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Original article

Indicators of iron status are correlated with adiponectin expression in adipose tissue of patients with morbid obesity

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Abstract

Aim. – The aim of this study was to assess interactions between glucose and iron homoeostasis in the adipose tissue (AT) of obese subjects.

Methods. – A total of 46 obese patients eligible for bariatric surgery were recruited into the study. Anthropometric and biochemical characteristics were assessed, and biopsies of subcutaneous (SCAT) and visceral adipose tissue (VAT) performed. The mRNA levels of genes involved in iron and glucose homoeostasis were measured in their AT and compared with a pool of control samples.

Results. – Gene expression of hepcidin (*HAMP*) was significantly increased in the SCAT and VAT of obese patients, while transferrin receptor (*TFRC*) expression was reduced, compared with non-obese controls, suggesting a higher iron load in obese patients. Also, mRNA levels of adiponectin (*ADIPOQ*) were decreased in both SCAT and VAT in obese patients, and correlated negatively with hepcidin expression, while adiponectin expression was positively correlated with *TFRC* expression in both SCAT and VAT. Interestingly, *TFRC* expression in VAT correlated negatively with several metabolic parameters, such as fasting blood glucose and LDL cholesterol.

Conclusion. – Iron content appears to be increased in the SCAT and VAT of obese patients, and negatively correlated with adiponectin expression, which could be contributing to insulin resistance and the metabolic complications of obesity.

Keywords: Adiponectin; Adipose tissue; Hepcidin; Insulin resistance; Iron; Obesity

1. Introduction

Obesity is often associated with impaired iron homoeostasis and, in particular, low iron status [1,2]. Serum iron and transferrin saturation are frequently lower, and ferritin and soluble transferrin receptor levels higher, in obese compared with normal-weight individuals [1,2]. Yet, despite higher ferritin levels, iron stores are depleted, which might explain the reduced phenotypic expression of genetic haemochromatosis in obese patients [3].

Systemic iron homoeostasis is finely regulated by hepcidin, a 25-amino-acid peptide encoded by the *HAMP* gene [4–6]. Hepcidin targets the transmembrane iron exporter ferroportin-1

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[solute carrier family 40 member 1 (*SLC40A1*)], inducing its internalization and degradation and, thus, impairing duodenal iron absorption and iron-recycling from macrophages [7]. Although hepcidin is mainly produced by the liver, recent investigations suggest that this iron-regulating hormone might be significantly expressed in adipose tissue (AT) [8]. More important, serum hepcidin is elevated in obese patients [9], thereby leading to reduced iron stores. However, it is unclear whether *HAMP* mRNA levels are increased in obese white AT (WAT) [9,10], while the role of such adipose production and its contribution to serum hepcidin levels remain to be elucidated.

Obesity is known to impair glucose homoeostasis through its association with insulin resistance. This disorder is characterized by AT dysfunction [11], which, in particular, manifests as impaired secretion of adiponectin, an adipokine with insulin-sensitizing effects [12,13]. Thus, reduction of serum adiponectin levels in some obese patients could be contributing to the development of metabolic complications [14,15].

Previous studies have suggested the presence of crosstalk between iron and glucose metabolism in AT. For instance, investigations of 3T3-L1 adipocytes showed that insulin treatment induced translocation of intracellular transferrin receptors to the plasma membrane and intracellular iron accumulation [16]. Furthermore, Gabrielsen et al. [17] demonstrated, in two mouse models, that adiponectin is negatively associated with adipocyte iron content. In this study, iron levels were inferred from measurements of transferrin receptor 1 (*Tfrc*) mRNA expression, the levels of which are modulated by the presence of iron-responsive elements in the 3′ untranslated region of mRNA [18]. Mice fed a high-iron diet had higher adipocyte iron, lower adiponectin mRNA and lower plasma adiponectin levels, and more insulin resistance, as assessed by hyperinsulinaemic–euglycaemic clamp test [17]. The aim of the present study was to investigate in humans the correlation between iron parameters in subcutaneous (SCAT) and visceral AT (VAT), and the metabolic alterations seen in patients with severe obesity.

2. Methods

The present investigations were ancillary to a single-centre (CHU de Nantes, France) prospective interventional study (NCT01156519, registered at www.clinicaltrials.gov), and focused on obese patients who underwent bariatric surgery.

2.1. Patients and samples

A total of 38 women and 8 men were included in the present study. Inclusion criteria were defined as:

- age ≥ 18 years;
- eligible for weight-loss surgery, with a body mass index (BMI) ≥ 40 kg/m^2, or ≥ 35 kg/m^2 with at least one comorbidity, as recommended by international guidelines [19,20].

Exclusion criteria were:

- sepsis;
- recent surgery (within < 30 days);
- any recent severe acute conditions requiring hospitalization (within < 30 days);
- steroid treatment.

All participants provided their written informed consent before entering the study. The protocol was approved by the local ethics committee and performed according to French legislation.

Clinical data were collected at baseline (at the time of surgery). Venous blood samples were drawn after an overnight fast for determination of various plasma parameters reflecting liver [aspartate/alanine aminotransferases (AST/ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT)] and kidney (creatinine) function, carbohydrate and lipid homoeostasis [glucose, HbA_{1c}, total cholesterol, low-density/high-density lipoprotein (LDL/HDL) cholesterol, triglycerides], inflammation (C-reactive protein, CRP) and iron [ferritin, serum iron, transferrin, soluble transferrin receptor (sTfR)] metabolism. Plasma levels of insulin, leptin, adiponectin and hepcidin were also assessed. During weight-loss surgery, samples of SCAT (periumbilical) and VAT were collected from all eligible patients, and stored at −80 °C for further investigations.

2.2. Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA was isolated from frozen AT samples using TRIzol reagent (Life Technologies, Saint-Aubin, France), and an aliquot (500 ng) was reverse-transcribed using High-Capacity cDNA Reverse-Transcription Kits (Life Technologies), according to the manufacturer's protocol. Real-time quantitative PCR (RT-qPCR) was then performed with 384-well plates in a 7900 HT Fast Real-Time PCR System (Life Technologies), using SYBR Green to monitor cDNA amplification. Expression of human ribosomal protein large P0 (RPLP0) was used as the reference, and the $2^{-\Delta\Delta Ct}$ method was employed to obtain the relative expression of each selected gene. A pool of AT total RNA samples from 18 apparently healthy subjects (Ozyme, Montigny-le-Bretonneux, France) was used as the control. There was no available information as to whether they were from SCAT or VAT. The sequences of primers used to assess mRNA expression of adiponectin (*ADIPOQ*), hepcidin (*HAMP*), interleukin-6 (*IL6*), peroxisome proliferator-activated receptor γ (*PPARG*), transferrin receptor (*TFRC*) and ferroportin 1 (*SLC40A1*) are available on request.

2.3. Western blot analysis

Total protein extracts were obtained from SCAT and VAT samples by cell lysis, using a radioimmunoprecipitation assay (RIPA) buffer. After electrophoresis of about 30 μg of proteins, Western immunoblotting was performed with anti-SLC40A1 antibody (Santa Cruz Biotechnology, Heidelberg, Germany) and