



DIFFERENCES IN ADIPONECTIN GENE EXPRESSION: ADIPONECTIN GENE EXPRESSION IS HIGHER IN GLEUTEAL THAN IN ABDOMINAL ADIPOSE TISSUE

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ABSTRACT

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Objective: Obesity down regulated the adiponectin gene expression, but differences in adiponectin gene expression in gluteal adipose tissue (GAT) and in abdominal adipose tissue (AAT) are not well known. Therefore, the purpose of this study was to examine the differences in adiponectin gene expression in GAT and in AAT.

Material & Methods: Nineteen healthy obese middle aged men (aged 41.4±6.1 years; mean ± SD) Adipose tissue (AT) biopsies from the subcutaneous abdominal and subcutaneous gluteal depots were obtained.

Results: Adiponectin gene expression was 22.7% higher in subcutaneous GAT than in subcutaneous AAT.

Conclusion: Adiponectin gene expression is higher in GAT than in AAT, suggesting GAT to be more important for circulating adiponectin levels.

Key words: Adiponectin, Gene expression, Gluteal adipose tissue, Abdominal adipose tissue.

"Fat distribution could contribute in adiponectin gene expression difference."

INTRODUCTION

Evidence is accumulating that AT releases a number of active metabolic compounds, including proinflammatory cytokines.¹ These compounds include leptin, adiponectin, resistin, angiotensinogen, tumour necrosis factor- α (TNF- α), plasminogen activator inhibitor type-1, interleukin (IL)-6 and adiponectin. Adiponectin, the product of the transcript-1 (apM1) gene, which is exclusively and most abundantly expressed in AT, is a 244 amino-acid protein structurally homologous to collagen VIII, collagen X and complement fraction C1q.² Epidemiological evidence has indicated that adiponectin gene expression and circulating adiponectin levels were reduced in patients with insulin resistance and type 2 diabetes,³ cardiovascular disease,⁴ essential hypertension,⁵ obesity⁶ and in smokers.⁵ Several clinical studies have observed that adiponectin mRNA levels are reduced in obesity, in which mean adipocyte size is increased, and increased in lean individuals with smaller adipocyte size.⁷ In a study of 1,356 women ages 60–85, those with excessive peripheral fat had less atherosclerosis (determined by aortic calcification scores), and the quartile with both the highest amount of central fat and peripheral fat seemed to be partially protected by the high percentage of peripheral fat mass as reflected in a number of measured risk factors.⁸ Preferential accumulation of AT in the gluteofemoral region “gynoid obesity” has been associated with protective effects especially with respect to cardiovascular disease risk.⁹ As adiponectin is suggested to play an important role in relation to atherosclerosis, higher adiponectin production from GAT could be one mediator of the protective properties of this AT depot. However, Lihn et al (2004) and Fisher et al (2002) no difference was found in adiponectin mRNA levels in gluteal compared to abdominal subcutaneous AT.^{10,11}

Thus, AT depot differences seem to play an important role in relation to health risk, and since data on adiponectin in relation to AT depot differences are conflicting, we wanted to compare of adiponectin mRNA expression in subcutaneous abdominal AT and subcutaneous gluteal AT.

MATERIALS AND METHODS

2.1. Subjects

Nineteen sedentary obese middle-aged men with a mean (\pm SD) body mass index of 31.6 ± 3.6 kg/m², volunteered to participate in this study. All the subjects were asked to complete a personal health and medical history questionnaire, which served as a screening tool. The subjects were nonsmokers and free from unstable chronic condition including dementia, retinal hemorrhage and detachment; and they have no history of myocardial infarction, stroke, cancer, dialysis, restraining orthopedic or neuromuscular diseases.

2.2. Anthropometric and body composition measurements

Height and weight were measured, and body mass index (BMI) was calculated by dividing weight (kg) by height (m²). Waist circumference was determined by obtaining the minimum circumference (narrowest part of the torso, above the umbilicus) and the maximum hip circumference while standing with their heels together. The waist to hip ratio (WHR) was calculated by dividing waist by hip circumference (cm).¹² Fat mass and lean body mass were assessed by bioelectrical impedance analysis using a Body Composition Analyzer (Biospace, Inbody 3.0, Jawn, Korea).

2.3. Tissue Samples

Subcutaneous abdominal and subcutaneous gluteal AT biopsies were obtained from all of the subjects. Paired AT biopsies from the subcutaneous abdominal and subcutaneous gluteal depots were obtained by open biopsy. The skin is anesthetized slowly with a small intracutaneous injection of 1% xylocaine without epinephrine.¹³ The abdominal biopsies were taken 5 cm lateral from umbilicus, and the gluteal biopsies were taken 5 cm beneath crista iliaca superior.¹⁰ All the biopsies were washed thoroughly with isotonic saline and transported in sterile containers to the laboratory within 30 min after removal and were either immediately frozen in liquid nitrogen for later RNA extraction (fresh AT) or were used for subsequent culture.

2.4. Real-Time PCR

RNA was reverse-transcribed with reverse

transcriptase and random hexamer primers according to the manufacturer's instructions (AccuPower™ Green Star qPCR PreMix, BIONEER). Then, PCR-mastermix containing the specific primers, Hot Star Taq DNA polymerase, and SYBR-Green PCR buffer was added. All the samples were determined as duplicates, and for a negative control the same setup was used except for the addition of reverse transcriptase. No PCR product was detected under these latter conditions. The adiponectin primers amplified a product of 301 bp: 5'-CATGACCAGGAAACCACGACT-3' and 5'-TGAATGCTGAGCGGTAT-3'. As a house-keeping gene β -actin was amplified using the following primers: 5'-ACGGGGTCACCCACACTGTGC-3' and 5'-CTAGAAGCATTGCGGTGGACGATG-3'. Real-time quantization of adiponectin to β -actin mRNA was performed using a SYBR-Green PCR assay (Rotrogen, 6000, Corbet). Adiponectin mRNA and β -actin mRNA were amplified in separate tubes and the thermal cycling protocol was 94°C for 40s, 94°C for 30s, and extension at 72°C for 30s and 72°C for 10 min. During the extension step increase in fluorescence was measured in real-time. Data were obtained as CT values (threshold cycle). Relative gene expression was calculated using the Pfaffl formula.¹⁴

$$\text{Ratio} = \frac{(E_{\text{target}})^{\Delta\text{CT}_{\text{target}}(\text{control sample})}}{(E_{\text{ref}})^{\Delta\text{CT}_{\text{ref}}(\text{control sample})}}$$

2.5. Ethics

The subjects were given both verbal and written instructions outlining the experimental procedure, and written informed consent was obtained. Biopsy is the invasive method; thus we anesthetized the skin with a small intracutaneous injection of xylocaine. The study was approved by the University of Guilan Ethics Committee.

2.6. Statistical analysis

For comparison of adiponectin mRNA levels between the two depots paired t-test was used. Values are presented as mean \pm SD and a P value <0.05 was considered statistically significant. Data analysis was performed using SPSS software for windows (version

16, SPSS, Inc., Chicago, IL).

RESULTS

Characteristics of the subjects were presented in Table 1. In nineteen obese subjects' fat biopsies were taken from the subcutaneous region of both the abdominal and the gluteal area. The results showed that adiponectin mRNA expression was higher in subcutaneous GAT than in subcutaneous AAT (1.89 ± 0.95 versus 1.46 ± 0.52 ; $P < 0.05$) (Figure 1).

Table 1. Characteristics of the subjects (mean \pm SD)

Characteristics	
Weight (kg)	88.07 \pm 10.3
Body mass index (kg/m ²)	31.6 \pm 3.6
Fat mass (kg)	28.1 \pm 6.4
Body fat (%)	31.3 \pm 3.7
Lean body mass (kg)	57.2 \pm 4.6
WHR	0.98 \pm 0.05

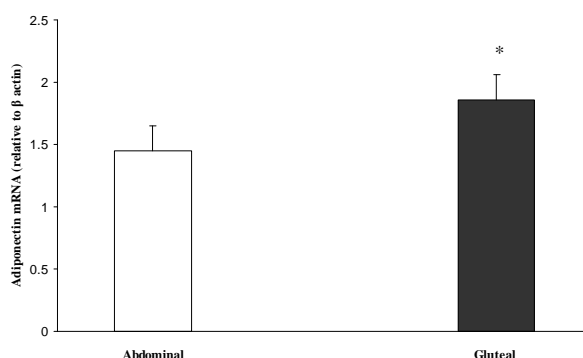


Fig 1. Adiponectin mRNA investigated in fresh adipose tissue from the subcutaneous abdominal (white bar) and gluteal (black bar) AT depots in nineteen subjects. Adiponectin mRNA levels are expressed relative to β -actin mRNA levels (Mean \pm SD).

DISCUSSION

Cross-sectional studies have shown peripheral AT to be positively correlated with plasma adiponectin levels.^{15,16} Therefore, we evolved the hypothesis that higher adiponectin gene expression in GAT could play a role for the protective effects on cardiovascular disease and insulin sensitivity seen in relation to "gynoid" obesity. The results of present study showed that adiponectin gene expression was 22.7% higher in

subcutaneous GAT than in subcutaneous AAT. This finding is in contrast with studies by Lihn et al (2004) and Fisher et al (2002) showing no difference in neither adiponectin mRNA levels nor in adiponectin protein content between gluteal and abdominal subcutaneous AT.^{10,11} Differences in number and size of adipocyte and body composition could contribute in adiponectin levels because a gynoid body fat distribution are known to have smaller and more numerous adipocytes than android fat distribution.¹⁷ It was illustrated that adiponectin mRNA increased 100-fold during adipose differentiation process⁵ and a significant down-regulation observed in fat tissues from obese humans and pigs.¹⁸⁻²⁰

Combs et al (2004) reported that overexpression of the adiponectin gene in fat tissue results in more lipid accumulation in the adipocytes.²¹ These effects in adipocytes are somewhat unexpected based on epidemiological data and suggest a new role for adiponectin in adipocyte biology.⁷ Several clinical studies have observed that circulating adiponectin levels are reduced in obesity,^{6,18} in which mean adipocyte size is increased, and increased in lean individuals with smaller adipocyte size.⁷ On the surface, Fu et al (2005) reported that adiponectin overexpression accelerates adipogenesis and augments cellular lipid accumulation.⁷ These observations can be reconciled that adiponectin functions as an adipocyte differentiation factor. Fu et al (2005) proposed that adiponectin acts locally at the tissue level to maintain adipocyte size and mass around an equilibrium set point. After weight loss, smaller adipocytes secrete more adiponectin, which has the effect of promoting adipocyte differentiation and lipid accumulation, thus returning adipocytes to their baseline size. Conversely, with weight gain, the reduction in adiponectin secretion leads to decelerated lipid accumulation and a reduction in adipocyte size to the baseline level. In a complementary manner, adiponectin could help to maintain equilibrium adipocyte size via metabolic effects as an autocrine/paracrine factor in AT.⁷

CONCLUSION

In conclusion, adiponectin mRNA is higher in GAT than in AAT suggesting body composition, fat distribution and adipocyte number and size could contribute in adiponectin gene expression difference.

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Authors Contributions:

MM: Participated in the design of the study; coordination and in the collection of data, performed the data analysis; drafted the manuscript and approval of the article.

HM and FRN: Participated in the design of the study and drafted the manuscript.

SHN,HN and MN: Participated in the collection of data.

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