Original Article

MOLECULAR METABOLISM

Activated macrophages control human adipocyte mitochondrial bioenergetics via secreted factors

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ABSTRACT

Objective: Obesity-associated WAT inflammation is characterized by the accumulation and local activation of macrophages (MΦs), and recent data from mouse studies suggest that macrophages are modifiers of adipocyte energy metabolism and mitochondrial function. As mitochondrial dysfunction has been associated with obesity and the metabolic syndrome in humans, herein we aimed to delineate how human macrophages may affect energy metabolism of white adipocytes.

Methods: Human adipose tissue gene expression analysis for markers of macrophage activation and tissue inflammation (CD11c, CD40, CD163, CD206, CD80, MCP1, TNFα) in relationship to mitochondrial complex I (NDUFB8) and complex III (UQCRC2) was performed on subcutaneous WAT of 24 women (BMI 20–61 kg/m^2). Guided by these results, the impact of secreted factors of LPS/IFNγ- and IL10/TGFβ-activated human macrophages (THP1, primary blood-derived) on mitochondrial function in human subcutaneous white adipocytes (SGBS, primary) was determined by extracellular flux analysis (Seahorse technology) and gene/protein expression.

Results: Stepwise regression analysis of human WAT gene expression data revealed that a linear combination of CD40 and CD163 was the strongest predictor for mitochondrial complex I (NDUFB8) and complex III (UQCRC2) levels, independent of BMI. IL10/TGFβ-activated MΦs displayed high CD163 and low CD40 expression and secreted factors that decreased UQCRC2 gene/protein expression and ATP-linked respiration in human white adipocytes. In contrast, LPS/IFNγ-activated MΦs showed high CD40 and low CD163 expression and secreted factors that enhanced adipocyte mitochondrial activity resulting in a total difference of 37% in ATP-linked respiration of white adipocytes ($p = 0.0024$) when comparing the effect of LPS/IFNγ- vs IL10/TGFβ-activated MΦs.

Conclusion: Our data demonstrate that macrophages modulate human adipocyte energy metabolism via an activation-dependent paracrine mechanism.

Keywords Cytokines; Oxidative phosphorylation; Glycolysis; Cellular metabolism

1. INTRODUCTION

White adipose tissue (WAT) plays a major role in energy homeostasis by storing nutrient energy, releasing fatty acids as bioenergetic substrates, and secreting endocrine mediators and factors [1–3]. Due to low mitochondrial content and activity as compared with brown adipocytes or myotubes, oxidative energy metabolism in WAT has been neglected for a long time. However, accumulating evidence suggests that mitochondrial activity is crucial for a normal WAT function since mitochondria are important for lipid storage [4,5] and secretory functions [6–8]. This relevance has been further corroborated by clinical studies showing strong associations of decreased mitochondrial content and oxygen consumption of WAT/adipocytes with metabolic complications such as insulin resistance, type 2 diabetes and cardiovascular diseases [9–13].

One key hallmark in the development of obesity-associated metabolic disorders is the chronic, low-grade inflammation of WAT [14,15]. Central players in obesity-associated inflammation and its

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comorbidities such as insulin resistance are adipose tissue macrophages (ATMΦs). They increase in number during the progression of obesity and subsequently become the most abundant cell type in WAT besides adipocytes and preadipocytes [15,16]. Although macrophages are mainly studied in the context of pathologies, ATMΦs execute important physiological functions such as metabolic adaption to cold, stress, and exercise [17,18]. Additionally, inflammation appears to be crucial for healthy WAT expansion and remodeling processes [19]. Notably, it has been suggested that the obesity-associated inflammation of human WAT may compromise mitochondrial function [16,20–22]. These findings indicate an important role for the immune system in controlling WAT mitochondrial function, strongly suggesting the need to understand the global effects of macrophages on the bioenergetics of adipocytes, in particular in humans. To elucidate the crosstalk and functional interactions between macrophages and adipocytes, it is pivotal to identify cytokine-mediated pathways as potential novel therapeutic targets to modify WAT mitochondrial function in metabolic disorders.

In WAT of obese humans, several different ATMΦ subtypes have been identified expressing macrophage activation markers not only of the M1 spectrum but also the M2 spectrum [23–26]. CD40, related to the M1 spectrum, displays upregulated gene expression by pro-inflammatory stimuli such as LPS and IFNγ [27]. The amount of $CD40^+$-macrophages is higher in obese WAT and decreases after weight loss induced by gastric surgery [28]. As a co-stimulatory factor, CD40 plays a crucial role in adipose tissue inflammation in mice [29–31] and has been suggested as a promising target in obesity-associated insulin resistance [32,33]. CD163, a monocyte/macrophage specific marker related to the M2 spectrum [34], is increased by IL10 [35], in obese human WAT [22,26], and mRNA levels associate with insulin-resistance [36,37].

In the present study, we performed adipose tissue gene expression analysis on CD40 and CD163 together with other known obesity-associated WAT inflammation and macrophage activation markers (CD11c, CD80, CD206, MCP1, TNFα) in relation to mitochondrial markers of the electron transport chain (ETC; NDUFB8 and UQCRC2) to gain insights into potential relevant connections of inflammation and mitochondrial bioenergetics *in vivo*. We performed quantitative analysis of respirometry and extracellular acidification measurements of human adipocytes (SGBS and primary cells) after exposure to microenvironments (conditioned media) created by either LPS/IFNγ- or IL10/TGFβ-activated human THP1, or primary macrophages (MΦs).

2. MATERIALS AND METHODS

2.1. Materials

Cell culture media and supplements were from Thermo Fisher Scientific (Invitrogen, Waltham, MA, USA). Human recombinant cytokines for macrophage activation were purchased from Peprotech (Rocky Hill, NJ, USA). All other chemicals and reagents were obtained from Sigma Aldrich (St. Louis, MO, USA) if not otherwise stated.

2.2. Experimental subjects and study approval

Human subcutaneous adipose tissue samples (mamma, abdominal) were obtained from 24 Caucasian women undergoing plastic surgery (cohort A: $n = 16$ (only abdominal), cohort B: $n = 8$ (mamma and abdominal)). Mean age of patients was 40 years (range 21–73 years) and their mean BMI was 31 kg/m^2 (range 20–61 kg/m^2). All procedures were performed in accordance with the Declaration of Helsinki guidelines and approved by the ethics committee of the University of Ulm. Patients gave a written informed consent in advance.

2.3. Macrophage culture and conditioned media

THP1 cells (purchased from ATCC, Manassas, VA, USA) tested for mycoplasma contamination were cultured and differentiated as reported previously [38,39] with the following modifications. THP1 cells (1×10^6/ml) were differentiated by incubation with 100 ng/ml PMA for 24 h in growth medium. After differentiation, adherent THP1 cells were stimulated with 10 ng/ml LPS plus 10 ng/ml IFNγ (LPS/IFNγ-activated THP1) or 10 ng/ml IL10 and 1 ng/ml TGFβ (IL10/TGFβ-activated THP1). PMA and all cytokines were added to a control dish not containing THP1 cells. After 24 h, control (cell-free), LPS/IFNγ-activated THP1 and IL10/TGFβ-activated THP1 containing dishes were washed thoroughly with PBS and serum-free medium was added. After 24 h, conditioned media (CM) and control media (cell-free, CF) were collected and cleared by centrifugation. Cells were collected for RNA analysis. Human $CD14^+$ blood monocytes from 4 healthy donors were purchased from Zenbio (Research Triangle Park, NC, USA) and differentiated with 10 ng/ml CSF1 for 7 days to adherent macrophages (MΦ) before same stimulation as for THP1 cells was performed. After 48 h, cells were thoroughly washed, and serum-free medium was added. After 24 h, LPS/IFNγ-activated MΦ-CM, IL10/TGFβ-activated MΦ-CM and control media were collected and cleared by centrifugation.

2.4. Adipocyte culture

SGBS adipocytes were cultured and differentiated for 10 days as published [40]. Human subcutaneous fat biopsies were obtained during gastric sleeve surgery from obese donors (11 for RNA analysis and 3 for energetic pathway studies) after an overnight fast. Preadipocytes were isolated and differentiated *in vitro* to adipocytes as previously described [41]. The study adhered to The Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants gave informed written consent to the study, and the study protocol was approved by the local ethics board. On day 10 of adipogenic differentiation, primary human cells or SGBS adipocytes were stimulated for 48 h with cell-free control media or CM of activated THP1 or primary MΦs (LPS/IFNγ-activated THP1-CM, IL10/TGFβ-activated THP1-CM or LPS/IFNγ-MΦ-CM, IL10/TGFβ-MΦ-CM) followed by RNA, protein and bioenergetic analysis.

2.5. Gene expression analysis

Total RNA was prepared using RNeasy Lipid tissue kit (Qiagen, Hilden, Germany). After cDNA synthesis (Superscript-II Reverse Transcriptase, Invitrogen) expression of specific genes was analyzed by real-time-PCR using SYBR® Green (Invitrogen) and the ViiA™ 7 Dx Instrument (Applied Biosystems, Foster City, CA, USA). Specific primers were obtained from Sigma (Sequences are available upon request). The mRNA levels of genes were normalized to Hypoxanthine-Phosphoribosyl-Transferase (*HPRT*) using ΔCt and if applicable to control group by ΔΔCt method.

2.6. Analysis of surface markers

THP1 cells after activation with LPS/IFNγ or IL10/TGFβ were harvested on ice, incubated with Fc block (Miltenyi, Bergisch-Gladbach, Germany) followed by incubation with primary antibodies against CD40 (clone 5C3, FITC, eBioscience) or CD163 (clone GHI/61.1, vioblue, Miltenyi, PE, eBioscience), CD11c (Miltenyi, clone MJ4-27G12.4.6, FITC), CD80 (Miltenyi, clone 2D10, PE) or CD206 (Miltenyi, clone DCN228, FITC) in the dark (4 °C). After 30 min, cells were washed and analyzed by flow cytometry (MACSQuant VYB, Miltenyi). Mean fluorescence intensity (MFI) was analyzed using MACSQuantify software (Miltenyi), normalized to MFI of corresponding isotype control.

