Human Mesenteric Adipose Tissue Plays Unique Role Versus Subcutaneous and Omental Fat in Obesity Related Diabetes

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Key Words

Obesity • Diabetes • Adipose tissue • Lipolysis

Abstract

Background/Aims: Obesity is a common and rapidly growing health problem today. Obesity is characterized by the increase of body fat and an excess of total body fat and, in particular, visceral fat accumulation, is considered to be a risk factor for type 2 diabetes mellitus. To determine whether the malfunction of the mesenteric adipose tissue plays an important role in the diabetic related metabolic syndrome, in this study, lipolysis and gene expression in the subcutaneous, omental and mesenteric adipose tissue of the diabetic subjects were evaluated. **Methods:** Lipolysis and real time PCR were utilized to determine adipocyte function. **Results:** Basal adipose tissue glycerol release is higher in diabetics than that of the non diabetics in all three fat depots. Isoproterenol (ISO) significantly increases glycerol release in subcutaneous, omental and mesenteric adipose tissues of non diabetic subjects but it stimulated glycerol release was significantly impaired in all three fat depots of the diabetic subjects. Gene

expression studies indicate that leptin, Peroxisome proliferator-activated receptor-gamma (PPAR-gamma), Fatty acid translocase (FAT/CD36) and 11 β -hydroxysteroid dehydrogenase (HSD) gene expression were significantly up regulated in the mesenteric adipose tissue of the diabetic patients. **Conclusion:** Human mesenteric adipose tissue in obese diabetic subjects has high basal glycerol release and impaired isoproterenol stimulated glycerol release. The obesity-related gene expressions in the mesenteric adipose tissue are up regulated, suggesting that the alterations of these genes in mesentery adipose depot may play a critical role in insulin resistance of type 2 diabetes and metabolic syndrome.

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Introduction

Obesity is one of the greatest public health concerns today. Obese patients face an increased risk of mortality and morbidity due to obesity associated diseases such as Type II diabetes mellitus, hypertension, obstructive sleep apnea, coronary artery diseases and cancer [1-3]. Obesity is characterized by increases in body and adipose tissue

weight resulting from increasing numbers of adipocytes [4-6]. The major function of adipose tissue is to store excess energy as neutral fat during periods of nutritional excess and these stored fats are used as metabolic energy during periods of nutritional deficiency, such as starvation. However, excess accumulation of fats, particularly in visceral adipose tissue including omental and mesenteric, was shown to lead to subsequent metabolic syndromes [7, 8]. The delivery of free fatty acids to the liver from visceral adipose tissue may contribute to pathological symptoms such as hyperinsulinemia, hypertriglycemia and glucose intolerance [8]. Lipolysis rate has been identified to be different from various fat depots, with higher lipolytic activity in the visceral adipose tissue than in the subcutaneous one. Numerous studies have also emphasized that visceral fat deposition is correlated to the metabolic complications of obesity, such as hypertension, cardiovascular disease, and non insulin dependent diabetes mellitus [7, 9, 10]. However, few studies have been performed to examine the specific role of the mesenteric adipose tissue on metabolic syndrome development due to the fact that the mesenteric adipose tissue is very hard to obtain. Therefore, whether malfunction of the mesenteric adipose tissue plays an important role in insulin resistance and metabolic syndrome remain uncertain. In this study, three adipose depots were obtained from gastric bypass surgery and functions of these adipose depots from non-diabetic and diabetic subjects were evaluated. Our results indicate that mesenteric adipose tissue of the diabetic subjects has high basal glycerol release and is less sensitive to the lipolytic effects of catecholamines. Larger differences in gene expression were also identified between omental and mesenteric adipose tissues, suggesting that malfunction of the mesenteric adipose tissue may play a more important role in the diabetic-related metabolic and vascular complications.

Materials and Methods

Subjects

Total 18 obese patients were selected from gastric bypass surgery which include ten type 2 diabetes and eight without type 2 diabetes. Patients underwent medical screening that included medical history, physical examination, standard laboratory hematology, blood chemistry, urine and anthropometric measurements. Body weight was stable for at least 3 months prior to the surgery. The study was approved by the Medical Ethics Committee of University of Alabama at Birmingham.

Serum profile

Blood will be collected in a heparinized tube for measurement of serum hormones. Glucose levels were measured by the glucose oxidase method with the YSI 2300 STAT Plus glucose analyser (YSI, Yellow Springs, OH, USA). Serum triglyceride levels were measured by an enzymatic method (Roche Diagnostics, Montreal, QC, Canada).

Adipose tissue collection

Fat tissues were obtained from abdominal subcutaneous, omental and mesenteric depots from obese subjects underwent gastric bypass operation.

Glycerol level measurement

One hundred milligrams of the adipose tissue were cut into small pieces and suspended in 900 μ L of Krebs-Ringer bicarbonate buffer. After one hour incubation of equilibrium, the samples were incubated with shaking at 37°C under an atmosphere of 5% CO₂-95% O₂ in the final concentration of 10⁻⁷ M of ISO (Sigma) for 60 minutes. Glycerol was measured using a colorimetric triglyceride kit from Sigma (Infinity Triglyceride Reagent), which uses glycerol kinase and glycerol phosphate oxidase. Data for NEFA release are expressed as nmol/mg protein [11].

RNA preparation

Total RNA was isolated from three different adipose depots using TRIZOL Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The integrity of the RNA was verified by ethidium bromide staining of ribosomal RNA bands separated on a 1% agarose gel. The RNA was stored at -70°C until use.

mRNA quantification

Quantitative real-time polymerase chain reaction (PCR) was used to determine relative mRNA levels. First, complementary DNA was synthesized from 1 μ g of total RNA using random hexamers and TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA). Sequence-specific primers and probes for leptin, adiponectin, PPAR- γ , FAT/CD36 and HSD were designed according to guidelines for quantitative TaqMan real-time PCR using Primer Express 1.0 software (Applied Biosystems). The probes were labeled at the 5'-end with the reporter dye 6-carboxy-fluorescein and at 3'-end with the quencher 6-carboxy-tetramethyl-rhodamine. All real-time PCR reactions were carried out using the TaqMan Universal PCR Master Mix according to the manufacturer's protocol (Applied Biosystems). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers will be added to same amount of RT reaction for PCR, as internal control, and the 180 bp product of GAPDH will be detected in each PCR reaction. The samples will be run in triplicate on an ABI Prism 7000 sequence detection system (Applied Biosystems) using the following cycling parameters: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Each reaction well contained cDNA, TaqMan PCR Master Mix, 900 nmol L⁻¹ of each primer and 250 nmol L⁻¹ of TaqMan probe. TaqMan reagent-based chemistry uses a fluorogenic

Fig. 1. Different adipose depot basal glycerol release. Panel A. NEFA in human abdominal subcutaneous (SC), omental (OM) and mesenteric (MS) adipose tissue was measured from the diabetic and non diabetic obese adults. Panel B. Effect of isoproterenol on glycerol release in abdominal subcutaneous (SC), omental (OM) and mesenteric (MS) adipose tissue. Samples of adipose tissue were incubated, and NEFA was measured as described in methods. All values are the mean $\pm$ SEM (n=8). Statistical significance is represented by asterisks. *P < 0.05 vs. control.

probe to enable detection of a specific PCR product as it accumulates during PCR cycles. When both quencher and reporter are attached to the probe, reporter dye emission is quenched. During each extension cycle at 60 °C, the polymerase cleaves the reporter dye from the probe. After being separated from the quencher, the reporter dye emits its characteristic fluorescence. A standard curve for each primer pair will be obtained using serial dilutions of WAT cDNA. A standard curve for serial dilutions of GAPDH rRNA will be similarly generated. The Ct represents the threshold cycle number at which the fluorescence signal is linearly increasing above background. The Ct readings for unknown samples will be used to calculate the amount of each target gene relative to GAPDH. The relative standard curve method (Applied Biosystems) will be used to calculate the amplification difference between the before and after naringenin treatment for each primer set.

Calculations and statistics

Data are presented as mean $\pm$ S.E.M. The means of parameters within groups were statistically analyzed using the paired Student's t-test. Means of parameters between groups (obese vs. diabetic obese) were compared using the two-tailed, independent Student's t-test. The significance level was set at 0.05.

Results

General parameters

Clinical and biochemical characteristics of the 10 obese subjects with type 2 diabetes and 8 obese subjects without type 2 diabetes who underwent bariatric surgery are shown in Table 1. Degree of obesity was similar in the two groups.

Basic glycerol release

Free fatty acid (FFA) has been shown to be main factor related to insulin resistance [8]. To determine

	Obese without diabetes (n=8)	Obese with diabetes (n=10)
Age	48 $\pm$ 5	50 $\pm$ 3
BMI (kg/m ²)	46.1 $\pm$ 3.1	44.9 $\pm$ 6.5
BP (mmHg) Systolic	135 $\pm$ 23.1	143 $\pm$ 12.5
Diastolic	91 $\pm$ 7.5	95 $\pm$ 3
Triglyceride (mg/dL)	125.3 $\pm$ 21.3	223 $\pm$ 31.4**
Total cholesterol (mg/dL)	188.9 $\pm$ 12.9	243.4 $\pm$ 31.4**
Glucose (mg/l)	101 $\pm$ 8.9	178 $\pm$ 11.3**

Table 1. Changes of BMI, lipids and blood glucose in obese patient. ** P < 0.01 compared with non-diabetic control group.

whether the malfunction of the human mesenteric adipose tissue plays unique role in obesity related diabetes, we compared basal lipolysis determined by glycerol release in three different adipose tissues of the diabetic subjects. Subcutaneous, omental and mesenteric adipose tissues from the patients underwent gastric bypass were obtained and adipose tissue glycerol release was determined. Our results indicate that glycerol release in subcutaneous (SC), omental (OM) and mesenteric (MS) of the diabetic subjects was significantly increased. Particularly, glycerol release from MS was significantly increased in the diabetic subjects compared to that of the obese subjects without diabetes (Figure 1A).

Effects of isoproterenol on FFA release

Lipid mobilization and release of glycerol are modulated by the sympathetic nervous system. Catecholamines are the most potent regulators of glycerol release in human adipocytes through stimulatory β 1- and β 2-adrenoreceptors or inhibitory α 2-adrenoreceptors

Fig. 2. Determination of leptin and adiponectin mRNA levels at three different adipose depots. Real time PCR was utilized to determine gene expression. Leptin and adiponectin mRNA levels were quantified by densitometry and standardized to GAPDH signal. Panel A represents leptin gene expression in three different adipose tissues. Panel B represents adiponectin gene expression in three different adipose tissues. Values represent mean $\pm$ SEM (n=8). The values for leptin mRNA levels in mesenteric adipose tissue were statistically higher than that of the omental and subcutaneous adipose tissues. $P < 0.05$.

[12]. To examine whether the isoproterenol induced glycerol release in these three different depots was altered in the diabetic subjects, the ability of the isoproterenol induced adipocyte glycerol release was examined. One hundred milligrams of the adipose tissue were cut into small pieces and suspended in 900 μ L of Krebs-Ringer bicarbonate buffer. After one hour incubation of equilibrium, the samples were incubated with the final concentration of 10^{-7} M of ISO (Sigma) for 60 minutes. Glycerol content in medium was measured. Our results indicate that isoproterenol significantly increased glycerol release in three adipose depots in non diabetic patients and the subcutaneous adipose tissue has the highest response. However, isoproterenol stimulated glycerol release was significantly impaired in diabetic patients. Isoproterenol induced glycerol release was blunted in all three fat depots, especially, the mesenteric adipose tissue (Figure 1B).

Fat mass and obesity related gene expressions among subcutaneous, omental and mesenteric adipose tissues of the diabetic subjects

A) Genes with an endocrine function. Due to the fact that ISO-induced adipocyte glycerol release was significantly impaired in the obese diabetic subjects, we then examined leptin and adiponectin gene expressions which are associated with adipocyte adipogenesis. Total RNA was obtained from three different adipose depots and real time PCR was utilized to determine leptin and adiponectin gene expressions. Our results indicate that leptin expression was significantly upregulated but adiponectin was down regulated in omental and

mesenteric depots of the diabetic subjects (Figure 2). To determine whether leptin and adiponectin protein were also altered, we examined serum leptin and adiponectin levels. Our results indicate that serum leptin level was significantly increased in both obese and diabetic subjects (43 ± 4 ng/ml non-diabetic vs 56 ± 7 ng/ml diabetic, $p < 0.05$) but adiponectin level was significantly decreased (8.2 ± 1.5 μ g/ml non-diabetic vs 5.9 ± 1.3 μ g/ml, diabetic, $p < 0.05$), indicating that leptin and adiponectin gene expressions are positively associated with protein expression.

B) Genes with an autocrine/paracrine function.

To determine whether the expressions of the PPAR- γ and FAT/CD36 in the mesenteric adipose tissue were altered in type 2 diabetics, these gene expressions were determined using real time PCR. Our results indicate that the PPAR- γ mRNA levels in MS was significantly increased in the diabetic subjects compared to that of subcutaneous and omental adipose tissues ($P < 0.001$) (Figure 3). Particularly, PPAR- γ was significantly upregulated in the mesenteric adipose tissue. Consistent with PPAR- γ gene expression, FAT/CD36 mRNA levels were also significantly upregulated in the mesenteric adipose tissue.

11 β -HSD1 has been identified to play an important role in the regulation adipocyte size and function. To examine whether 11 β -HSD1 plays a key role in free fatty acid accumulation in three different adipose depots, 11 β -HSD1 gene expression was evaluated using real time PCR. Our results indicate that 11 β -HSD1 mRNA was moderately increased at SC and OM but significantly upregulated in MS of the diabetic subjects (Figure 4).

Fig. 3. Determination of PPAR- γ and FAT/CD36 mRNA levels at three different adipose depots. Real time PCR was utilized to determine FAT/CD36 mRNA expression. mRNA levels were quantified by densitometry and standardized to GAPDH rRNA signal. Panel A represents PPAR gene expression in three different adipose tissues. Panel B represents FAT/CD36 mRNA expression in three differential adipose tissues. Values represent mean $\pm$ SEM (n=8). The values for PPAR- γ and FAT/CD36 in MS were statistically higher than that of the omental and subcutaneous adipose tissues. $P < 0.05$.

Discussion

In this study, we have identified that basal glycerol release is increased in the diabetic patients in all three adipose depots but isoproterenol induced glycerol release was significantly impaired. Leptin, PPAR- γ , FAT/CD36 and HSD gene expressions were significantly up-regulated in the mesenteric adipose tissue of the diabetic patients.

There is now growing evidence showing that abnormal fat metabolism may be the culprit of insulin resistance syndrome and type II diabetes. Visceral fat tissue is an important contributor to plasma FFA levels, as its adipocytes are more lipolytically active than subcutaneous adipocytes and are more sensitive to fat-mobilizing enzymes [8, 13, 14]. Previous studies indicate that basal lipolysis was similar in intraabdominal tissues from normal men and women [12]. However, FFA concentrations and FFA flux are higher in obese individuals than in lean persons and visceral (i.e. predominantly mesenteric and omental) adipose tissue mass, but not total or subcutaneous adipose tissue mass, correlates with insulin resistance [8, 15, 16]. Whether specific abdominal fat compartments, for example visceral abdominal fat (VAT) compared to subcutaneous abdominal fat, carry greater metabolic and cardiovascular risks remains more controversial [17, 18], especially in subjects with DM2. Previous study indicates that adipocytes from obese subjects show increased lipolytic responses to catecholamines and different depot of the adipose tissues has different response in catecholamine-induced lipolysis [19]. Our results demonstrate that isoproterenol induced

Fig. 4. The expression levels of 11 β -HSD1 mRNA in the subcutaneous, omental and mesenteric adipose tissues from obese subjects. 11 β -HSD1 mRNA levels were quantified by densitometry and standardized to GAPDH signal. Values represent mean $\pm$ SEM (n=8). The values for 11 β -HSD1 mRNA in the mesenteric adipose tissue were statistically higher than that of the omental and subcutaneous adipose tissues. $P < 0.05$.

glycerol release was significantly increased in obese non diabetic subjects which are consistent with the typical features of visceral fat which show increased sensitivity to the lipolytic action of catecholamine [20-22]. However, our results indicate that the function of the mesenteric adipose tissue is different from that of the subcutaneous and omental adipose tissues in the diabetic subjects.

Glycerol release is greater in the mesenteric adipose tissue of the diabetic subjects than that of the omental or subcutaneous depots and the sensitivity of the mesenteric fat to catecholamine-induced glycerol release is also significantly impaired in Type 2 diabetes. The explanation for this may be that basal glycerol release in the diabetic subjects was very high and the maximal glycerol release induced by isoproterenol is therefore limited. High level of basal glycerol release in the mesenteric fat in the diabetic subjects may play a key role in insulin resistance and metabolic syndrome. To determine whether the genes related to adipose lipolysis are also altered in the different adipose depots, we have examined the gene expression of the adipose tissue at the diabetic patients. Several genes have been involved in adipose tissue function [8, 23, 24]. Leptin acts as a critical link between adipose tissue and hypothalamic regulation of appetite and energy homeostasis, as well as glucocorticoid metabolism. A significant increase in leptin mRNA from the mesenteric adipose tissue was observed and the leptin mRNA expression in the mesenteric adipose tissue is higher than that of other depots, which may contribute directly to the increase in circulation levels and leptinemia. Adiponectin is an adipocyte specific protein possessing insulin sensitizing, anti-atherogenic and anti-inflammatory properties [25, 26]. A significant decrease in adiponectin mRNA from the mesenteric adipose tissue was observed and the adiponectin level in serum was also decreased, indicating that leptin and adiponectin mRNA expression is positively associated with the protein expression. Our current results are consistent with previous reports that serum leptin level is high but adiponectin level is low in the diabetic subjects [27, 28].

Previous results indicated that the expression of PPAR- γ mRNA is increased in adipose tissue of the obese subjects [29, 30]. To determine whether PPAR- γ is involved in the mesenteric adipose tissue function, we examined PPAR- γ gene expression in the mesenteric adipose tissue. Our results are consistent with the previous report that there is no difference in transcription factor PPAR- γ gene expression between the subcutaneous and omental adipose tissue [31]. However, our results indicate that PPAR- γ gene is highly expressed in the mesenteric adipose tissue and PPAR- γ gene expression is significantly upregulated, particularly in the mesenteric adipose tissue in the diabetic subjects,

supporting that PPAR- γ plays an important role in the mesenteric adipose tissue lipolysis.

Long-chain fatty acids (LCFAs) cross the plasma membrane via a protein-mediated mechanism involving one or more LCFA-binding proteins. Among these, FAT/CD36 has been identified as key LCFA transporter in the heart, skeletal muscle and adipocytes, where it is regulated acutely and chronically by insulin [32-34]. In skeletal muscle, FAT/CD36 expression and/or subcellular distribution is altered in obesity and type 2 diabetes [35]. Our current study indicates that basal FAT/CD36 expression is not different in the subcutaneous, omental and mesenteric adipose tissues in the non-diabetic subjects. However, FAT/CD36 expression is upregulated in three adipose depots of the diabetic subjects. Especially, FAT/CD36 is significantly upregulated in the mesenteric adipose tissue, suggesting that the mesenteric adipose tissue FAT/CD36 may respond in a more dynamic manner to metabolic disturbances than subcutaneous and omental adipose tissues. 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) is a key enzyme found in adipose tissue, the liver and the central nervous system. It provides sufficient intracellular cortisol levels when circulating levels are low by converting inactive circulating cortisone to active cortisol [36]. Obese subjects had elevated 11 β -HSD1 mRNA levels and enzyme activity, suggesting that it may be involved in the metabolic disturbance [8, 37]. Our results indicate that 11 β -HSD1 mRNA is significantly upregulated in three adipose depots of the diabetic subjects. Particularly, mesenteric adipocytes has the highest 11 β -HSD1 mRNA expression which is consistent with previous report [37]. The positive association of 11 β -HSD1 mRNA in mesenteric adipose tissue with diabetes may reflect a key role in visceral fat accumulation during diabetic development. Increasing 11 β -HSD1 mRNA in mesenteric adipose tissue could suggest that high level of 11 β -HSD1 may be the reason of the upregulation of leptin, PPAR- γ and FAT/CD36 gene expression.

In conclusion, fat depot origin affects the capacity of human adipocyte lipolysis. Mesenteric adipocytes have different responses to ISO compared to that of the subcutaneous and omental adipocytes in the diabetic subjects. The alterations of adipose tissue lipolysis and gene expression in mesentery adipose depot may play a critical role for insulin resistance of type 2 diabetes and metabolic syndrome.

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