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The Relationship between Body Mass Index(BMI), Adipocyte Size and Leptin and Angiotensin II Secretion in Human Adipose Tissue

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ABSTRACT

Adipose tissue has now been recognized as a rich source of metabolically active molecules that include leptin and angiotensinogen(AGT), the precursor of angiotensin II(Ang II). Both of which have been implicated in the pathogenesis of metabolic alteration and hypertension associated with obesity. In this study, we examined the relationship between body mass index(BMI), adipocyte size, leptin, Ang II secretion and mRNA expression in human adipose tissue obtained from female subjects. Leptin and Ang II were analyzed using specific radioimmunoassay kits following a 48hour tissue culture. Leptin and Ang II secretion varied from 1.4 − 72.1ng/g and 0.8 − 57.3pg/g of tissue respectively. These large individual variations limit significant correlation between BMI, leptin and Ang II secretion. Ang II secretion was significantly higher in the obese than the non-obese ($p < 0.05$) and positively correlated with BMI. However, no difference in leptin secretion between the obese and the non-obese was observed and leptin secretion showed negative correlation with BMI. No difference in leptin and AGT mRNA expression in adipose tissue between the obese and the non-obese was observed. Although several limitations of this study, we found increased Ang II secretion in obese patients compared with non-obese patients, and positive correlation between AGT and BMI. Observed difference in AGT expression between the obese and the non-obese in this study might be of importance in relation with obesity related hypertension. (*J Community Nutrition* 8(2): 69~75, 2006)

KEY WORDS: adipose tissue · angiotensin II · leptin · gene expression.

Introduction

Obesity is a complex quantitative trait influenced by both genetic and environmental factors. Adipose tissue has been recognized as a real endocrine organ expressing and secreting many factors that act at different levels with both autocrine/paracrine and endocrine functions(Faloia et al. 2000; Mohamed-Ali et al. 1998). A majority of these secreted proteins are involved in obesity linked complications. Hypertension develops in almost 60% of obese individuals(Sharma et al. 2001). In particular, several studies have reported the existence of the renin-angiotensin system(RAS) including angiotensinogen(AGT), angiotensin II receptor(AT1 and AT2), angiotensin converting enzyme(ACE) and renin in adipose tissue(Crandall et al. 1999; Engeli et al. 1999; Giacchetti et al. 2000; Giacchetti et al. 2002; Jones et al. 1997). The RAS has been well established as a major determinant of blood pressure and hypertension. RAS in adipose tissue is associated with adipocyte differentiation, fat tissue growth and involved in the pathogenesis of the complications of obesity(Faloia et al. 2000). Ang II acts as trophic factor of white adipose tissue development by enhancing the formation of GPDH-expressing cells from preadipocytes(Saint-Marc et al. 2001). Obese hypertensives have significantly higher levels of AGT, AT1, AT1 receptor and ACE mRNA levels in the visceral adipose tissue(VAT) than normotensives(Faloia et al. 2002; Giacchetti et al. 2002). All together, these results suggest that adipose tissue RAS could be involved in pathogenesis of metabolic alteration and hypertension(Engeli et al. 2000; Shrama et al. 2001). Although the relationship between an

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increase in adipose tissue and a rise in blood pressure has long been recognized, the mechanism linking these two phenomena is yet to be fully understood. In our study we focus on the secretion of these two important obesity/hypertension associated proteins, leptin/Ang II in the same tissue sample from the same subjects. Because several studies have been conducted separately on leptin and Ang II secretion in adipose tissue, so far their secretion in the same tissue sample has not been reported. In this study, we measured two secreted proteins, leptin the obesity gene product that is a well known marker of adipocity and Ang II, which is known as a regulating factor of hypertension. We also analyzed leptin and AGT mRNA and examined the relationship between body mass index (BMI), adipocyte size, leptin and Ang II secretion and their mRNA expression in the same human adipose tissue.

Materials and Methods

1. Subjects

Subcutaneous abdominal adipose tissue was obtained from 10 healthy normal to obese women. The age of subjects ranged between 27 − 64 years, with body mass index (BMI) of 25.8 ± 4.2kg/m^2 (range: 21.8 − 32.7kg/m^2). All patients were non-diabetic and non-hypertensive, with no known metabolic abnormalities. The study was approved by the Institutional Review Board for Human subjects and by the Committee for Research Protocols at the University of Tennessee, Knoxville.

2. Human adipose tissue cultures

Human adipose tissue was processed as previously described (Moustaid et al. 1996); the adipose tissue was immediately transported to the laboratory in Hank's solution. The adipose tissue was washed with Hank's solution and cut into small pieces (< 10mg each). The adipose tissue was preincubated for 24h for acclimatization and then incubated with serum-free media for 48h prior to the treatment; the tissue fragments were placed in dishes (1g adipose tissue/35 mm well) containing 5ml of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% fetal bovine serum, Hepes (15mM), glucose (5 or 25mM) as indicated, penicillin (100U/ml), streptomycin (100 μg/ml), and gentamicin (50 μg/ml) for 48h. A 48h incubation period was chosen based on linear stimulated leptin secretion into the medium which is in accordance with other reports (Casabiell et al. 1998; Fain et al. 2000; Kim et al. 2002; Menendez et al. 2000; Williams et al. 2000). Sets of six dishes were maintained for each treatment and sets of four dishes for the time-course experiments. The dishes were placed in a humidified incubator at 37℃ and at an atmosphere of 5% CO_2. After incubation, the culture media were collected from the dishes and stored at −80℃ until analyzed for angiotensin II and leptin. The adipose tissue was immediately homogenized for extracting protein and stored at −80℃ until analyzed. Untreated adipose tissue was used for total RNA extraction.

3. Assay of leptin and Ang II in the media

Leptin and Ang II levels in the media were determined using the commercially available leptin radioimmunoassay kit (Linco Research Ltd., St. Charles, MO, USA) and Ang II radioimmunoassay kit (Phoenix peptide Co., CA USA) from 100 μL of sample each. All experiments were conducted in duplicates.

4. Determination of fat cell size and number

Isolated fat cells were prepared by collagenase treatment according to the Rodbell's method (1964) and as previously described Moustaid et al. (1996). Isolated cell were sized using the Coulter Counter (Beckman, Inc.).

5. Real Time-Polymerase Chain Reaction (RT-PCR)

Total RNA from adipose tissue was isolated by the Trizol method. Total RNA from adipocytes and preadipocytes were extracted in GTC and purified following $CsCl_2$ density gradient centrifugation as our previously described (Kim et al. 2002). Further, all RNA were subjected to a phenol-chloroform extraction and ethanol precipitation. Preadipocytes harvested from the collagenase digestion were cultured in DMEM supplemented with 10% FBS and antibiotics as mentioned earlier until they reached confluence. The mRNA expression was quantified by real time PCR (Smartcycler) using specific primers and probes designed for human leptin and angiotensinogen gene sequences.

6. Statistical analysis

Values are expressed as means ± SEM. The SAS statistical packet (SAS/8.0, SAS) was used for the calculation. Leptin and Ang II secretion were expressed as the total of leptin and Ang II secreted into the media by a given sample

(in ng/ml or pg/ml) with respect to total volume and divided by the amount of fat tissue in grams, i.e. pg Ang II/g tissue and ng leptin/g tissue. The Student's unpaired t-test was used to compare the data between the obese and the non-obese. For determining the correlation between the variables, Pearson's correlation coefficients were used. Values of $p < 0.05$ were considered significant.

Results

1. Adipocyte size

Adipose tissue was obtained from ten healthy female subjects undergoing elective cosmetic surgery. The BMI pattern among the subjects was as follows: three obese (BMI > 30), five overweight (BMI = 25 − 30) and two normal (BMI < 25). The adipocyte size measurement positively correlated with BMI ($r = 0.3885$, $p = 0.06$). The mean adipocyte size was $76.2 \pm 2.2\ \mu$m in non-obese and $81.2 \pm 3.3\ \mu$m in obese, however there was no significant difference in adipocyte size between the obese and the non-obese.

2. Leptin and angiotensin II secretion from human adipose tissue, relationship to BMI and cell size

As shown in Fig. 1, leptin secretion from adipose tissue ranged from 1.3 − 72.1ng/g of tissue showing a negative but not significant relation with BMI. Ang II secretion from adipose tissue ranged from 0.8 − 57.3pg/ g of tissue and showed positive but not significant correlation with BMI. Three of subjects who were non-obese had significantly higher leptin levels compared to the other lean and obese subjects. After excluding responsive patients with extreme value, there is a positive correlation between BMI and leptin ($r = 0.7989$, $p < 0.05$) or Ang II ($r = 0.7092$, $p < 0.05$). The correlation between the secreted proteins and adipocyte size showed the same trends as with BMI.

3. Comparison of leptin and angiotensin II secretion from adipose tissue between the obese and the non-obese

As shown in Fig. 2, there was no significant difference in leptin secretion from adipose tissue between the obese and the non-obese. However, Ang II secretion from adipose tissue

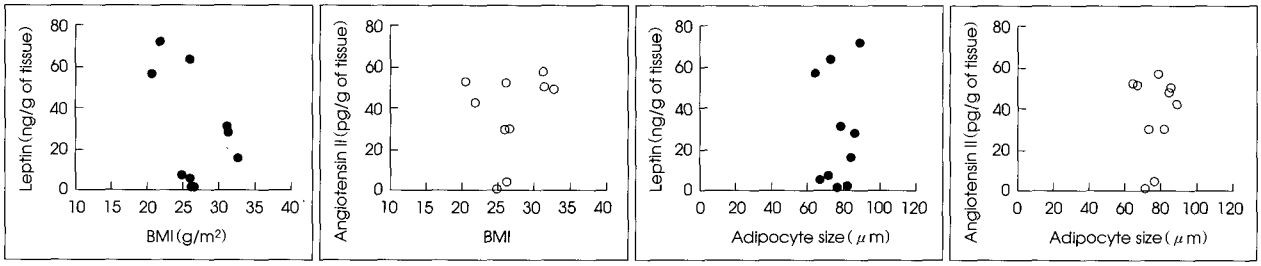

Fig. 1. Leptin and angiotensin II secretion from adipose tissue. Adipose tissue was incubated with DMEM for 48hrs. Leptin and angiotensin II secretion to medium was measured by using RIA kit, respectively. Values are scattered by BMI. All values were scattered in upper panel ($n = 10$). For determining the correlation between the variables Pearson's correlation coefficients were used. Values of $p < 0.05$ were considered significant.

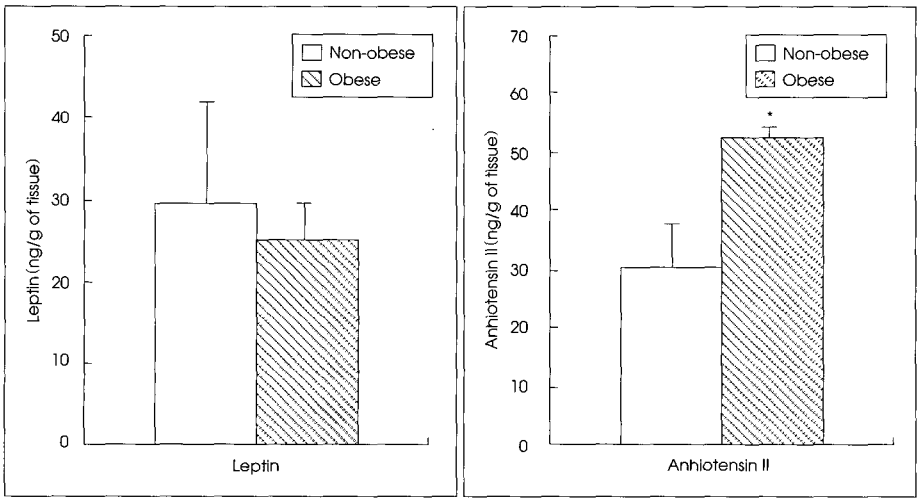

Fig. 2. Leptin and angiotensin II secretion on adipose tissue from obese and non-obese subjects. Adipose tissue from each subject was incubated with DMEM for 48 hrs. Leptin secretion to medium was measured by using RIA kit, respectively. The subjects with BMI > 30 were defined to obese ($n = 3$) and the subjects with BMI < 30 were considered non-obese ($n = 7$). All incubations were performed in six dishes from each subject. Results are expressed as mean ± SEM. The Student's unpaired t-test was used to compare the data. Values of $p < 0.05$ were considered significant.

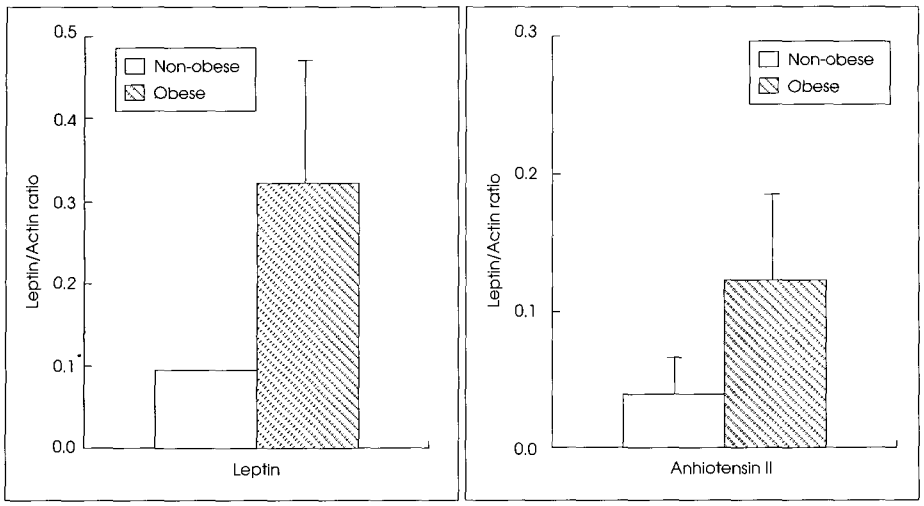

Fig. 3 Leptin and angiotensinogen mRNA expression in adipose tissue for obese and non-obese. The adipocytes were isolated from obese and non-obese subjects. RNA was prepared and analyzed. The intensity levels of the target mRNA were determined as described in materials and methods. Adipose tissue sample from one non-obese (BMI = 20.6) and two obese (BMI = 31.1 and 32.2, respectively) were used.

was significantly higher in the obese than the non-obese.

4. Comparison of leptin and angiotensinogen mRNA expression in adipose tissue between the obese and the non-obese.

There was no significant difference in leptin mRNA expression on adipose tissue between the obese and the non-obese. As shown in Fig. 3, both of leptin and AGT mRNA in adipocyte were similarly 3-fold higher in the obese than the non-obese.

Discussion

The direct relation of obesity to hypertension in humans has been well established (Faloia et al. 2000). Obesity or excess adipose tissue deposition directly influences manifestation of several health concerns including cardiovascular discords. Recent advances in adipose tissue metabolism reveal the importance of the fat tissue not just as a storage organ but also as a secretory tissue releasing many proteins into the blood that influences whole body metabolism. Although several studies have been conducted separately on leptin and Ang II secretion in adipose tissue, so far their secretion in the same tissue sample has not been reported. In our study we focus on the secretion of these two important obesity/hypertension associated proteins from the same tissue sample and their influence on an individual's status with regards to BMI, adipocyte size and gene expression. This study was designed to examine the relationship between body mass index (BMI), adipocyte size and leptin and Ang II secretion from abdominal subcutaneous adipose tissue from women. We also determined relation with leptin and Ang II secretion from abdominal adipose tissue and compared the difference on those variables between the obese and the non-obese.

It is commonly admitted that both the net amount of fat and the average size of individual adipocytes are the main regulators of circulating leptin (Caro et al. 1996; Hamilton et al. 1995; Montague et al. 1997). Adipocyte size is an important determinant of leptin synthesis, as larger adipocytes contain more leptin than smaller adipocytes in the same individual (Hamilton et al. 1995). Leptin levels have been shown to be positively correlated with body fat both in humans (Considine et al. 1995; Havel et al. 1996; Maffei et al 1995; Umemura et al. 1997) and rodents (Frederich et al. 1995; Maffei et al. 1995; Tamura et al. 1996). It is reported, however, that for any given amount of fat stores in obese humans, leptin levels vary markedly (Maffei et al. 1995).

Our data shows that adipose size was positively correlated with BMI and Ang II secretion and also positively correlated with BMI, although there was no statistic significance. The comparisons between the obese and the non-obese also showed the same results that Ang II secretion was significantly higher in the obese than the non-obese. We compared AGT mRNA expression between the obese and the non-obese, however there was no significant difference. These results are consistent with results from others (Bloem et al. 1995; Schorr et al. 1998). Bloem et al. (1995) reported an independent relationship of serum angiotensinogen with body mass index ($p = 0.0001$). Schorr et al. (1998) reported the relationships between plasma AGT and BMI and plasma leptin in young, normotensive, non-obese men with/without family history of hypertension. Plasma AGT was signify-

cantly correlated with both BMI ($r = 0.29$, $p < 0.01$) and plasma leptin ($r = 0.40$, $p < 0.001$). While plasma AGT and blood pressure were positively correlated only in subjects with a positive family history of hypertension ($r = 0.33$, $p < 0.05$), plasma leptin was related to blood pressure in both groups ($r = 0.26$, $p = 0.01$). They concluded that circulating AGT levels are related to adipose mass in young, normotensive, non-obese men. In our study, the Ang II had a significant positive correlation with BMI only after excluding the subjects with extreme value. The disagreements with other reports might be due to the small size of subjects. Disparity in blood pressure among individuals belonging to different racial groups has been documented (Bloem et al. 1995; Forrester et al. 1996). Therefore, consistency among individuals can only be obtained when a large section of the population is considered for any study and, further, definite significant criterion cannot be pin-pointed when human studies are considered.

In our study, the leptin secretion was extremely wide range by individuals with no significant correlation with BMI. However, it showed significant positive correlation with leptin secretion and BMI after excluding the subjects with extreme values. Other researchers reported adipocyte mRNA levels and circulating rate of leptin are increased in obese subjects, although there is considerable inter-individual variability in leptin expression and plasma concentrations at each body mass index (Hamilton et al. 1995; Lonnqvist et al. 1995; Lonnqvist et al. 1997). In our study, leptin secretion and mRNA expression were elevated in the obese than the non-obese, this confirms the data from other studies (Lonnqvist et al. 1995; Lonnqvist et al. 1997). Lonnqvist et al. (1997) reported leptin mRNA level in adipose tissue was 2 times higher in the obese than the non-obese. They reported BMI accounted for 50 − 60% of variations in leptin secretion and leptin mRNA accounted for about 40% of the variations in secreted leptin. They speculate that, in obesity, increased leptin mRNA enhances leptin production in fat cells, so that the leptin secretion rates from adipose tissue increase. In our study, Ang II showed also similar relationships with BMI and adipocyte size as like leptin. And mRNA expression of AGT, precursor of Ang II also increased in the obese than the non-obese.

In our study we used subcutaneous fat, although subcutaneous region is the major fat deposit (~80% of the total fat mass), regional variations in adipose tissue function are reported (Lonnqvist et al. 1997; Masuzaki et al. 1995; Harmelen et al. 2000). Dusserre et al. (2000) reported depot-related difference in the production of molecules, leptin and Ang II as well as in its mRNA expression. The leptin mRNA levels were higher in subcutaneous fat, whereas AGT mRNA levels were higher in visceral fat. For AGT, this difference was more pronounced in individual with BMI lower than 30 k/m^2 and in the more obese subjects, this difference is less apparent, mainly due to significantly increased expression of AGT in subcutaneous adipose tissue without a change in its expression in the visceral fat (Dusserre et al. 2000). Also several studies have been reported that there were sex-related differences also existing (Menendez et al. 2000). In this study, we used adipose tissue from just females. Although this study has several limitations that small sample size, only female subjects and subcutaneous fat depot as well as no blood pressure data, it's difficult to obtain a general conclusion. We found increased Ang II secretion in obese patients compared with non-obese patients, and positive correlation between AGT and BMI. Leptin mRNA expression was shown higher in the obese than in the non-obese and after excluding extreme value, leptin secretion showed positive correlation with BMI. Although the mechanisms underlying these correlations are unclear, several possibilities of relationships among the different factors can be speculated. So taken together, observed difference in AGT expression between the obese and the non-obese might therefore be of importance in relation with obesity related hypertension.

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