Omentum Adiposity is Linked with Resistin Gene Expression

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Keywords: Resistin; CD68; Gene expression; Adipose tissue; Drug

Abbreviations: TG: Triglycerides; T2DM: Type 2 Diabetes Mellitus; BMI: Body Mass Index; OM: Omental Adipose Tissue; SC: Subcutaneous Adipose Tissue; IDF: International Diabetes Federation; GUSB: β-Glucuronidase; TRBP: TATA-Binding Protein; MO: Morbidly Obese; HbA1c: Glycated hemoglobin; AST: Aspartate Aminotransferase; ALT: Alanine Transaminase; T2D: Thiazolidinediones; RSG: Including Rosiglitazone

Introduction

Recent studies have shown the potential contribution of visceral adipose tissue, via its secretory function, to the pathogenesis of obesity and related disease such as diabetes, hypertension, and cardiovascular disease. Adipocytes secrete adipokines, such as resistin [1]. In obesity fat cell size and number increases. Lipolysis in adipocytes results from breakdown of adipocyte triglycerides (TG) into nonesterified fatty acids and glycerol through the action of lipases and TG hydrolases [2,3]. Adipose tissue is a critical endocrine organ involved in regulating obesity, insulin resistance and type 2 diabetes mellitus (T2DM) [4,5]. Resistin is a circulating hormone, expressed primarily in adipocytes, known to antagonize insulin action in mice [6]. Resistin belongs to a family of secreted proteins (resistin like molecules) that share an unusual cysteine-rich motif at the amino terminal ending [7]. Janke et al. have reported higher expression of the resistin gene in human preadipocytes that decreased during adipogenic differentiation to very low levels [8]. Consequently, resistin expression was weakly detectable in mature human adipocytes. The expression of the resistin gene has been reported to be lower in the obese, diabetes, tubby, and viable yellow mouse models of genetically determined obesity, suggesting that formation of resistin may not be related to insulin resistance in these models [9]. The expression of the resistin gene in fat cells and adipose tissue from overweight subjects has been reported to be almost absent [10], making the question of the role of resistin in human obesity and diabetes controversial.

Resistin and some adipokines, such as TNF-α and IL-6 are produced and secreted by both adipocytes and macrophages in T2DM [6,11]. Increased production of these adipokines occurs with particularly visceral obesity, by both the adipocytes and the nonfat cells, mostly macrophages that infiltrate the adipose tissue [12,13]. While resistin’s role as an inflammatory marker in human and rodent physiology is well known, its role in obesity and T2DM is still under investigation. It is important to note that human resistin is expressed in much higher levels in macrophages than in adipocytes [14]. Several human studies have failed to show relationship of circulating resistin levels with Body Mass Index (BMI) or other metabolic parameters [8,15,16]. In contrary, there are reports detecting increased serum resistin levels in obesity and/or type 2 diabetes [17-19]. It has been suggested by Nagaev et al. that oral antidiabetic treatment, namely thiazolidinediones greatly downregulated resistin gene expression, and neutralization of the resistin protein enhanced blood glucose uptake and insulin sensitivity [10]. The aim of this study was to evaluate the site-specific differences in adipose tissue resistin gene expression among individuals with and without T2DM. Conventional drug therapy relationships with adipose tissue resistin expression were also detected.

Abstract

Background: This study demonstrated site-specific adipose tissue resistin gene expression differences in individuals with and without type 2 diabetes mellitus. The relationship between conventional drug therapy and adipose tissue resistin gene expression was also determined. Paired omental and subcutaneous adipose tissues were excised during elective surgery from morbidly obese and obese patients.

Methods: Resistin mRNA expressions were determined by qPCR. All tissue sections also were also analyzed for their resistin and CD68 protein expressions by immunohistochemistry.

Results: No significant difference for omental and subcutaneous adipose tissue resistin mRNA expression levels were found among morbidly obese and obese study groups. The omental adipocytes resistin mRNA expressions increased with macrophage number both in the omental and subcutaneous fat. Resistin mRNA expressions of the omental and subcutaneous fat were in positive correlation. As the omental adipocytes radius decreased, the macrophage number increased in subcutaneous fat. In the omentum the adipocytes diameter and areas increased, in correlation with macrophage number. The anti-diabetic drug use was found to increase adipocyte size both in the omentum and subcutaneous fat.

Conclusions: The higher resistin gene expression in the omental fat may induce the increase in size and number of adipocytes, thus leading to elevation in omental fat mass.
Materials and Methods

Subjects and tissue sampling

Omental adipose tissue (OM) and subcutaneous adipose tissue (SC) biopsies were obtained from 10 morbidly obese patients (6 women, 4 men) and 5 overweight controls (2 women, 3 men) during elective surgery. The morbidly obese (age: 43.20 ± 4.35) and overweight (age: 44.20 ± 3.95) study groups were matched for age and sex. Obesity and T2DM were diagnosed according to International Diabetes Federation (IDF) guidelines [20]. Among morbidly obese patients, 6 (60%) were suffering from T2DM, 4 (40%) from hypertension, 3 (30%) from dyslipidemia and 6 (60%) from metabolic syndrome. Subjects with cancer, collagen diseases, endocrinopathies, secondary hypertension (renal artery stenosis, glomerulonephritis), and diabetic microangiopathic complications were excluded from the study. The drug use for the morbidly obese subjects were: 40% for hypertensives, 60% for antidiabetics; 30% for antiobesity drugs. None of the overweight subjects were using any drugs. When the data of morbidly obese patients were analyzed, a great majority of the group was observed to have insulin resistance.

All the individuals in the study group gave their written informed consent, and the project was approved by the local ethics committee. All the fat tissues were collected during operation in the form of biopsy. One half of the tissue samples were immediately transferred into liquid nitrogen, and subsequently stored at -80ºC prior to total RNA isolation, the other part was fixed in 10% neutral buffered formaline for immunohistochemistry.

Biochemical measurements

All biochemical measurements were determined by using enzymatic reference methods.

RNA isolation and real-time PCR analysis

The total RNA from OM and SC fat tissues were extracted with High Pure RNA Tissue Kit (Roche, Germany) according to the manufacturers instructions. Total RNA samples were diluted in DNase-RNase free sterile water and stored at -80ºC until use. Transcriptor HiFi cDNA synthesis kit was used for cDNA synthesis in DNase-RNase free sterile water and stored at -80ºC until use. The drug use for the morbidly obese subjects were: 40% for hypertensives, 60% for antidiabetics; 30% for antiobesity drugs. None of the overweight subjects were using any drugs. When the data of morbidly obese patients were analyzed, a great majority of the group was observed to have insulin resistance.

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Table 1: The demographic and biochemical characteristics of the obese groups.

<table>
<thead>
<tr>
<th></th>
<th>Morbid Obese (n=10)</th>
<th>Obese (n=5)</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SE; Median (Range)</td>
<td>Mean ± SE; Median (Range)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>47.4 ± 3.14; 42.4 (40-72)</td>
<td>28.61 ± 1.27; 27.7 (26-33)</td>
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<tr>
<td>Fasting glucose (mg/dl)</td>
<td>139.40 ± 24.22; 112.0 (78-326)</td>
<td>101.2 ± 7.13; 105.0 (78-121)</td>
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<tr>
<td>HbA1c (%)</td>
<td>6.83 ± 0.84; 6.0 (5-13)</td>
<td>5.08 ± 0.17; 5.10 (5-6)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>48.00 ± 20.86; 24.0 (14-209)</td>
<td>35.00 ± 12.83; 17 (13-75)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>55.67 ± 16.51; 34.0 (16-160)</td>
<td>38.60 ± 16.01; 19 (7-90)</td>
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<tr>
<td>Hematocrit (%)</td>
<td>40.03 ± 2.07; 39.8 (32-48)</td>
<td>38.36 ± 3.67; 38.4 (24-49)</td>
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<tr>
<td>Free T3 (pmol/L)</td>
<td>3.16 ± 0.11; 3.23 (2.7-3.5)</td>
<td>3.19 ± 0.12; 3.05 (2.9-3.2)</td>
</tr>
<tr>
<td>Free T4 (ng/dl)</td>
<td>1.02 ± 0.06; 1.0 (0.8-1.2)</td>
<td>1.00 ± 0.07; 1.10 (0.9-1.3)</td>
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<tr>
<td>TSH (µIU/mL)</td>
<td>4.31 ± 1.05; 3.3 (3.0-10.6)</td>
<td>4.01 ± 1.03; 3.0 (2.75-8.9)</td>
</tr>
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Morbid obese: BMI≥40, Obese: BMI≥25. Values are represented as mean ± SE
TSH: Thyroid-stimulating hormone, p<0.001

Immunohistochemistry staining for resistin and CD68

Resistin and macrophage specific CD68 immunohistochemical staining were performed by streptavidin-biotin-peroxidase method on 10% neutral buffered formalin-fixed, paraffin embedded adipose tissue specimens. The sections of 4µm thickness were placed onto slides coated with poly-L-Lysine, (PLL, Sigma, St. Louis, MO) then deparaffinized in xylene and rehydrated in graded alcohol. Histostain Plus Bulk Kit (Zymed, USA) was used for immunoperoxidase staining. Immunohistochemistry procedure was performed using a combination of microwave oven heating for antigen retrieval and standard streptavidin-biotin-peroxidase method. Endogenous peroxidase activity was blocked by hydrogen peroxide (3%). Each section was then incubated for 15 minutes at room temperature with blocking solution. Sections were incubated with resistin (dilution 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or CD68 (prediluted; LabVision Corp., USA) for 1 hour at room temperature, then washed with PBS. Specific staining was performed with biotinilated universal secondary antibody, horseradish peroxidase streptavidin-complex, and amino-ethyl-carbazole as chromogen. As for negative control, distilled water was performed instead of primary antibody.

Morphometric analysis

Morphometric analysis for CD68 positive cells was performed for sections, using a light microscope. Automatic image analysis of sections for calculation of tissue areas was performed with Leica IM50 (version4.0) morphometric analysis software (Leica, Germany). Cell counts were performed using a x20 objective in 10 different fields. Resistin immunoreactivity was evaluated semiquantitatively according to the staining intensity.
Semiquantitation of immunoperoxidase staining

Immunostaining was evaluated using a LeicaDM2500 light microscope (LeicaMicrosystems,Wetzlar,Germany). Resistin staining was evaluated in 10 randomly selected all fields of tissue sections using a x20 objective. The immunostaining of cells was scored as: no staining (−), weak staining (+), moderate staining (++) and strong staining (+++).

Statistical analysis

Data are expressed as numbers and percentages for discrete variables and as mean ± SE, for continuous variables. Baseline differences between study groups were examined by Mann-Whitney U test. Variables were analyzed by Spearman’s correlation test using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) for windows. Statistical significance was taken as p<0.05.

Results

Basic subject characterization

The demographic and biochemical characteristics of the study groups as a function of obesity are shown in Table 1. Fasting glucose, glycated haemoglobin (HbA1c), aspartate aminotransferase (AST), alanine transaminase (ALT), hematocrit, Free T3, Free T4 and thyroid stimulating hormone (TSH) levels were not statistically different in the morbidly obese (MO) and obese study groups. BMI was higher in morbidly obese patients than that of overweight patients (p<0.001).

Immunohistochemistry results

In morbidly obese group resistin immunopositivity was observed to be more intense in adipocytes cytoplasm and also non-fatty cells in intracellular area of the omental and subcutaneous tissues in comparison to the obese group (Figure 1 and Table 2). The omental resistin immunopositivity was more intense in the intercellular area in the morbidly obese compared to obese group (Figure 1 and Table 2). No immune reaction was observed in negative controls.

The macrophage specific CD68 immunopositive cells were observed in the intercellular area (Figure 2). The CD68 immunopositive macrophage number was found to be higher in morbidly obese group compared to obese both in omental and subcutaneous fat tissues significantly. In morbidly obese group, especially in the omentum, the CD68 immunopositive macrophage number was higher to that of subcutaneous fat tissue (Figure 2 and Table 3).

Resistin gene expression in omental adipose and subcutaneous adipose tissues

The resistin expression rates in omental and subcutaneous adipose tissues by using TBP as reference gene are given in Table 4. In detail, omental and subcutaneous adipose tissue resistin expression levels were not found to differ significantly among morbidly obese and obese study groups. The omental and subcutaneous resistin expression rates were not found to differ significantly in morbidly obese and obese groups. The morbidly obese patients omental fat adipocytes (0.290 ± 0.220) were found to have higher resistin expression rates in comparison to subcutaneous adipocytes (0.060 ± 0.050), where expression was nearly absent. Resistin gene expression was absent in omental and subcutaneous adipocytes of obese patients (Table 4).

The resistin gene and CD68 positive macrophage number correlations both in omentum and subcutaneous fat were evaluated. The omental adipocytes resistin expressions were found to increase

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**Figure 1:** Resistin immunopositive fat cells and non fat cells are seen in subcutaneous (A) and omental (B) adipose tissues of obese group, and in subcutaneous (C) and omental (D, E) adipose tissues of morbid obese group. Negative control (F). (Bar: 20μ)

**Figure 2:** CD68 immunopositive cells are seen in subcutaneous adipose tissue of obese (A) and morbid obese (B) and also in omental adipose tissue of obese group (C). CD68 labelled cells (↑) between degenerated fat cells (D) and enlarged fat cells (↑)(E) are seen in the omentum of the morbid obese. The omentum of morbid obese group (F,▲) has more CD68 positive cells between fat cells compare to the obese group (B). (Bar: 20μ)
The antidiabetic drug use was found to change CD68 expression in human preadipocytes which decreased during adipogenic differentiation to very low levels. Consequently, resistin gene expression was hardly detectable in mature human adipocytes [8]. In our study, the measured resistin gene expressions we considerably low in type 2 diabetic and morbidly obese patients fat tissues. Our gene expression results were consistent with immunohistochroemical results obtained for morbidly obese fat tissues. No resistin gene expression was found in our obese patients subcutaneous or omental fat tissues, whereas weak resistin immunopositivity was found in both sites. The inconsistency of the gene and protein expression data in our obese group may be due to the undetectable low expression of resistin gene below the cut-off values.

It is well established that thiazolidinone (TZDs), including rosiglitazone (RSG), function as potent insulin-sensitizing agent by raising insulin-dependent glucose uptake and reducing hepatic glucose output [20-24]. Steppan et al. have also shown that TZD reduced resistin gene expression in 3T3-L1 adipocytes and adipose tissue in vivo [6]. In agreement to the results of McCrernan et al. obtained from a rodent model [25], our data indicate that, resistin secretion from SC abdominal adipocytes increases under hyperinsulimnic conditions, and this rise may be as a result of oral antidiabetic drug use. The findings of Patel et al. verifying that resistin expression and secretion in human macrophages in vitro together with the identification of a putative peroxisome proliferator-activated receptor- binding site in the promoter of the resistin gene strongly support the idea that the resistin gene can be directly transcriptionally regulated by the peroxisome proliferator activated receptor-mediated mechanism [14]. In the present study, together with the decrease of omental adipocytes radius measurements, the CD68 positive macrophage number increased in subcutaneous fat. As the adipocytes diameter and area increased, the CD68 positive macrophage number also raised in our samples omental fat tissues. The oral antidiabetic drug use was found to enlarge adipocyte size, both in the omental and subcutaneous fat tissues. The greater size and number of adipocytes observed in the antidiabetic drug or external insulin users in our study may demonstrate that, these type of drug use leads to weight gain during diabetes treatment. This observation is the major finding on pharmacogenomic results of our study.

In rodent models in vivo and in the murine 3T3-L1 adipose cell line, Lazar and co-workers demonstrated that recombinant resistin reduced glucose uptake [6]. In addition, obesity and insulin resistance are known to be associated with a chronic mild inflammation as determined by increased plasma C-reactive protein, IL-6, IL-8, and TNF levels in patients and different animal models of obesity [26,27]. This systemic inflammatory response is located in adipose tissue [28]. Inflammatory adipokine expression has been reported to be increased in obesity in mice [29-31] and humans [32]. To determine whether resistin exerts similar effects in human tissue, we examined the effects of human resistin on glucose and metabolic diseases in human omental and subcutaneous adipose tissues. According to our results, the degree of obesity was found to decrease with increased resistin gene expression in the omental fat tissue.

Since adipocytes have special roles in systemic insulin sensitivity [33], it is possible that inflammatory cytokines may trigger T2DM. Because inflammatory adipokines are mostly produced in obese adipose tissue, predominantly by nonfat cells such as macrophages adipose tissue macrophages are assumed to contribute to the pathogenesis of T2DM and thus the metabolic syndrome [12,31]. This suggestion is...
supported by findings that obesity related adipose tissue inflammation is characterized by an increment of adipose tissue macrophages. In our study we detected significant increase in omental macrophages and resistin protein in morbidly obese patients fat tissues.

Conclusion

According to our results, the degree of obesity has been observed to change parametrically as a response to resistin gene expression in the omental fat. Therefore, by benefiting from this relationship in our future studies alternative drugs or gene therapy might be a chance for improvement of morbidity obesity.

Acknowledgement

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References

