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OMENTAL GENE EXPRESSION OF ADIPONECTIN CORRELATES WITH DEGREE OF INSULIN SENSITIVITY BEFORE AND AFTER GASTRIC BYPASS SURGERY

Jiegen Chen, PhD¹, Anna Spagnoli, MD², and Alfonso Torquati, MD, MSCI¹

¹Department of Surgery, Duke University

²Department of Pediatrics, University of North Carolina

Abstract

Background and Aim—Circulating adiponectin is known to correlate negatively with insulin resistance in patients with obesity and diabetes. The aim of this study was to assess the effect of gastric bypass (GB) surgery on adiponectin gene expression in subcutaneous and omental adipose tissues.

Methods—Adipose tissues and plasma were obtained from 25 subjects undergoing GB surgery, 15 non-obese subjects, and 12 subjects after GB surgery. Real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) was used for analysis of the adipose tissues. Adiponectin expression was normalized for GAPDH and expressed as percentage of subject-matched subcutaneous expression which was given an arbitrary value of 100%. Insulin resistance was assessed by the homeostatic model assessment (HOMA). Circulating adiponectin was assayed by ELISA.

Results—Omental adiponectin gene expression was 5-fold higher in subjects after GB when compared with age matched morbidly obese subjects before GB ($P<0.01$). There were not statistical differences in omental adiponectin gene expression observed in subjects after GB and age matched non-obese subjects. For the entire cohort of subjects, there was a significant negative correlation between omental adiponectin expression and insulin resistance expressed by HOMA values ($r=-0.62$, $P<0.001$). Circulating adiponectin was significantly lower ($p<0.05$) in the obese group than in the non-obese and post-GB groups.

Conclusion—Omental adiponectin gene expression significantly increase after GB surgery reaching levels equal to age matched non-obese subjects. Omental adiponectin expression has a significant negative correlation with the insulin resistance status.

Keywords

adiponectin; gastric bypass surgery; bariatric surgery; omentum; adipose tissue; insulin resistance

INTRODUCTION

Large epidemiologic studies reveal that the risk for insulin resistance increases from the very lean to the very obese [1]. However, not all types of obesity are associated with increased

Correspondence to: Alfonso Torquati MD, MSCI, Associate Professor of Surgery, Department of Surgery, Duke University, 407 Crutchfield Street, Durham, NC 27704, alfonso.torquati@duke.edu.

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risk of metabolic and cardiovascular complications [2]. Individuals with peripheral fat distribution in the gluteo-femoral regions are less prone to develop type 2 diabetes (T2DM) and cardiovascular disease (CVD) than individuals with abdominal fat distribution [2]. Furthermore, the amount of intra-abdominal fat strongly correlates with insulin resistance and can account for most of the variability in insulin sensitivity in the obese population [3]. Abdominal visceral fat is an important link between the many facets of the metabolic syndrome (i.e. glucose intolerance, hyperinsulinemia and hypertriglyceridemia) and other features such as hypertension and altered high-density lipoprotein (HDL) and very-low-density lipoprotein (VLDL) levels [4, 5]. The “portal paradigm”, built up from several clinical observations, stated that the complications of obesity are mainly attributable to increases in visceral adipose tissue mass [6]. As compared with subcutaneous adipocytes, visceral adipocytes have high basal lipolysis, are highly sensitive to catecholamines and are poorly sensitive to insulin [7]. These traits are magnified when visceral adipocytes become hypertrophic [8]. As a result, enlarged visceral fat stores tend to flood the portal circulation with free fatty acids (FFA) at metabolically inappropriate times when FFA are unlikely to be oxidized, thus exposing tissues to excessive FFA levels and giving rise to insulin resistance syndrome and beta-cell failure [9]. However the central and sole role of FFA in the pathogenesis of insulin resistance has been challenged by the discovery of leptin, the first peptide hormone demonstrated to be secreted by adipose tissue, and by the discovery of the protein now generally known as adiponectin, produced almost exclusively by adipocytes [10]. Since that time it has been recognized that adipose tissue is the site of expression of a large number of peptides that relate to metabolic regulation, including known cytokines such as TNF- α and IL-6 and known proteins with other functions such as angiotensinogen (potentially involved in raising blood pressure), plasminogen activator inhibitor-1 (potentially involved in generation of a prothrombotic state) and some of the acute-phase proteins that are markers of inflammation [11]. These proteins secreted by adipose tissue are now generally known as adipokines. Adipose gene expression of TNF- α and PAI-1 are elevated with obesity [12–14], while the expression levels of adiponectin are lower [15, 16]. Reduction in visceral fat mass correlates with decreases in the serum levels of many of these pro-inflammatory adipokines and increase of adiponectin [17]. Therefore, the aim of this study was to test the hypothesis that significant and sustained weight loss, induced by gastric bypass (GB) surgery, increase adiponectin gene expression in the omental fat depot.

METHODS

The study approved by the local Institutional Review Board was conducted at the Vanderbilt University Medical Center. Participants were enrolled among subjects status post GB surgery (with a least 12 months follow up) undergoing an elective abdominal surgical procedure (e.g. cholecystectomy, abdominal wall hernia repair, and/or abdominoplasty), subjects with class II and III obesity undergoing GB surgery, and non-obese (BMI: 20–29) control subjects requiring an elective abdominal surgery.

Obese and normal weight controls were selected to match age distribution of the post-GB group. The study employed the following exclusion criteria: diagnosis of neoplastic disease, inflammatory bowel disease, acute cholecystitis, and pregnancy.

During the surgical procedure, immediately after entering the abdominal cavity, two <0.5-cm³ biopsies of abdominal subcutaneous and intra-abdominal omental fat tissue were obtained. The tissue samples were washed in PBS solution and then soaked in RNAlater preservative solution (Qiagen, Courtaboeuf, France) and stored at –80°C until analysis. All the samples were stored in the same –80°C freezer and then analyzed together. Total RNA was extracted from the adipose tissue biopsies using the RNeasy total RNA Mini kit (Qiagen). The integrity of total RNA was checked by electrophoresis through an agarose gel.

Adiponectin gene expression was studied by quantitative, real-time RT-PCR using the specific protocol for the iCycler iQ Detection System (BioRad, Hercules, CA, USA) with SYBR green fluorophore. Reactions were performed in a total volume of 20 μ L—including 10 μ L 2x SYBR Green PCR Master Mix (Applied Biosystems), 5 μ L of each primer at 5 μ M concentration, and 1 μ L of the previously reverse-transcribed cDNA template. The PCR primer sequence for adiponectin and GAPDH was chosen based on the sequences available in GenBank (www.ncbi.nlm.nih.gov/Genbank). Melting curves were used to determine the specificity of the gene products, which was subsequently confirmed by running the PCR products on agarose gels. The threshold cycle (CT) value for each reaction, reflecting the amount of PCR needed to identify a target gene was calculated. Specimens were run in duplicate and the CT values averaged. GAPDH was used as internal control housekeeping gene to normalize the PCRs for the amount of RNA added to the reverse transcription reactions. The data was analyzed using the $2^{-\Delta\Delta CT}$ method [18] where $\Delta\Delta CT = (CT_{\text{adiponectin}} - CT_{\text{GAPDH}})_{\text{omentum}} - (CT_{\text{adiponectin}} - CT_{\text{GAPDH}})_{\text{subcutaneous}}$ that allows to determine the fold change in the target gene in the omentum compared to subcutaneous fat from the same subject.

A fasting blood sample was also obtained from each subjects immediately before the induction of general anesthesia and immediately spun in a refrigerated (4°C) centrifuge at 3,000 rpm for 10 minutes. The extracted plasma was used for glucose and insulin level determinations. Plasma glucose concentration was determined by the glucose oxidase method. Immunoreactive insulin was determined in plasma with a double-antibody system. Insulin resistance was assessed by the homeostatic model assessment (HOMA): [fasting plasma glucose (mmol/l) $\times$ fasting insulin (μ U/ml)/22.5]. The extracted plasma was used for adiponectin determinations by high-sensitivity enzymatic assay (Millipore, Inc.).

Cryopreserved human subcutaneous adipose stem cells (hASCs) purchased from Zen-Bio, (Zen-Bio Inc., RTP, NC) were maintained and differentiated according to the supplier's specifications. Differentiated adipocytes maintained in AM-1 medium (Zen-Bio Inc., RTP, NC) were exposed for 24 hours to 5, 10 and 100 ng/ml of des-(1–3)-IGF-I (des-IGF-I) (Diagnostic System Laboratories), then harvested by Trizol reagent (Invitrogen Corp., Carlsbad, CA).

Statistical Analysis

Comparisons of continuous variables between the groups were completed by unpaired Student's t tests. Categorical variables were compared using the chi-square test or Fisher's exact test. Time course experiments were analyzed by ANOVA. The SPSS statistical software program (version 17.0, SPSS, Chicago, USA) was used for all analyses. All tests were 2-tail. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Abdominal adipose tissues and plasma were obtained from 25 subjects undergoing GB surgery, 15 non-obese subjects, and 12 subjects after gastric bypass at the average follow-up of 19 months after the procedure.

As shown in Table 1, the three groups showed no significant differences in terms of age, gender and racial distribution. The post-GB group had a mean BMI significantly higher ($P < 0.01$) than the non-obese control group but significantly lower than the obese group ($P < 0.001$). The demographics, postoperative weight loss and comorbidities resolution observed in the post-GB cohort are similar to those observed in the general population of post-GB patients followed in our practice.

The differential adiponectin gene expression in the two adipose tissues was significantly different among the three groups. As shown in the Figure1, omental adiponectin gene expression was 5-fold higher in morbidly obese subjects after GB surgery when compared with matched morbidly obese subjects and non-obese controls ($P<0.01$). The difference in omental adiponectin gene expression observed in subjects after GB surgery and matched non-obese control was not statistically significant.

The parameters relative to the glucose metabolism and circulating adiponectin of the three groups are shown in Table 2. Subjects status post-GB and non-obese controls had similar levels of plasma glucose and insulin levels. Consequentially, the insulin sensitivity calculated as HOMA index in these groups was analogous. As expected, obese individuals undergoing GB surgery had significant insulin resistance as demonstrated by their higher HOMA indexes ($P<0.001$ vs. non-obese and post-GB groups). Circulating adiponectin levels were significantly higher ($p<0.05$) in the obese group than non-obese and post-GB groups.

For the entire cohort of subjects, there was a significant negative correlation between omental adiponectin expression and insulin resistance expressed by HOMA-IR values ($r=-0.62$, $P<0.001$).

To investigate temporal regulation of adiponectin mRNA expression during adipogenesis, human ASCs were differentiated into mature adipocyte over a 13 days period. As shown in Figure 2A, adiponectin expression was significantly increased during adipogenesis ($p<0.05$) reaching the highest level at day 13 (mature adipocyte status). To define the role of inflammation cytokines on adiponectin expression, adipocytes were incubated with 12.5 ng/ml of TNF α . As shown in Figure 2B, TNF α significantly decreased ($P<0.05$) adiponectin expression in adipocytes, in a time-course fashion.

DISCUSSION

In the current study, adiponectin gene expression was determined in plasma, subcutaneous and omental adipose tissues of individuals after GB surgery and compared with age matched obese and non-obese individuals. The results of our study data clearly indicate that adiponectin mRNA expression is selectively up-regulated in the omental adipose tissue of subject after significant weight loss induced by GB surgery. The levels of adiponectin gene expression in the omentum are significantly higher than the one observed in age matched obese subjects and similar to those observed in non-obese matched subjects. This is a very important finding because adiponectin is the most abundant adipose tissue secretory protein and has a mechanistic role in the pathogenesis of insulin resistance and atherosclerosis [11, 15]. In fact, adiponectin has insulin-sensitizing effects and inhibits expression of key atherosclerotic proteins and actions such as TNF- α [19]. Therefore, the selective up-regulation of adiponectin in the omental fat depot might be one of the main cause for the improved glucose metabolism [20] and reduced CVD risk [21] observed in patients who underwent GB surgery. A previous study has demonstrated that the negative correlation of adiponectin levels and visceral adiposity is stronger than between adiponectin levels and subcutaneous adiposity [22]. However in their paper they were unable to identify the mechanism by which plasma levels were reduced in individuals with visceral fat accumulation. Our data clarify that decreased expression in the omental fat of obese individual is responsible for such a change.

Previous studies have shown that plasma adiponectin levels are negatively associated with the insulin-resistant state irrespective of gender or ethnicity [23] and significant reduction of fat mass following bariatric surgery is associated with increased plasma adiponectin concentrations [17, 24, 25]. Changes in adiponectin gene expression are mostly related to

size and differentiation status of adipocytes. In obese individual adipocytes are large and not well differentiated. These cells produce lower levels of adiponectin and higher levels of PAI-1 and TNF α . After significant weight loss adipocytes became smaller and well differentiated. This new status is characterized by high levels of adiponectin and low levels of inflammatory adipokines [26]. Circulating levels of adiponectin are direct expression of the adipose tissue expression because this protein is secreted exclusively by mature adipocytes [19]. However, there are no studies aimed to evaluate which adipose tissue depot is the major contributor to the increased levels of circulating adiponectin observed after GB surgery. In studies involving medical weight loss intervention, adiponectin adipose tissue gene expression either increased [27] or did not change [28–30]. However in all these studies, the resulting weight loss was less than 15% of body weight. A recent study from Coughlin CC et al [31] was the first to examine the effect of weight loss induced by bariatric surgery on adiponectin transcription in the adipose tissue. However, differently from our study adiponectin gene expression was determined only in the subcutaneous fat that is not the one linked to the metabolic syndrome [19]. Their data show that marked weight loss induced by GB surgery increases adiponectin gene expression in both upper- and lower-body subcutaneous fat. This increase in adipose tissue adiponectin production resulted in an increase in plasma adiponectin concentrations, which likely contributed to the decrease in insulin resistance observed after weight loss. Our study now clarifies that the omental fat contributes more than the subcutaneous fat to the circulating levels of adiponectin. However because our study was not designed to gather data about compartmental distribution of fat in the obese groups, a potential difference between the groups in central adiposity may have affected adiponectin levels.

An equally intriguing finding was that our results demonstrate statistically significant negative correlations between omental adiponectin and insulin sensitivity as estimated by the HOMA index. Previous studies [32, 33] have shown negative correlations between circulating adiponectin and insulin resistance. However, our study is the first to demonstrate that the adiponectin produced in the omental adipose tissue is the major contributor to this correlation. This finding is consistent with the data recently presented by Lin et al [17]. These authors have shown that 6 months after GB surgery, decreases in visceral fat measured by computed tomography were more strongly related to increases in adiponectin and decreases in CRP than were changes in general adiposity or subcutaneous adipose tissue.

In summary, omental adiponectin gene expression significantly increased after GB surgery reaching levels equal to age and gender matched non-obese control subjects. Omental adiponectin expression has a significant negative correlation with insulin sensitivity status.

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Figure 1.

Relative Adiponectin RNA levels normalized to GAPDH RNA levels in subcutaneous and omental adipose tissue collected from obese patients, patients at least 12 months after RYGB, and non-obese controls. Data presented as mean $\pm$ SEM.; * = $P < 0.01$ vs. non-obese control and post-GB.

Figure 2.

A. Adiponectin mRNA levels during adipogenesis. B. Time-course effect of TNFα on adiponectin mRNA expression in cultured adipocytes. Data presented as mean $\pm$ SEM.

Table 1

Demographic and anthropometric measurements

Variable	Non-Obese n=15	Post-GB n=12	Obese n=25
Gender (M/F)	6/9	1/11	6/19
Age (years)	46 ± 12	43 ± 12	43 ± 9
BMI (kg/m ²)	24.5 ± 2.7	29.7 ± 5.1 *	48.0 ± 11.3 **
Race	13C, 2AA	11C, 1AA	22C, 2AA, 1H
Type II Diabetes	0/15	1/12	8/25

* = P <0.01 vs. Non-obese Control and Obese

** = P <0.001 vs. Non-obese Control and Post-RYGB

C=Caucasian; AA=African-American; H=Hispanic

Table 2

Plasma Metabolism Parameters

Variable	Non-Obese n=15	Post-GB n=12	Obese n=25
Insulin (μ IU/mL)	7.1 $\pm$ 1.9	7.2 $\pm$ 2.3	18.8 $\pm$ 6.2 *
Glucose (mmol/L)	4.8 $\pm$ 0.4	5.1 $\pm$ 0.5	6.4 $\pm$ 0.6 *
HOMA	1.6 $\pm$ 0.3	1.7 $\pm$ 0.6	5.4 $\pm$ 1.7 *
Adiponectin (μ g/ml)	4.7 $\pm$ 2.7	4.8 $\pm$ 2.7	2.4 $\pm$ 1.4 **

* = P <0.01 vs. Non-obese Control and Post-RYGB

** = P <0.05 vs. Non-obese Control and Post-RYGB