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The *TGR5* gene is expressed in human subcutaneous adipose tissue and is associated with obesity, weight loss and resting metabolic rate

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ABSTRACT

Bile acids have emerged as a new class of signaling molecules that play a role in metabolism. Studies in mice have shown that the bile acid receptor *TGR5* mediates several of these effects but the metabolic function of *TGR5* in humans is less well established. Here we show that human adipose tissue *TGR5* expression is positively correlated to obesity and reduced during diet-induced weight loss. Adipose tissue *TGR5* expression was also positively correlated to resting metabolic rate. Our study indicates that human adipose tissue contributes to the *TGR5* mediated metabolic effects of bile acids and plays a role in energy expenditure.

1. Introduction

Bile acids are synthesized in the liver and play a central role in dietary lipid emulsification in the intestine. The majority of the secreted bile acids is taken up by the distal parts of the small intestine and return to the liver and the gall bladder via the enterohepatic circulation. Beyond their well established function in dietary lipid emulsification, bile acids are signaling molecules with metabolic effects mediated by specific receptors. The farnesoid X receptor (FXR) is the most studied receptor for bile acids. FXR is a nuclear receptor that regulates bile acid synthesis as well as lipid and glucose metabolism [1]. The G protein-coupled receptor *TGR5* (also denoted G protein-coupled bile acid receptor 1 or GBPAR1) has also been found to be a functional bile acid receptor [2]. In 2006, Watanabe et al. showed that bile acid administration augmented energy expenditure in mice [3], and that these effects were mediated via the *TGR5* and its effects on the thyroid hormone activating enzyme iodothyronine deiodinase type II (DIO2) in brown adipose tissue (BAT). In addition, they showed that bile acid treatment of human skeletal myocytes increased both DIO2 activity and oxygen consumption, indicating that similar mechanisms are relevant also in humans. More recently, *TGR5* activation has been shown to induce glucagon-like peptide-1 (GLP-1) release in mice, indicating a direct role for *TGR5* in glucose homeostasis [4].

Despite these advances in understanding of *TGR5*, much less in known about *TGR5* function in humans and its expression in other metabolically relevant organs such as adipose tissue. The aim of this study was therefore to investigate the potential role of adipose tissue *TGR5* in human metabolism.

2. Materials and methods

The regional ethics committee in Gothenburg approved these studies.

2.1. Sib Pair study

The swedish obese subjects (SOS) Sib Pair study consists of 154 nuclear families with BMI discordant sibling pairs (BMI difference $\geqslant 10\ kg/m^2$), resulting in a study population consisting of 732 subjects [5]. The subjects were extensively phenotyped [5], including anthropometric measurements and determination of resting metabolic rate (RMR) in a ventilated hood [6]. Subcutaneous adipose

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tissue needle biopsies were obtained and used for gene expression analysis. For the current study, complete data from 353 siblings and 86 parents were available for the analysis.

2.2. Very low calorie diet (VLCD) study

The very low calorie diet (VLCD) study was performed to investigate gene expression changes in adipose tissue of obese subjects during weight loss induced by caloric restriction. Twenty-eight obese subjects (20 women and 8 men, age 39.7 ± 12.7 years, BMI 36.3 ± 3.7 kg/m^2) were treated with VLCD (450 kcal/day) for 12 weeks [7,8]. Subcutaneous adipose tissue needle biopsies were obtained at the start of the VLCD treatment (day 0) and three times during the VLCD treatment (weeks 2, 6, and 12). After 12 weeks of VLCD treatment, the mean weight loss was 19%.

2.3. Perithyroid and perirenal adipose tissue studies

Perthyroid adipose tissue surgical samples were obtained from 24 patients undergoing surgery in the thyroid region for malignancies or endocrine disorders. Clinical characteristics of the patients have been described previously [9]. Perithyroid adipose tissue biopsies containing BAT was identified by expression analysis of uncoupling protein 1 (*UCP1*) and histological analysis. Samples with high *UCP1* expression was classified as BAT positive (BAT+, $n = 9$). Paired subcutaneous white adipose tissue biopsies from the surgical incision area were also obtained from the same nine patients (2 men and 7 women, age 47 ± 21 years, BMI 23 ± 2 kg/m^2).

Biopsies of perirenal adipose tissue were obtained from 55 healthy kidney donors. The perirenal adipose tissue samples were screened for *UCP1* expression and samples with high *UCP1* expression (samples from 4 men and 6 women) were classified as BAT positive (BAT+) samples. These samples constituted the BAT+ group. A control group ($n = 10$) with low perirenal adipose tissue *UCP1* expression (classified as BAT− samples) was matched to the BAT+ group based on sex, age and BMI. The subjects in the BAT+ group had an average age of 42 ± 14 years and a BMI of 26 ± 2. The subjects in the BAT− group had an average age of 44 ± 9 years and a BMI of 26 ± 3.

2.4. Gene expression analysis

Total RNA was isolated from adipose tissue using the RNeasy lipid tissue midi kit (Qiagen, Chatsworth, CA) or the phenol–chloroform extraction method of Chomczynski and Sacchi [10].

Gene expression in adipose tissue from the Sib Pair study and in the perithyroid adipose tissue was analyzed study using Human Genome U133 plus 2.0 arrays (Affymetrix, Santa Clara, CA). Gene expression in the perirenal adipose tissue samples were analyzed by (Affymetrix Gene 1.0 ST arrays at the Uppsala Array Platform, Uppsala, Sweden). All arrays were analyzed according to the manufacturer's instructions. Expression data were analyzed using the RMA algorithm (Affymetrix). *TGR5* expression was assessed using probe sets 1552501_a_at and 8048249 for Human Genome U133 plus 2.0 and Expression assay Gene 1.0 ST arrays, respectively.

Adipose tissue total RNA from the VLCD study was reversed transcribed using the High Capacity cDNA RT kit (Life Technologies, Paisley, UK) according to the manufacturer's protocol. Reagents for real-time PCR analysis of *TGR5* (Hs00544894_m1) and low-density lipoprotein (LDL) receptor-related protein 10 (LPR10) (Hs00204094_m1) were purchased from Life Technologies and used according to the manufacturer's instructions. cDNA was used for real-time PCR in the Applied Biosystems PRISM 7900HT Sequence Detection System (Life Technologies) using default cycle parameters. A standard curve was plotted for each primer-probe set with a serial dilution of cDNA synthesized from pooled RNA. All samples and standards were analyzed in triplicate and *LRP10* gene expression was used as a reference gene [11].

2.5. Gene ontology (GO) enrichment analysis

GO enrichment analysis was performed using the DAVID web–accessible program [12,13] (http://david.abcc.ncifcrf.gov). Transcripts significantly correlated with *TGR5* expression were identified in the Sib Pair study using Spearman correlation. Accounting for multiple testing, a threshold of $p < 9.1 \times 10^{-7}$ was used as a cut off for declaring statistical significance (Bonferroni correction). This yielded a total of 1755 negative and 1767 positivity correlated transcripts which were included separately in the GO enrichment analysis. The analysis was limited to the GOTERM_BP_FAT, GEOTERM_CC_FAT and GEOTERM_MF_FAT options and Human Genome U133 Plus 2.0 was used as a background.

2.6. Statistical analysis

Statistical analyses were performed using the SAS software package (v. 9.1.3, SAS Institute Inc., Cary, NC) or and PASW Statistics (Chicago, IL, USA). Quantitative data were transformed towards normal distribution using Box–Cox power transformations. Outliers beyond three standard deviations from the trait mean were excluded. Correlations between clinical traits and *TGR5* expression were analyzed using the MIXED procedure in SAS. In the linear mixed models, we used a "sandwich estimator" of the covariance matrix to adjust for non-independence among family members. This asymptotically yields the same parameter as ordinary least squares or regression methods while standard errors and, consequently, hypothesis tests are adjusted for the family relatedness. Comparisons in gene expression between BAT and WAT were performed using Student's *t*-test (paired or unpaired as appropriate). Gene expression during weight loss was analyzed using a one-way repeated measures analysis of variance (ANOVA) with Greenhouse–Geisser correction. Comparisons between time points were assessed by Bonferroni corrected post-hoc tests.

3. Results

3.1. TGR5 adipose tissue expression

Initially, we investigated the link between *TGR5* adipose tissue expression and obesity. Among the Sib Pair families, *TGR5* expression was positively correlated with BMI ($r = 0.40$, $p < 0.0001$), independent of age, sex, family membership and generation. In line with this finding, obese subjects treated with VLCD displayed a drastic reduction of adipose tissue *TGR5* expression determined by a repeated measures ANOVA ($F(1.4, 38) = 38$, $p < 0.0005$; Fig. 1). Compared to day 0, adipose tissue *TGR5* expression was approximately half as low during the entire dieting period and post-hoc tests using the Bonferroni correction showed that *TGR5* levels were reduced compared with day 0 at all subsequent time points measured (weeks 2, 6 and 12, $p < 0.0005$ for each test; Fig. 1).

3.2. Gene ontology enrichment analysis

To gain insights into functions in adipose tissue that may be related to *TGR5* expression, Gene ontology enrichment analysis was performed using transcripts positively or negatively correlated to *TGR5* expression in subcutaneous adipose tissue in the Sib Pair offspring study. Transcripts negative correlated to *TGR5* expression displayed highly significant (adjusted *p*-values down to 3×10^{-20}) enrichment of several GO terms relating to mitochondria and ribosome (Table 1). Enrichment analysis of transcripts