Comparison of Human Abdominal Subcutaneous Versus Omental Preadipocyte Differentiation in Primary Culture

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Excess intra-abdominal fat is associated with a higher risk for type 2 diabetes mellitus and cardiovascular disease, yet little is known about what influences regional adipose tissue accumulation. Adipocytes arise from specialized fibroblast-like preadipocytes within the adipose tissue stromal-vascular compartment. The aim of our study was to determine if there are variations in preadipocyte differentiation between abdominal subcutaneous (SC) and omental (OM) preadipocytes. Abdominal SC and OM preadipocytes were isolated from adipose tissue obtained from 18 subjects (7 men, 11 women), undergoing elective abdominal surgery, by collagenase treatment and filtration/centrifugation. Preadipocytes were placed in culture and then differentiated for 3 weeks in a serum-free medium containing insulin, dexamethasone, isobutylmethylxanthine, and carbaprostacyclin. The cells were then harvested for measurement of cytosolic glycerol phosphate dehydrogenase (GPDH), a marker of terminal differentiation. Data are expressed as a differentiation index (DI), which was the log of the SC/OM ratio of GPDH values for each patient (calculated as 0 for an equivalent SC *v* OM responses). The mean DI for the group (n = 18) was 0.04, with a 95% confidence interval (CI) of -0.11 to 0.20. The mean DI for men was 0.07 (95% CI, -0.06 to 0.19), and that for women was 0.03 (95% CI, -0.21 to 0.27). This indicates that SC versus OM preadipocyte differentiation responses were not significantly different from each other, either for the group as a whole or when divided by gender. Overall, 8 subjects had a DI favoring SC preadipocyte differentiation, compared to 11 subjects with a DI reflecting greater OM preadipocyte differentiation. There was no correlation of the DI with body mass index or age. Our results indicate that preadipocytes from the abdominal SC adipose tissue depot do not uniformly differentiate more than those from the OM depot.

THE RISING PREVALENCE of obesity in industrialized countries is of concern, since obesity is associated with insulin resistance, leading to type 2 diabetes mellitus and cardiovascular disease.[1] In particular, excess intra-abdominal fat, as opposed to that in the subcutaneous (SC) region, is most strongly linked to the metabolic and vascular complications of obesity.[2] High levels of free fatty acids released from excess intra-abdominal fat are hypothesized to perturb hepatic, skeletal muscle, and pancreatic β cells function, leading to augmented glucose output, overproduction of atherogenic lipoproteins, reduced peripheral glucose disposal, and reduced insulin secretion.[3] Intra-abdominal omental (OM) adipocytes possess a variety of phenotypic attributes that distinguish them from SC adipocytes, and these may contribute to their deleterious effect on glucose and lipid metabolism. For example, OM adipocytes contain more β-adrenergic and fewer α_2-adrenergic receptors, perhaps accounting for the heightened sensitivity they display in response to lipolytic stimuli.[4,5]

Given the association of excess intra-abdominal fat and insulin resistance, it is important to learn more about what governs the deposition of adipose tissue in the intra-abdominal versus the Sc region. The accumulation of adipose tissue depends in part on new adipocyte formation as well as enlarging adipocyte volume.[6,7] Little is known about depot-specific characteristics of preadipocytes, the specialized fibroblasts within the stromal-vascular fraction of adipose tissue. These progenitor cells, when appropriately cued, differentiate into mature adipocytes.[8] An activating mutation in a critical adipogenic transcription factor, PPARγ2, has been found to associate with human obesity, underlining the potential importance of preadipocyte differentiation in the pathogenesis of obesity.[9] The aim of our study was to examine whether anatomic region-specific variation could be observed in the differentiation of cultured human preadipocytes.

MATERIALS AND METHODS

Isolation of Human Preadipocytes

Abdominal SC and OM adipose tissue samples were obtained from 18 patients (7 men, 11 women) undergoing a variety of elective abdominal surgical procedures (eg, gastric and colonic resections; abdominal hysterectomies) at the Ottawa Hospital. The experimental protocol was approved by the Ottawa Health Research Institute Research Ethics Committee. The patients were not acutely ill, and were weight-stable. SC and OM adipose tissue biopsy specimens were obtained at surgery and immediately transported to the laboratory. Preadipocytes were isolated from adipose tissue by first dissecting away fibrous tissue and blood vessels, followed by collagenase digestion, filtration, and centrifugation. Floating mature adipocytes were discarded, and the stromal-vascular cell pellet was subjected to additional filtrations (25 μm final mesh size) to yield the stromal-vascular preadipocyte fraction.[10,11]

Primary Cell Culture and Preadipocyte Differentiation

Preadipocytes were seeded at 5×10^4 cells/cm^2 and maintained overnight in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Life Tech, Burlington, Canada). All media used were supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 50 U/mL nystatin. The next morning, cell cultures were placed in serum-free medium consisting of DMEM/F12

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Table 1. Patient Characteristics, Wet Weight of Adipose Tissue Samples, and Number of Preadipocytes per Gram of Adipose Tissue

Patients	Age (yr)	BMI	SC Fat (g)	SC #/g (×100,000)	OM fat (g)	OM #/g (× 100,000)
All						
Mean	56.0	27.0	8.3	3.3	9.2	4.2
SD	12.5	4.5	3.4	1.9	4.6	3.1
Women						
Mean	58.2	25.5	7.8	3	7.6	4.5
SD	12.4	4.3	3.9	1.2	3.9	3.3
Men						
Mean	54.4	28.5	8	3.7	11.9	3.5
SD	13.4	4.7	2.1	2.7	4.1	2.5

(1:1) supplemented with 33 μmol/L biotin, 17 μmol/L pantothenate, 10 μg/mL transferrin, 0.2 nmol/L triiodothyronine (Sigma, St Louis, MO), 1 μmol/L insulin (Roche Biochemical, Laval, Canada), 0.2 μmol/L cPGI2 (carbaprostacyclin; Cayman, Ann Arbor, MI), and for the first 4 days only, 1 μmol/L dexamethasone (Steraloids, Wilton, NH), and 0.1 mmol/L isobutylmethylxanthine (IBMX). Control preadipocyte cultures were treated under parallel conditions, except insulin was at 0.1 μmol/L, and cPGI2, dexamethasone, and IBMX were omitted. The medium was replaced every 4 days, and the cell cultures were examined by phase-contrast microscopy by 2 independent observers, and then harvested after 3 weeks for quantitative assessment of terminal adipocyte differentiation by measuring the specific activity (U/mg protein/min) of cytosolic glycerol phosphate dehydrogenase (GPDH).[12]

Statistical Analysis

Two-tailed *t* tests or analysis of variance (ANOVA), as indicated, were performed using GraphPad InStat version 3.00 for Windows 98 (GraphPad Software, San Diego, CA).

RESULTS

The patient characteristics are listed in Table 1. There were no significant differences in age or BMI between men and women ($P > .50$; two-tailed *t* test). The amount of adipose tissue obtained is shown in grams of wet weight (g), and the resulting yield of isolated preadipocytes per gram of adipose tissue (#/g) agrees with published data for stromal-vascular preadipocytes generated by collagenase digestion and filtration.[13] There were no differences in the number of preadipocytes isolated per gram of adipose tissue between men and women, or between SC and OM fat depots ($P > .50$; ANOVA).

Overall, the induction of differentiation was satisfactory, as indicated qualitatively by the morphological extent of differentiation, which ranged from 30% to 70% of cells. To quantify the adipogenic response, the GPDH specific activity (U/mg/min) of SC preadipocytes (mean ± SD) subjected to differentiation increased by approximately 6.5-fold to 1,397 ± 1,355, compared to 285± 407 for cells maintained in control medium. The GPDH specific activity for OM preadipocytes induced to differentiate rose to 1,336 ± 1,235, compared to 117 ± 214 in control cells. The much lower levels of GPDH measured under control conditions was consistent with the expected minimal extent of differentiation (5% of cells). There were no significant differences between the GPDH values between control SC versus OM preadipoccytes, or between differentiated SC versus OM cells ($P > .40$; ANOVA).

The GPDH specific activities ranged widely due to the heterogeneity of the patients. Therefore, for each individual patient, we compared the degree of differentiation of SC versus OM preadipocytes by calculating the ratio of SC/OM GPDH values. An equivalent differentiation response of SC and OM preadipocytes would be calculated as 1. In order to analyze 95% confidence intervals (CI), it is necessary to log transform the data, so that ratios above (SC > OM) or below (SC < OM) 1 have the same arithmetic impact. For example, a SC preadipocyte GPDH level that is twice as high as the OM preadipocyte level will give a value of 0.3 (log transformation of 2), whereas the opposite situation will give a value of -0.3 (log

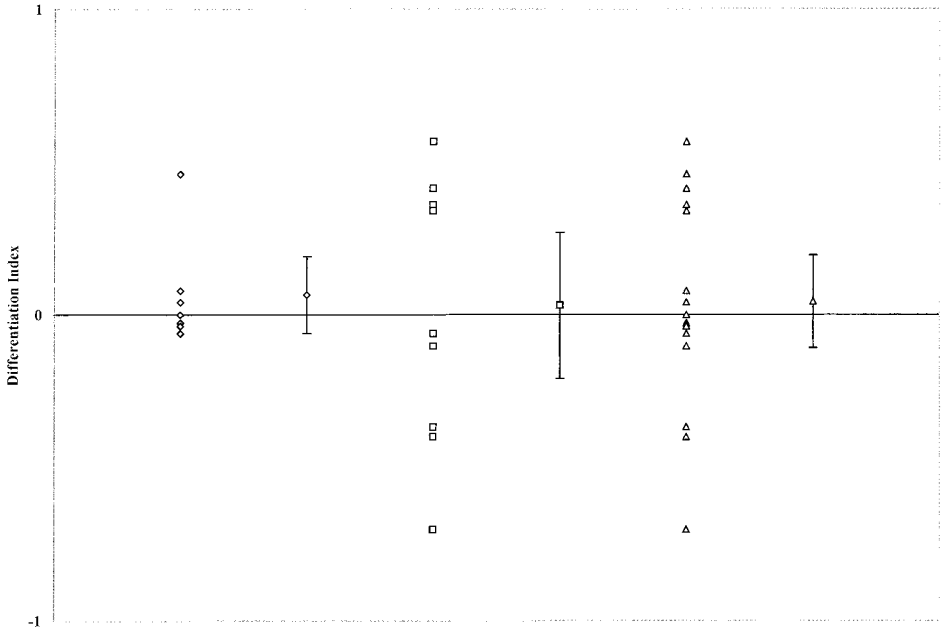

Fig 1. Comparison of SC *v* OM preadipocyte differentiation responses. Human SC and OM preadipocytes were isolated, cultured, and differentiated for 3 weeks as described. Cultures were then processed for measurement of GPDH as described, and the log of the ratio of SC/OM values for each pair was calculated (DI). The data are plotted as individual data points for each group, followed by the mean value and corresponding 95% CI. (◇) Males, (□) females, (△) pooled.

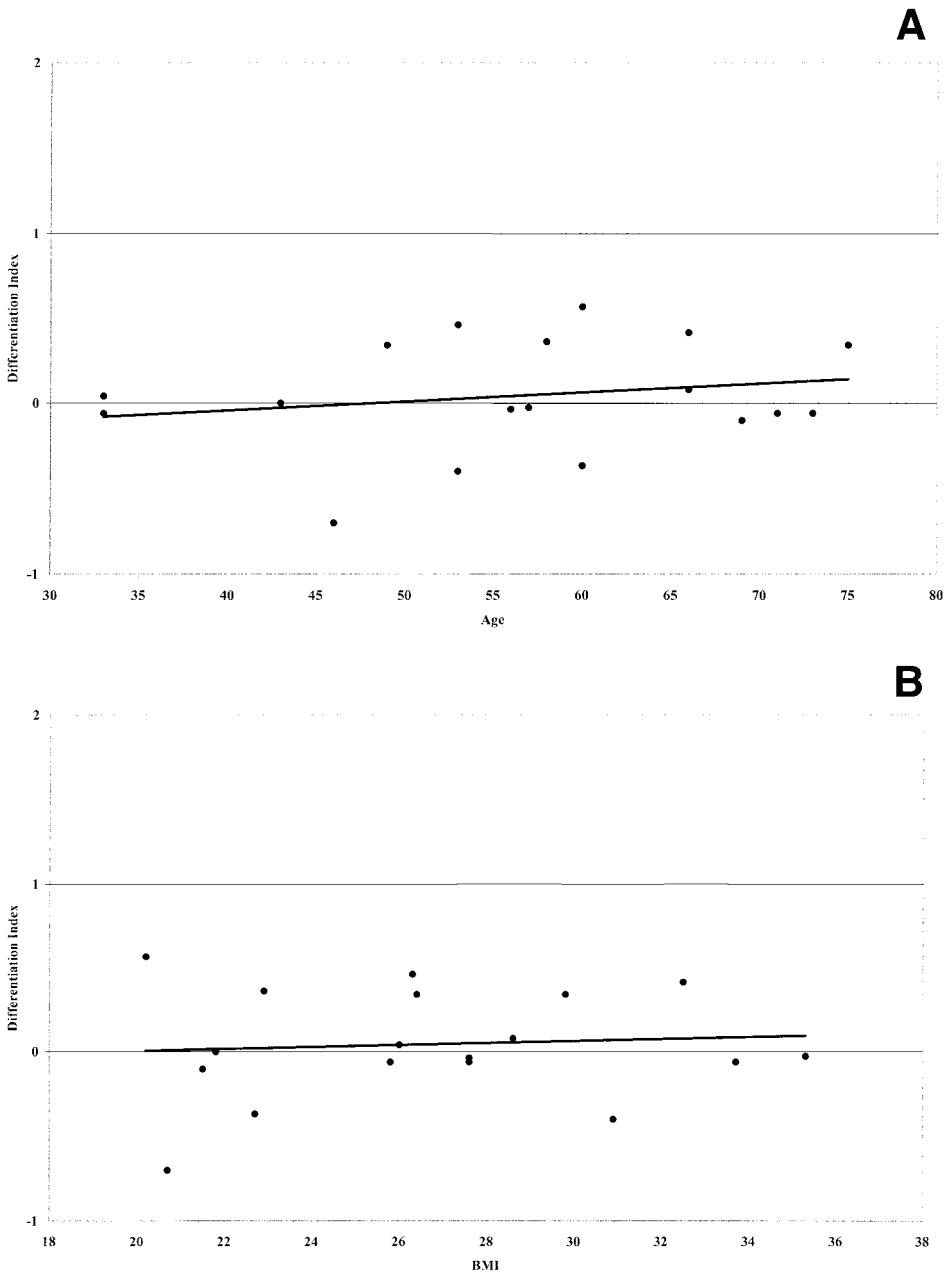

Fig 2. Lack of correlation between (A) DI and age, and (B) DI and BMI. DI was calculated as described in the text.

tranformation of 0.5), which are equidistant from 0 (log transformation of 1). This log-transformed ratio of SC/OM GPDH values is termed the differentiation index (DI), and 95% CI values crossing 0 indicate the DI is not significantly different than 0.

As shown in Fig 1, the mean DI for the entire group was 0.04 (95% CI, -0.11 to 0.20). It was 0.07 (95% CI, -0.06 to 0.20) for men, and was 0.03 (95% CI, -0.21 to 0.27) for women. None of these values was significantly different from 0. This indicates that there was no significant difference between the SC versus OM preadipocyte differentiation response, even when expressed as a ratio for each individual patient. Overall, 8 subjects had a DI favoring SC preadipocyte differentiation, compared to 11 subjects with a DI reflecting greater OM preadipocyte differentiation. There was no significant relationship of the DI with age ($r = .20$; $P > .10$) or body mass index ($r = 0.23$; $P > .10$) (Fig 2).

The measurement of cytosolic protein concentration, required for the calculation of the GPDH specific activity, also provides an index of cell viability during the prolonged culture conditions. There were no significant changes in cytosolic protein (mean ± SD), consistent with what we have previously reported for human preadipocyte cultures.[12] For SC preadipocytes, values were 0.06 ± 0.046 and 0.072 ± 0.035 mg/mL for control and differentiated cells, respectively, whereas for OM preadipocytes, values were 0.08 ± 0.045 and 0.065 ± 0.048

mg/mL for control and differentiated cells, respectively ($P > .50$, ANOVA).

DISCUSSION

We have examined whether the adipogenic response of preadipocytes is influenced by anatomic site-specific factors. Our data indicate that, under a defined differentiation program, abdominal SC and OM preadipocytes from mature men and women differentiate similarly in primary culture.

Depot-specific adipogenic differences, specifically those between SC and OM preadipocytes, have been investigated previously in 3 other studies, but only to a limited extent. In contrast to our data on men and women, one report based on 14 women (obese and non-obese) showed that abdominal SC preadipocytes have a 4.5-fold greater differentiation capacity than those of the intra-abdominal OM region.[14] The serum-based differentiation medium used by these investigators was suboptimal, precluding GPDH measurements, and the few cells differentiating were instead counted by microscopy. In addition, their study subjects had a mean age that was approximately 20 years younger than our patients. Given that we likely had a greater proportion of postmenopausal female subjects, based on age criteria, the differences might also reflect hormonal influences on adipogenesis. A later study by the same group, but restricted to SC preadipocytes from 24 obese women, demonstrated that abdominal SC preadipocytes had a 1.6-fold greater rate of adipose conversion than those from the SC femoral depot, measured by GPDH assay.[15] In those female subjects, mean age and BMI were 43 and 41, respectively, compared to 59 and 27 in our study. Their results suggest that weak regional differences may exist in the capacity of adipose tissue to form new fat cells, but OM preadipocytes were not studied.

In another report specifically comparing maturation of abdominal SC and OM preadipocytes obtained from a number of control ($n = 7$) and obese ($n = 8$) subjects, no differences were observed, in agreement with our data.[16] Their culture medium differed from ours, and the number of differentiating cells (~6.5%) was very low.

Recently, it has been shown that thiazolidinediones (ligands and activators of PPARγ) permit the differentiation of multiply-passaged preadipocytes from abdominal SC adipose tissue, but with a reduced response from OM preadipocytes.[17] There were no region-specific differences in PPARγ protein expression detected that might have explained this disparity. Our culture conditions differ from the ones employed in that study, in that we did not allow preadipocyte proliferation prior to placing the cells in differentiation medium, in order to facilitate differentiation.[10] The difference between SC and OM preadipocytes observed by these authors might be on the basis of a region-specific preadipocyte response to the potent thiazolidinediones used.

Our study was based on a late and specific marker of adipocyte differentiation, GPDH.[8,12] Because of the limited number of preadipocytes available to us using our nonproliferating culture conditions, we were unable to assess whether early adipogenic markers, or the rate of differentiation, might differ between depots. Future work is needed to answer these issues, as well as to examine the cellular responses to other differentiation protocols. Nevertheless, our results indicate that SC preadipocytes are not routinely more susceptible than OM preadipocytes to adipogenic stimuli. Given the number of patients in our study, the statistical power was sufficient ($\beta < 0.2$) to allow us to detect such differences of the order observed in other studies.[14,15] However, it is possible that smaller differences between the adipogenic responses of preadipocytes from these two depots might exist.

Further studies will be needed to determine if there are intrinsic functional differences between preadipocytes from different depots, and their responses to potent thiazolidinedione drugs. Such differences might be due to distinct inherent characteristics particular to these cells. Alternatively, variations in local paracrine/cytokine influences on preadipocytes in vivo, unique to each adipose tissue depot, might have a persistent effect on their behavior in primary culture. Differences in innervation, vascularity, and overall cellular composition of these depots might also prove relevant in this regard. Recent clinical trials on type 2 diabetic subjects suggests that thiazolidinediones may both reduce intra-abdominal fat and augment subcutaneous fat depots.[18-20] Other investigators have suggested that defects in adipogenic capacity may be important in the pathogenesis of type 2 diabetes mellitus in the obese.[21,22] More knowledge of depot-specific characteristics of preadipocytes at the molecular and cellular level may identify targets that could be developed into novel therapies for obesity.

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