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Lipocalin 2 produces insulin resistance and can be upregulated by glucocorticoids in human adipose tissue

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ABSTRACT

The adipokine lipocalin 2 is linked to obesity and metabolic disorders. However, its role in human adipose tissue glucose and lipid metabolism is not explored. Here we show that the synthetic glucocorticoid dexamethasone dose-dependently increased lipocalin 2 gene expression in subcutaneous and omental adipose tissue from pre-menopausal females, while it had no effect in post-menopausal females or in males. Subcutaneous adipose tissue from both genders treated with recombinant human lipocalin 2 showed a reduction in protein levels of GLUT1 and GLUT4 and in glucose uptake in isolated adipocytes. In subcutaneous adipose tissue, lipocalin 2 increased IL-6 gene expression whereas expression of PPARγ and adiponectin was reduced. Our findings suggest that lipocalin 2 can contribute to insulin resistance in human adipose tissue. In pre-menopausal females, it may partly mediate adverse metabolic effects exerted by glucocorticoid excess.

1. Introduction

The central role of adipose tissue in the regulation of systemic metabolism is well established. The wide range of signaling peptides and proteins produced by adipocytes, collectively known as adipokines, can contribute to the development of insulin resistance, type 2 diabetes and cardiovascular diseases (Fantuzzi, 2005). Production of adipokines is governed by a multitude of hormonal, metabolic and inflammatory factors (Karim et al., 2015; Balistreri et al., 2010). Numerous adipokines have been identified so far. However, a complete understanding of the diverse effect of these factors, and their interactions still remain intangible. Knowledge about the pathophysiological role of adipokines could help in defining new biomarkers to predict, diagnose and monitor metabolic diseases. They may also provide novel pharmacological mechanisms for prevention and treatment of such diseases.

The adipokine lipocalin 2 (Lcn2) is a 25 kDa protein also known as neutrophil gelatinase-associated lipocalin, siderocalin, or 24p3. It was first isolated from human neutrophils (Kjeldsen et al., 1993); however, it is also expressed and secreted abundantly by white adipose tissue (Kratchmarova et al., 2002; Lin et al., 2001; Wang et al., 2007).

Pro-inflammatory cytokines such as IFN γ, TNF α (Zhao et al., 2014) and agents promoting insulin resistance can induce Lcn2 gene expression. For example, dexamethasone, a synthetic glucocorticoid analog increases the Lcn2 mRNA levels in adipose tissue from mice (Yan et al., 2007) and in omental adipose tissue from female subjects (Fain et al., 2010). In contrast, our microarray analysis of human subcutaneous and omental adipose tissue from male subjects did not show any effect on dexamethasone-mediated Lcn2 gene expression (Pereira et al., 2014). Taken together, these studies suggest a gender-specific differential effects of glucocorticoids on adipose tissue function, that has been suggested previously (Lundgren et al., 2008).

Abbreviations: BCA, Bicinchoninic acid protein; BMI, body mass index; BSA, Bovine serum albumin; DMEM, Dulbecco's modified eagle medium; EDTA, Ethylenediaminetetraacetic acid; FBS, Fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT1, Glucose transporter-1; GLUT4, Glucose transporter-4; IL-6, Interleukin-6; TNF α, Tumor necrosis factor α; KRH, Krebs–Ringer media; Lcn2, Lipocalin 2; PBS-T, Phosphate Buffered Saline with Tween 20; PEST, Penicillin-streptomycin; PPARγ, Peroxisome proliferator-activated receptor gamma; rhLcn2, Recombinant human lipocalin 2; SVC, Stromal vascular cells; WST-1, Water-soluble tetrazolium-1.

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Previous studies in animal models have suggested a role for Lcn2 in systemic insulin sensitivity and glucose homeostasis (Wang et al., 2007; Yan et al., 2007). Lcn2 gene knockdown in adipose tissue attenuates insulin resistance related to obesity and aging (Law et al., 2010). On the other hand, Lcn2 gene expression is elevated in subcutaneous and visceral adipose tissue of obese patients (Catalan et al., 2009; Auguet et al., 2011) and obese (Wang et al., 2007) and diabetic mice (Yan et al., 2007). In addition, obese and type 2 diabetic patients show an increased levels of Lcn2 in both circulation and adipose tissue (Wang et al., 2007; Huang et al., 2012). Moreover, in diabetic patients, an elevated serum concentration of Lcn2 is reduced after treatment with the PPARγ agonist rosiglitazone (Wang et al., 2007). Thus, previous studies suggest an important link between Lcn2 and systemic metabolism. However, its role in human adipose tissue, glucose and lipid metabolism, as well as the underlying mechanisms, has hitherto not been described.

The present study aims to investigate the effect of glucocorticoids on Lcn2 expression in human subcutaneous and omental adipose tissue in both males and females. In addition, we also studied in this context, the effect of Lcn2 on glucose and lipid metabolism in human adipose tissue as well as the mechanisms involved. Finally, we assessed the effect of Lcn2 on the expression of pro-inflammatory cytokines and also explored for the first time the effect of Lcn2 on PPARγ and its downstream target gene adiponectin in human subcutaneous adipose tissue.

2. Materials and methods

2.1. Subjects

Paired human subcutaneous and omental adipose tissue samples were obtained from non-diabetic subjects (7M/14F, 19–76 years, BMI 23.9–55.8 kg/m^2) that underwent kidney donation (n = 10) at the Sahlgrenska University Hospital or bariatric surgery (n = 11) at the Uppsala University Hospital. Subcutaneous and omental adipose tissue were incubated with dexamethasone (Sigma Chemical, St. Louise, MO, USA) to study its effect on Lcn2 mRNA expression (n = 19). In addition, subcutaneous adipose tissue was obtained from non-diabetic volunteers (8M/26F, 20–77 years, BMI 20.2–37.3 kg/m^2) by needle aspiration after administration of local anesthetic lidocaine (Xylocain; AstraZeneca, Sweden) at the lower part of the abdomen. Subcutaneous adipose tissue samples obtained from needle biopsy were used to study the effect of recombinant human lipocalin 2 (rhLcn2) (Rand D Systems, Abingdon. UK) on glucose uptake (n = 9), lipolysis (n = 11), gene (n = 11) and protein (n = 8–9) expression of the glucose transporters GLUT1 and GLUT4 and gene (n = 11) expression of interleukin-6 (IL6), tumor necrosis factor α (TNFα), PPARγ and its downstream target gene adiponectin. In addition, the effect of dexamethasone on Lcn2 gene expression (n = 5) was also explored. Fasting blood samples were taken to measure the levels of plasma glucose, insulin, and lipids at the Department of Clinical Chemistry at the respective hospitals, and anthropometric measurements were done. Subjects with diabetes and other endocrine disorders, cancer or other major illnesses, as well as ongoing treatment with beta-adrenergic blockers, systemic glucocorticoids or immune-modulating therapies, were excluded from the study. The study was approved by the Regional Ethics Review Boards in Gothenburg (Dnr 336-07) and Uppsala (Dnr 2013/330). A written informed consent was obtained from all study participants. Anthropometric and biochemical characteristics of subjects included in the study are presented in Table 1. Furthermore, details of subjects used to study the effect of dexamethasone on Lcn2 gene expression are mentioned in Supplementary Table 1.

Table 1
Characteristics of subjects included in the study.

Variable	Surgical biopsy	Needle biopsy
Sex (male/female; n)	7M/14F	8M/26F
Menopausal state (pre/post)	8/5	10/16
Age (years)	43 ± 13	50 ± 19
BMI (kg/m^2)	37 ± 12.2	25.3 ± 5.7
Waist circumference (cm)	116 ± 25.5	91.1 ± 10.4
Subcutaneous adipocyte diameter (μm)	105.5 ± 12.1	112.2 ± 8.0
Omental adipocyte diameter (μm)	94.6 ± 20	–
HbA_{1c} (mmol/mol, IFCC)	33.6 ± 4.0	35.2 ± 4.7
Plasma glucose (mmol/L)	5.4 ± 0.6	5.8 ± 0.7
Serum insulin (mU/L)	13.8 ± 9.5	8.7 ± 3.5
HOMA-IR	3.2 ± 2.7	2.3 ± 1.0
Plasma triglycerides (mmol/L)	1.3 ± 0.6	1.2 ± 0.7
Plasma total cholesterol (mmol/L)	4.7 ± 1.0	5.5 ± 1.0
Plasma LDL-cholesterol (mmol/L)	3.0 ± 0.8	3.3 ± 0.9
Plasma HDL-cholesterol (mmol/L)	1.2 ± 0.4	1.5 ± 0.2

Data are mean ± SD; BMI, body mass index; HbA_{1c}, glycosylated hemoglobin (Normal range 27–46 mmol/mol, IFCC standard); HOMA-IR, homeostatic model assessment of insulin resistance index (fasting blood glucose × fasting insulin/22.5); LDL, low-density lipoprotein; HDL, high-density lipoprotein.

2.2. Adipose tissue incubation and isolation of adipocytes

Subcutaneous and omental adipose tissue obtained from surgical biopsies was cut into small pieces (approximately 5–10 mg). Tissue was then incubated with DMEM containing 6 mM glucose (Invitrogen Corporation, Paisley, USA), 10% BSA (fetal bovine serum, Invitrogen) and 1% penicillin-streptomycin (PEST, Invitrogen) in the presence or absence of dexamethasone at concentrations ranging from 0.003 to 3 μM for 24 h at 37 °C and 5% CO_2. At the end of incubation period, tissue was collected and snap frozen for further analysis of gene (n = 15–23) expression. Similarly, the tissue was incubated only with 0.3 μM dexamethasone to study its effects on Lcn2 protein levels (n = 4). Subcutaneous adipose tissue obtained by needle biopsy was incubated in DMEM medium with or without rhLcn2 100 ng/ml for 24 h as described above. After incubation, a part of the adipose tissue sample was snap frozen for the gene (n = 11) and protein (n = 9) expression and part were digested with collagenase (Sigma) as previously described (Lundgren et al., 2007). Isolated adipocytes were then filtered through a 250 μm pore size nylon mesh and used to perform glucose uptake (n = 9), lipolysis (n = 11) and cell size measurement. In addition, isolated adipocytes were also used to study the effect of short-term incubation with rhLcn2 (30 min) on glucose uptake capacity (n = 6). Subcutaneous adipose tissue obtained from needle biopsy was also incubated with dexamethasone as described above and used to study its effect on Lcn2 gene expression (n = 5).

2.3. Glucose uptake

Glucose uptake in adipocytes was performed according to the method described previously (Yu et al., 1997). Briefly, adipose tissue was digested with collagenase after 24 h of incubation as described above. Isolated adipocytes were then washed three times with 5-min intervals in glucose-free Krebs–Ringer media (KRH) supplemented with 4% BSA (Sigma), 150 nM adenosine (Sigma) and pH 7.4. Adipocytes were then diluted ten times in KRH media and incubated with or without insulin (25 and 1000 μU/ml, Actrapid, NovoNordisk, Bagsvaerd, Denmark) for 15 min, followed by an additional 45 min of incubation with D-[U-^{14}C] glucose (0.26 mCi/L, 0.86 μM, Perkin Elmer, Boston, MA, 161 USA). The reaction was stopped by transferring the cells into pre-chilled vials followed by separation from the medium by centrifugation through silicon oil (SERVA Electrophoresis GmbH, Heidelberg, Germany).