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TNF-α inhibits PPARβ/δ activity and SIRT1 expression through NF-κB in human adipocytes

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ABSTRACT

The mechanisms linking low-grade chronic inflammation with obesity-induced insulin resistance have only been partially elucidated. PPARβ/δ and SIRT1 might play a role in this association. In visceral adipose tissue (VAT) from obese insulin-resistant patients we observed enhanced p65 nuclear translocation and elevated expression of the pro-inflammatory cytokines *TNF-α* and *IL-6* compared to control subjects. Inflammation was accompanied by a reduction in the levels of SIRT1 protein and an increase in *PPARβ/δ* mRNA levels. Stimulation of human mature SGBS adipocytes with TNF-α caused similar changes in PPARβ/δ and SIRT1 to those reported in obese patients. Unexpectedly, PPAR DNA-binding activity and the expression of PPARβ/δ-target genes was reduced following TNF-α stimulation, suggesting that the activity of this transcription factor was inhibited by cytokine treatment. Interestingly, the PPARβ/δ ligand GW501516 prevented the expression of inflammatory markers and the reduction in the expression of PPARβ/δ-target genes in adipocytes stimulated with TNF-α. Consistent with a role for NF-κB in the changes caused by TNF-α, treatment with the NF-κB inhibitor parthenolide restored PPAR DNA-binding activity, the expression of PPARβ/δ-target genes and the expression of *SIRT1* and *PPARβ/δ*. These findings suggest that the reduction in PPARβ/δ activity and SIRT1 expression caused by TNF-α stimulation through NF-κB helps perpetuate the inflammatory process in human adipocytes.

1. Introduction

Obesity, insulin resistance and type 2 diabetes mellitus are closely associated with low-grade chronic inflammation characterized by abnormal cytokine production [1]. The adipocyte plays a crucial role in this process, since this cell is a source of cytokines and chemokines (TNF-α, IL-6, MCP-1), which are secreted as a result of the activation of several signaling cascades involved in obesity-induced insulin resistance [2]. A number of studies have implicated chronic activation of the pro-inflammatory transcription factor NF-κB as part of one of these signaling pathways that link inflammation with obesity and insulin resistance [3,4]. For instance, overexpression of the NF-κB activator IκB kinase (IKK)β in mice results in increased inflammatory cytokine production and the onset of diabetes [5]. Furthermore, in human adipose tissue, inhibition of NF-κB suppresses the release of pro-inflammatory cytokines [6]. This transcription factor can be activated by a wide array of exogenous and endogenous stimuli. In mammals the NF-κB/Rel family includes five known members: p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), c-Rel, and RelB. The most abundant form of NF-κB is a heterodimer consisting of p50 and p65. In unstimulated cells, NF-κB is sequestered in the cytoplasm in an inactive form through the interaction with the IκB inhibitory proteins. In the canonical activation pathway, activation of cells by specific stimuli, such as the pro-inflammatory cytokine TNF-α, results in phosphorylation of IκB by the IKK complex, leading to its degradation by the 26 S proteasome. This releases NF-κB, which then translocates to the nucleus, where it activates the transcription of a wide variety of genes, such as those of *TNF-α* and *IL-6* [7].

Abbreviations: CPT-1b, carnitine palmitoyl transferase 1; HDL, high-density lipoprotein; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor-κB; PDK4, pyruvate dehydrogenase kinase 4; PGC-1α, PPARγ co-activator 1α; PPAR, peroxisome proliferator-activated receptor; SIRT1, sirtuin 1; SAT, subcutaneous adipose tissue; TNF-α, tumour necrosis factor-α; VAT, visceral adipose tissue

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Since the specific mechanisms linking the presence of low-grade chronic inflammation and the development of obesity-induced insulin resistance have only been partially elucidated (for review see reference [8]), the discovery of new key factors involved in this association might provide new pharmacological targets for preventing obesity-induced insulin resistance. Among these new factors, Peroxisome Proliferator-Activated Receptors (PPARs) could play an important role. PPARs are members of the nuclear receptor superfamily of ligand-inducible transcription factors that regulate the expression of genes involved in many important biological processes [9]. They form heterodimers with retinoid X receptors (RXRs) and bind to consensus DNA sites composed of direct repeats (DRs) of hexameric DNA sequences usually separated by 1 bp (DR1) [10]. In addition, PPARs suppress inflammation through diverse mechanisms, for example by reducing the release of inflammatory factors or stabilizing repressive complexes at inflammatory gene promoters [11–14]. The PPAR family consists of three members: PPARα (NR1C1 according to the unified nomenclature system for the nuclear receptor superfamily), PPARβ/δ (NR1C2) and PPARγ (NR1C3) [10]. PPARα and PPARγ are the targets for hypolipidemic (fibrates) and anti-diabetic (thiazolidinediones) drugs, respectively. Finally, activation of the third isotype, PPARβ/δ, by high-affinity ligands (including GW501516) has been proposed as a potential treatment for insulin resistance [15–18].

Another potential player in the relationship between inflammation and obesity-induced insulin resistance is sirtuin 1 (SIRT1). This is a prominent member of the family of NAD^+-dependent enzymes that deacetylate lysine residues on various proteins. It has recently been proposed that SIRT1 could play a role in the protection against proinflammatory responses in adipose tissue [19]. In fact, SIRT1 activation represses proinflammatory gene expression through NF-κB deacetylation at lysine 310 and improves insulin signaling, whereas exposure to a high-fat diet downregulates SIRT1 in white adipose tissue [19]. Interestingly, it has recently been reported that PPARβ/δ activation can increase the expression of *SIRT1* [20].

The aim of this work was to study whether the presence of inflammation in visceral adipose tissue (VAT) from severely obese insulin-resistant patients leads to changes in the levels of PPARβ/δ and SIRT1 that can perpetuate this process. Our findings demonstrate that severely obese insulin-resistant patients show enhanced inflammation in VAT that is accompanied by a reduction in SIRT1 protein levels and an increase in *PPARβ/δ* mRNA levels. By treating human adipocytes with TNF-α we reproduced in vitro the increase in PPARβ/δ expression and the reduction in SIRT1 levels found in obese insulin-resistant patients. However, the increase in PPARβ/δ expression was linked to reduced expression of its target genes and PPAR-DNA binding activity. Interestingly, treatment with either the PPARβ/δ agonist GW501516 or a NF-κB inhibitor prevented the changes caused by TNF-α. These findings indicate that TNF-α reduces PPARβ/δ activity and SIRT1 expression through NF-κB activation. Given the role of SIRT1 and PPARβ/δ in inflammation and insulin signaling, the changes induced by TNF-α in these genes may help to perpetuate the inflammatory process in human adipocytes.

2. Materials and methods

2.1. Materials

GW501516 was provided by Alexis Biochemicals (Lausen, Switzerland). [γ-^{32}P]dATP (3000 Ci/mmol) was purchased from Perkin Elmer (Waltham, MA). All other chemicals, except where specified, were from Sigma-Aldrich (St. Louis, MO).

2.2. Subjects

The study included a cohort of 23 severely obese subjects (Body mass index, BMI 57.4 ± 7.3 kg/m^2) recruited at the Malaga Clinic Hospital (Malaga, Spain) (Table 1). For inter-group comparisons we selected an age- and gender-matched population of 35 overweight subjects (BMI 26.2 ± 3.6 kg/m^2) recruited at the University Hospital Joan XXIII (Tarragona, Spain) as a control group.

Patients were excluded if they had cardiovascular disease, arthritis, acute inflammatory disease, infectious disease, or were receiving drugs that could alter their lipid profile or metabolic parameters at the time of inclusion in the study. None of the morbidly obese patients was being treated with insulin therapy, oral antidiabetic agents, or diet. The weight of all persons had been stable for at least 1 month, and none had renal involvement. The Hospitals' Ethics Committees approved the study, and informed consent was obtained from all participants.

VAT and subcutaneous adipose tissue (SAT) were obtained during bariatric surgery in the severely obese patients or during elective abdominal surgery procedures in control subjects. The biopsy samples were washed in physiological saline and immediately frozen in liquid nitrogen. Biopsy samples were maintained at −80 °C until analysis.

2.3. Laboratory measurements

Blood samples were collected after a 12-h fast. The serum was separated and immediately frozen at −80 °C. Serum biological parameters were measured in duplicate. Serum glucose, cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides (Randox Laboratories Ltd., Antrium, UK) were measured by using standard enzymatic methods. Low-density lipoprotein cholesterol was calculated according to the Friedewald formula. Insulin was analyzed via an immunoradiometric assay (Biosource International, Camarillo, CA). HOMA-IR was calculated from fasting insulin and glucose according to the following equation: HOMA-IR = fasting insulin (μIU/ml) × fasting glucose (mmol/l)/22.5. Circulating plasma IL-6 levels were measured by ELISA (Diaclone, bioNova, Barcelona, Spain). Serum levels of TNFα were measured with human TNFα high sensitivity (BLK Diagnostics International, Barcelona, Spain).

2.4. Cell culture

The human Simpson–Golabi–Behmel Syndrome (SGBS) cell line of preadipocytes was induced to differentiate to mature adipocytes as described previously [21]. Before applying the different stimuli, cells were seeded in duplicate in 6- or 12-well tissue culture plates and differentiated into mature adipocytes. At day 14 of differentiation, adipocytes were cultured for 6 h in DMEM/F-12 (Lonza, Barcelona, Spain) without serum and in the presence of 0.2% BSA, preincubated with or without 10 μM GW501516 for 30 min and then stimulated with either 100 ng/ml

Table 1
Clinical and anthropometric characteristics of the patients.

	Control (n = 35)	Obese-IR (n = 23)	p
Age (years)	44.5 ± 8.3	40 ± 0.4	ns
Gender (male/female)	23/12	9/14	ns
BMI (kg/m^2)	26.2 ± 3.6	57.4 ± 7.3	<0.001
Waist circumference (cm)	90.8 ± 13	146.2 ± 23.5	<0.001
SBP (mm Hg)	122.4 ± 13.3	139 ± 23.6	ns
DBP (mm Hg)	70.6 ± 9.4	84.5 ± 14.2	0.004
Cholesterol (mmol/l)	4.8 ± 1	5.1 ± 1.1	ns
HDL-cholesterol (mmol/l)	1.3 ± 0.3	0.9 ± 0.6	ns
LDL-Cholesterol (mmol/l)	2.9 ± 0.9	3.5 ± 1.1	ns
Triglycerides (mmol/l)	1.2 ± 0.7	1.4 ± 0.8	ns
Glucose (mmol/l)	5.3 ± 0.7	5.6 ± 1	ns
Insulin (pmol/l)	5.9 ± 4.5	31 ± 18.8	<0.001
HOMA-IR	1.4 ± 1.1	8.1 ± 5.9	<0.001
sIL-6 (pg/ml)	1.9 ± 1.4	6.1 ± 4.5	<0.001
sTNFα (pg/ml)	2.5 ± 1.4	4.2 ± 2.5	ns

The results are given as the mean ± SD. DBP: Diastolic blood pressure. SBP: Systolic blood pressure. ns: not significant.