °C). The plasma samples were stored at −80 °C until analyses.

The needle biopsies and 2 ml of uncoagulated blood samples were taken 30 min before the start of the experimental infusions and 15 min before the end of infusions. Needle biopsies of SAAT were obtained approximately 10–15 cm laterally to the umbilicus under local anesthesia (1% Mesocain, Zentiva, Prague, Czech Republic), as previously described [23]. Approximately 1 g of SAAT was used for isolation of stroma-vascular fraction (SVF). SVF and 2 ml of venous blood were used for flow cytometry analyses. An aliquot of SAAT (approx. 0.2 g) was immediately frozen in liquid nitrogen and stored at −80 °C until gene expression analysis.

2.3. Determination of plasma levels of biochemical parameters

Homeostasis model assessment of the insulin resistance index (HOMA-IR) was calculated as follows: ((fasting insulin in mU/l) × (fasting glucose in mmol/l)/22.5). Plasma levels of FFA and TG were measured using enzymatic colorimetric kits (Randox, Crumlin, UK). The concentrations of VEGF-A, and MCP-1 in plasma were measured by ELISAs (eBioscience, San Diego, USA; R&D Systems, Minneapolis, USA). The concentrations of sVCAM, sICAM, IL-8, IL-6, and TNFα were measured by multiplex immunoassays (Milliplex Cardiovascular disease panel HCVD2MAG and High sensitivity T-Cell panel HSTCMAG, Merck-Millipore, USA). The intra-assay coefficients of variation for individual immunoassays obtained in our laboratory were: VEGF-A 7.5%, MCP-1 7.1% IL-8 3.6%, IL-6 5.3%, and TNFα 3.3%, sVCAM 2.7%, sICAM 5.8%. Plasma levels of other parameters were determined using standard clinical biochemical methods.

2.4. Isolation of SVF cells

SAAT was washed with saline, minced into small pieces and digested with type I collagenase (SERVA, Heidelberg, Germany) for 1 h in 37 °C shaking water bath and subsequently centrifuged at 200 g for 10 min and filtered through 100- and 40-μm sieves to isolate SVF cells.

2.5. Flow cytometry analyses

The whole blood and isolated SVF cells were analyzed immediately after isolation for flow cytometry analyses as described before [7]. Briefly, SVF cells were resuspended to final concentration 10^6 cells/ml in PBS solution containing 0.5% BSA and 2 mmol/l EDTA and 100 μl of this suspension was incubated with fluorescence-labelled monoclonal antibodies (FITC-conjugated antibody CD14, CD16, CD4; PE-conjugated antibody CD14, TLR4, CD3, CD36; PerCP-conjugated CD45 antibody and APC-conjugated