The correlation between adiposity and adiponectin, tumor necrosis factor α, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults?

M. Bahceci[1], D. Gokalp[1], S. Bahceci[2], A. Tuzcu[1], S. Atmaca[3], and S. Arikan[1]

Departments of [1]Endocrinology, [2]Histology and Embryology, and [3]Microbiology, Dicle University School of Medicine, Diyarbakir, Turkey

ABSTRACT. ***Objective:*** **Hypertrophic obesity correlates with metabolic complications of obesity. We evaluated adipocyte volume and its relationship with tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), adiponectin and high sensitivity C-reactive protein (hs-CRP) levels.** ***Subjects and methods:*** **Patients were divided into 4 groups; lean healthy controls [body mass index (BMI): 24.2±1.4 kg/m^2], non-diabetic obese patients (30.2±2.9), obese (30.1±3.2) and non-obese (22.2±1.5) Type 2 diabetic patients. TNF-α, hs-CRP, adiponectin and IL-6 levels were measured preoperatively and sc fat specimens were obtained during operation. Semi-thin sections were stained with toluidine-blue and evaluated by light microscopy. Fat volumes were calculated by Goldrick's formulation.** ***Results:*** **Mean adipocyte volumes were higher in obese diabetic patients than in other groups ($p<0.0001$). Mean TNF-α, hs-CRP and IL-6 levels were higher in obese diabetic patients than in control subjects, obese non-diabetic and non-obese diabetic patients ($p<0.0001$, $p<0.02$ and $p<0.01$, respectively). Mean TNF-α levels of non-diabetic obese patients were higher than the control group ($p<0.05$). Mean IL-6 levels of diabetic and non-diabetic obese patients were higher than control subjects ($p<0.02$ and $p<0.0001$, respectively). Mean adiponectin levels of control subjects were higher than non-diabetic obese, non-obese diabetic and obese-diabetic subjects ($p<0.0001$). Mean adiponectin levels of obese diabetic patients were lower than non-diabetic obese subjects ($p<0.008$). Mean hs-CRP levels were higher in diabetic patients whether they were obese or not. There was a positive correlation between adipocyte size and TNF-α ($p<0.01$), IL-6 ($p<0.03$) and hs-CRP levels ($p<0.004$), and negative correlation between adipocyte size, adiponectin levels ($p<0.0001$).** ***Conclusions:*** **TNF-α, IL-6 and hs-CRP levels were positively, adiponectin negatively correlated with adipocyte size. Therefore, adiposity may be an inflammatory condition.**

(J. Endocrinol. Invest. 30: 210-214, 2007)

©2007, Editrice Kurtis

INTRODUCTION

Obesity is the storage of excess fat, and it can be classified according to the number and size of fat cells. Two distinct types of human obesity have been identified on the basis of cellar character of the mass of adipose tissue: hyperplastic, with a small to moderate increase in adipose cell size, and hypertrophic, with a large increase in cell size alone (1). Hypertrophic obesity is more common in adults (2, 3). Hypertrophic obesity correlates with metabolic complications of obesity, including impaired glucose tolerance, adverse lipid profile, hypertension, and coronary heart disease (CHD) (2). However, there has been slow progress in understanding the basic pathophysiology that underlies co-morbid conditions associated with obesity (4). Being an important component of body composition, adipose tissue is able to produce hormone-like peptides, named adipokines or adipocytokines (5). Obesity, particularly visceral adiposity, is associated with chronic low-grade inflammation, as indicated by increased levels of the inflammatory markers C-reactive protein (CRP) and interleukin-6 (IL-6) in the circulation of obese subjects. IL-6 and tumor necrosis

Key-words: TNF-α, IL-6, hs-CRP, adiponectin, adipocyte volume, obesity, inflammation.

Correspondence: M. Bahceci, MD, Dicle University School of Medicine, Department of Endocrinology, 21280 Diyarbakir, Turkey.

E-mail: mbahceci@dicle.edu.tr

Accepted September 19, 2006.

factor α (TNF-α) are the two best-intensively studied cytokines in obesity and have been consistently found to be increased in the serum of obese subjects (4). Circulating levels of TNF-α and IL-6 are directly correlated with adiposity and insulin resistance (4). On the contrary Bastard et al. (6) claimed that IL-6 is correlated with adiposity and insulin resistance but TNF-α is not. TNF-α primarily induces insulin resistance in rodents and not in humans. Low adiponectin levels seem to be highly correlated to the deteriorated metabolism observed in obese Type 2 (T2) diabetic subjects. In addition, adiponectin is down-regulated by IL-6 and TNF-α (7). The concomitance of inflammation, hypertension, and dyslipidemia increases the likelihood of development of T2 diabetes and cardiovascular disease (CVD) (8). TNF-α can directly lead to insulin resistance by inducing serine phosphorylation of the insulin receptor, which inhibits insulin signaling (9). Therefore TNF-α is considered a likely mediator of the insulin resistance and T2 diabetes associated with high visceral adiposity (10).

We aimed at evaluating adipocyte volumes in different groups (healthy control, obese non-diabetic, non-obese T2 diabetic and obese T2 diabetic patients), and the relationship between adipocyte volumes and serum cytokine levels such as TNF-α, IL-6, adiponectin and CRP.

MATERIALS AND METHODS

Subjects

This study was performed in 100 patients (male: 32, female: 68, aged 25-67 yr, mean: 47.3±11.2 yr) who underwent elective abdominal operation in general surgery (hernia, cholecystectomy and gastroplasty; no.=76) and gynecology clinics (hysterectomy for myoma uteri; no.=24). Type 1 (T1) diabetic patients, patients with malignancy suspicion, patients with a history of acute myocardial infarction or angioplasty within the preceding 6 months and patients diagnosed with concomitant diseases (arthritis) or conditions that could possibly be associated with an acute-phase reaction (such as estrogen use) were excluded. Because viral infections such as viral rhinitis affect CRP, TNF-α and IL-6 measurements were performed at least 4 weeks after an infection. Patients were divided into 4 groups according to their specifications; Group 1: lean healthy controls [body mass index (BMI)<25 kg/m^2], Group 2: non-diabetic healthy obese subjects (BMI≥25kg/m^2), Group 3: non-obese T2 diabetic patients (BMI<25 kg/m^2), and Group 4: obese T2 diabetic patients (BMI≥25 kg/m^2). Informed written consent was obtained from all subjects.

Methods

Anthropometric evaluations and body fat analyses

Body weights were measured without shoes and in light clothing, and were recorded to the nearest 0.5 kg. Body heights were measured without shoes and/or cap, and were recorded to the nearest centimeter. BMI was expressed as weight (kilograms) per height (meters) squared.

Blood samples and laboratory evaluation

All blood samples were obtained after 10-12 h fasting period between 08:30-09:30 a.m. (24 h before operation). Blood samples were centrifuged and separated immediately. Blood samples were collected in tubes containing citric acid and stored -80 C until assayed. TNF-α and IL-6 levels measured with chemiluminescence in immulite one DPC. Plasma adiponectin levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA, Chemicon International, USA and Canada). The kit has a sensitivity of 0.1 ng/ml. The intra- and interassay coefficient of variation was 8.38% (3.75 ng/ml) and 9.82% (7.5 ng/ml). Hs-CRP concentrations were measured with immunometric assay by Immulite 2000 high sensitivity CRP kits (Diagnostic Products Corporation, Los Angeles, CA, USA). With this method, bilirubin and hemolysis do not affect CRP levels. Homeostasis model assessment index for insulin resistance (HOMA-IR) was calculated with the formula [HOMA-IR=(fasting plasma insulin (μU/ml)x fasting plasma glucose (mmol/l)/22.5] (11).

Adipocyte size and histologic evaluation

All sc fat tissue specimens (approximately 1x2 cm size) obtained from abdominal region during operation were put into formaldehyde solution and sent to the histology department. Fat tissue specimens were cut into small pieces and fixed in 2.5% glutaraldehyde solution. After 24 h, tissue specimens were washed in buffer solutions and put into osmium tetroxide solution. Then specimens were washed in buffer solution, passed through graded alcohol and propylene-oxide phase, fat tissue specimens were put into 1:1 araldite and embedded. Semi-thin sections were cut with ultramicrotome, stained with toluidine-blue and evaluated with Olympus BH-2 light microscopy in 40x10 objective. In each slide, by using ocular micrometer, diameters of a hundred adipocytes were counted and mean of adipocytes sizes were determined. Adipocyte volumes were calculated by Goldrick's (πd [3(SD)^2+d^2)] /6) formulation (12) (Fig. 1).

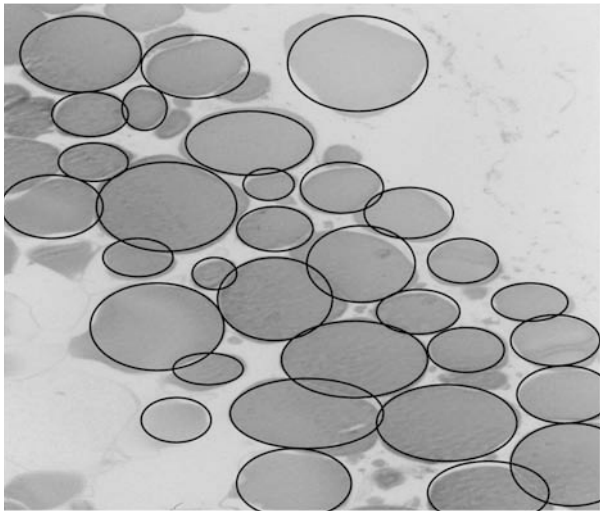

Fig. 1 - *Adipocytes in fat tissue sample (with toluidine blue at 40x10 magnification).*

Statistical analysis

All results were presented as mean±standard deviation (SD). Results of clinical or laboratory parameters were compared with one-way analysis of variance. Tukey's b test was used for sub-group analysis. Pearson's correlation coefficient was used to determine the relationship between adipocyte size and serum cytokine (TNF-α, IL6 and hs-CRP and adiponectin) levels. Values of $p<0.05$ were accepted as statistically significant.

RESULTS

Anthropometrical results

Mean body weight and BMI of obese patients (both diabetic and non-diabetic) were higher than control subjects and non-obese diabetic patients ($p<0.0001$). All anthropometrical results of subjects were shown in Table 1.

Comparison of adipocyte volumes, cytokines and HOMA-IR

Mean of adipocyte volume was higher in obese diabetic patients (OD) than in the other groups ($p<0.0001$). Mean adipocyte volume was higher in non-diabetic obese patients than in control subjects ($p<0.03$). Mean TNF-α, IL-6 and hs-CRP levels were higher in obese diabetic patients than in control subjects ($p<0.0001$). In addition, mean TNF-α levels of non-diabetic obese patients were higher than the control group ($p<0.05$). Mean IL-6 levels of non-obese diabetic and non-diabetic obese patients were also higher than control subjects ($p<0.02$). Mean adiponectin levels of control subjects were higher than all groups ($p<0.0001$). In addition mean adiponectin levels of the obese diabetic group were lower than the obese non-diabetic group ($p<0.008$). Mean hs-CRP levels of non-obese diabetic ($p<0.05$), non-diabetic obese ($p<0.05$) and obese diabetic patients ($p<0.0001$) were higher than control subjects. Mean fasting blood glucose (FBG), fasting insulin (FI) and mean HOMA-IR levels in obese diabetic patients were higher than in the other groups ($p<0.0001$, $p<0.01$ and $p<0.0001$, respectively). Adipocyte volume, TNF-α, IL-6, adiponectin, hs-CRP levels, adipocyte diameter (μm) and HOMA-IR values of subjects are shown in Table 2. We wanted to determine whether adipocyte volume is associated with cytokine and adiponectin levels or not. For this reason, we performed correlation analysis between adipocyte volume and serum cytokine levels. We found a positive correlation between adipocyte size and TNF-α ($r=0.247$, $p<0.01$), IL-6 ($r=0.207$, $p<0.03$), and hs-CRP levels ($r=0.167$, $p<0.004$), and a negative correlation between adipocyte size and adiponectin ($r=-0.446$, $p<0.0001$).

DISCUSSION

There were three main findings in this study. First, adipocyte volumes were correlated with BMI and body weight. These results implied that obesity was related to body weight and BMI. In fact, it was generally accepted that adult obesity was hypertrophic, and that it was related to adipocyte volume (1).
Second, mean of inflammatory marker levels (TNF-α, IL-6 and hs-CRP) was higher in obese diabetic patients than in control subjects, but also in non-diabetic obese and non-obese diabetic patients. Mean adiponectin levels of all groups were lower than control subjects and mean adiponectin levels of obese diabetic patients were also lower than non-diabetic obese subjects. These findings pointed out that obese T2 diabetics had higher inflammatory markers and lower adiponectin levels. Inflammation is thought to play an impor-

Table 1 - *All anthropometrical results of subjects.*

	C (no.=30)	OB (no.=30)	NOD (no.=20)	OD (no.=20)	*p*-value
Age (yr)	47.8±11.2	46.4±14.0	48.7±9.1	46.8±8.4	ns
Sex (F/M)	20/10	21/9	13/7	14/6	ns
Body height (cm)	164.9±6.7	163.4±6.6	167±5.6	163.7±6.5	ns
Body weight (kg)	59.1±7.2	80.5±9.3*	62.1±6.8	80.8±6.7*	<0.0001
BMI (kg/m^2)	24.2±1.4	30.2±2.9*	22.2±1.5	30.1±3.2*	<0.0001
Systolic BP (mmHg)	124.5±10.9	129.5±13.2	127.5±13.1	130±12.2	ns
Diastolic BP (mmHg)	77.7±6.8	81.8±8.8	78.5±7.1	81.3±7.4	ns
Adipocyte size (μg lipid/cell)	0.22±0.04	0.29±0.04*	0.21±0.02	0.56±0.11*	<0.0001

*OB and OD *vs* C and NOD. ns: non-significant; C: healthy control subjects; OB: obese non-diabetic patients; NOD: non-obese diabetic patients; OD: obese diabetic patients; F: female; M: male; BMI: body mass index; BP: blood pressure.

Table 2 - *Mean tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), high sensitivity C-reactive protein (hs-CRP), adipocyte volumes and homeostasis model assessment index for insulin resistance (HOMA-IR) levels of groups.*

	C (no.=30)	OB (no.=30)	NOD (no.=20)	OD (no.=20)	*p*-value
TNF-α (pg/ml)	6.8±2.0	12.6±4.8*	11.2±4.1*	19.6±17.1	<0.0001
IL-6 (pg/ml)	6.6±1.9	11.7±5.4**	10.4±3.1**	15.9±12.6	<0.0001
Adiponectin (ng/ml)	0.58±0.1	0.49±0.05***	0.46±0.1	0.41±0.1	<0.0001
hs-CRP (mg/l)	3.6±2.2	7.2±4.2*	8.3±4.0*	13.8±14.6	<0.0001
Adipocyte diameter (μm)	73.8±16.1	83.3±10.1	75.0±8.2	101.4±21.3	<0.0001
FBG (mg/dl)	95.5±11	101.1±9	149.1±19	190.5±24	<0.0001
FI (mU/ml)	7.7±3.2	10.2±3.6	7.2±4.6	9.5±3.7	<0.01
HOMA-IR	1.8±0.8	2.5±0.8	2.6±1.6	4.4±1.9	<0.0001

*$p<0.05$ C vs OB and C vs NOD; **$p<0.02$ C vs OB and C vs NOD; ***$p<0.008$ OB vs OD.
FBG: fasting blod glucose; FI: fasting insulin; C: healthy control; OB: obese non-diabetic; NOD: non-obese diabetic; OD: obese diabetic.

tant role in the progression and complications of atherosclerosis. In this manner CRP, a non-specific marker of inflammation, has been proven to be one of the strongest predictors of the risk of cardiovascular events in patients with CVD (13) as well as in patients without CVD (14-16). We also previously reported that T2 diabetic men without CHD had similar hs-CRP levels to non-diabetic men with CHD (17). This finding may also support the theory that obese T2 diabetic patients run a very high risk of CHD. Mean TNF-α, hs-CRP and IL-6 levels of non-obese diabetic patients were also higher than the control group, as were mean TNF-α, hs-CRP and IL-6 levels of non-diabetic obese patients. Therefore, adipocyte volume may be important for inflammatory condition even in subjects with obesity alone. However, mean TNF-α, hs-CRP and IL-6 levels of non-diabetic obese patients and non-obese diabetic patients were higher than the control group; the highest level was found in obese T2 diabetic patients. Insulin resistance is common in T2 diabetics. Therefore, we can say that adipokines were associated not only with obesity, but also insulin resistance and T2 diabetes mellitus itself.

Third, we found a positive correlation between adipocyte size and TNF-α, IL6, hs-CRP, and a negative correlation between adipocyte size and adiponectin levels, and this correlation existed not only in obese T2 diabetics but also in obese non-diabetic subjects. It can be claimed that larger adipocyte volume is associated with higher TNF-α, IL-6 and hs-CRP and lower adiponectin levels, and this result implied that hypertrophic obesity is associated with inflammation and low adiponectin levels. This is a very interesting finding and supports the idea that large adipocyte volume (hypertrophic obesity) may be correlated with complications of obesity and concomitant disease (2). TNF-α and IL-6 are the two best-studied adipokines in obesity and circulating levels of TNF-α and IL-6 are directly correlated with adiposity and insulin resistance in obese subjects (4). TNF-α can directly lead to insulin resistance by inducing serine phosphorylation of the insulin receptor, which inhibits insulin signaling (9). Therefore, TNF-α is considered a likely mediator of insulin resistance and T2 diabetes (10). Adipose tissue can be divided into two major types: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT represents the vast majority of adipose tissue in the organism (18). The number of macrophages present in WAT is directly correlated with adiposity (18-20). Macrophages are the major source of TNF-α and contribute approximately 50% of IL-6 (19). Although not completely demonstrated, the current working hypothesis is that adipokines, cytokines, and other factors produced and released by WAT are responsible for the chronic inflammatory state of visceral obesity (21). Our findings showed that increased adipocyte volume in sc fat tissue was associated with high cytokine, low adiponectin levels. For this reason, hypertrophic obesity may be associated with chronic low-grade inflammation, as indicated by increased levels of the inflammatory markers TNF-α, hs-CRP and IL-6 in the circulation of obese subjects (8).

CONCLUSIONS

TNFα, IL-6 and hs-CRP levels were higher, adiponectin levels were lower in obese patients with or without T2 diabetes, and hypertrophic obesity may be associated with inflammation. Adipocyte volume is positively correlated with TNFα, hs-CRP and IL-6

levels whereas negatively correlated with adiponectin levels, and this correlation was not only related to T2 diabetes but also obesity alone.

REFERENCES

1. Salans LB, Cushman SW, Weismann RE. Studies of human adipose tissue. Adipose cell size and number in nonobese and obese patients. J Clin Invest 1973, 52: 929-41.
2. Bray GA, Ryan DH. Clinical evaluation of the overweight patient. Endocrine 2000, 13: 167-86.
3. Aronne LJ. Classification of obesity and assessment of obesity-related health risks. Obes Res 2002, 10 (Suppl 2): 105S-15S.
4. Cottam DR, Mattar SG, Barinas-Mitchell E, et al. The chronic inflammatory hypothesis for the morbidity associated with morbid obesity: implications and effects of weight loss. Obes Surg 2004, 14: 589-600.
5. Bernshtein LM. Hormones of adipose tissue (adipocytokines): ontogenetic and oncologic aspects. Adv Gerontol 2005, 16: 51-64.
6. Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 2000, 85: 3338-42.
7. Bruun JM, Lihn AS, Pedersen SB, et al. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. Am J Physiol Endocrinol Metab 2003, 285: E527-33.
8. Yudkin JS. Adipose tissue, insulin action and vascular disease: inflammatory signals. Int J Obes Relat Metab Disord 2003, 27 (Suppl 3): S25-8.
9. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci USA 1994, 91: 4854-8.
10. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R. Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. Diabetes 1996, 45: 881-5.
11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985, 28: 412-9,
12. Goldrick RB. Morphological changes in the adipocyte during fat deposition and mobilization. Am J Physiol 1967, 212: 777-82.
13. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group, Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. Lancet 1997, 349, 462-6.
14. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation 1998, 97: 2007-11.
15. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000, 342: 836-43.
16. Danesh J, Whincup P, Walker M, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ 2000, 321: 199-204.
17. Bahceci M, Tuzcu A, Ogun C, Canoruc N, Iltimur K, Aslan C. Is serum C-reactive protein concentration correlated with HbA1c and insulin resistance in Type 2 diabetic men with or without coronary heart disease? J Endocrinol Invest 2005, 28: 145-50.
18. Curat CA, Miranville A, Sengenes C, et al. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes, Diabetes 2004, 53: 1285-92.
19. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue, J Clin Invest 112, 2003: 1796-808.
20. Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, J Clin Invest 112, 2003: 1821-30.
21. Kalantar-Zadeh K, Block G, Horwich T, Fonarow GC. Reverse epidemiology of conventional cardiovascular risk factors in patients with chronic heart failure. J Am Coll Cardiol 2004, 43: 1439-44.