Original Articles

Increased Lipolysis and Decreased Leptin Production by Human Omental as Compared With Subcutaneous Preadipocytes

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Site differences in adipose tissue function may have implications for insulin-resistant conditions. In mature adipose tissue, subcutaneous adipocytes have higher leptin secretion, similar tumor necrosis factor (TNF)-α secretion, and lower catecholamine-stimulated lipolysis as compared with omental adipocytes. In this study, lipolysis and leptin and TNF-α secretion were compared between human omental and subcutaneous preadipocytes. After 16 days of incubation in a minimal differentiation medium, leptin mRNA and secretion were found to be two to eight times higher in subcutaneous than omental preadipocytes ($P < 0.05$). On the other hand, norepinephrine-induced lipolysis was about two times higher in the omental than in the subcutaneous preadipocytes, whereas basal lipolysis did not differ between the two regions. TNF-α secretion was marginally but significantly higher in the omental than in the subcutaneous preadipocytes. Preadipocyte differentiation was equal in both regions and was augmented to the same extent by different thiazolidinediones (rosiglitazone, pioglitazone, or troglitazone) in the two depots. In the presence of rosiglitazone, leptin secretion remained about three times higher and norepinephrine-induced lipolysis about six times lower in subcutaneous as compared with omental preadipocytes ($P < 0.05$), whereas TNF-α secretion and basal lipolysis were similar in preadipocytes from the two regions. These findings remained unaltered even if rosiglitazone was removed from the medium. However, leptin mRNA showed no regional differences in rosiglitazone-treated cells. Thus, regional differences in adipocyte leptin secretion as well as in norepinephrine-induced lipolysis are marked and present during different stages of preadipocyte differentiation and seem to be determined by intrinsic (i.e., primary) factors. *Diabetes* 51:2029–2036, 2002

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DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; GPDH, glycerol-3-phosphate dehydrogenase; PPAR, peroxisome proliferator–activator receptor; TNF, tumor necrosis factor; TZD, thiazolidinedione.

Adipose tissue has at least two major functions, namely the storage and release of energy-rich fatty acids and the secretion of proteins, involved in the endocrine or autocrine regulation of energy metabolism and insulin sensitivity (rev. in 1,2). Of potential interest for insulin-resistant conditions is the fact that human adipose tissue is a heterogeneous organ, in terms of both endocrine (protein secretory) and metabolic function. Site differences in lipolysis (i.e., the breakdown of intracellular triglyceride stores) have been repeatedly demonstrated by independent groups (rev. in 3–5). Catecholamines stimulate lipolysis more markedly in the omental than in the subcutaneous adipose tissue, whereas insulin, adenosine, and prostaglandins E are less antilipolytic in the omental as compared with the subcutaneous adipose tissue (3–5). Furthermore, the basal (i.e., nonhormonal) lipolytic rate is higher in subcutaneous than in omental adipocytes (6–8). The omental, but not the subcutaneous adipose tissue, has a direct access to the liver through the portal vein, and some data suggest that there is excess free fatty acid release from the visceral adipose tissue in central obesity, as reviewed (3–5). The latter might interfere with liver metabolism and contributes to the development of disturbances such as glucose intolerance, hyperinsulinemia, and hypertriglyceridemia (3–5).

Two of the most intensely investigated proteins secreted by adipose tissue are leptin and tumor necrosis factor (TNF)-α. Leptin has a major role in the regulation of appetite and energy balance (9). On the other hand, TNF-α is a pro-inflammatory cytokine, with effects on lipid and glucose metabolism (10,11). A number of independent studies have shown that the leptin production rate is much higher in subcutaneous than in omental adipose tissue (12–17). This regional variation might play a role in controlling the size of different fat depots (12,17). In contrast, adipose TNF-α secretion seems not to be subject to depot-specific differences (18), although higher expression levels of both TNF-α and TNF-α receptors were found in subcutaneous versus omental adipose tissue (19).

Previous studies demonstrating heterogeneity in adipose lipolysis and endocrine function have been performed on mature adipocytes and on adipose tissue. These

kind of studies cannot readily determine whether regional differences in adipose tissue function are due to primary or secondary events, i.e., intrinsic differences in adipocytes or effects of external factors derived from the circulation or the central nervous system. An alternative way to obtain more precise information about the etiology is to study preadipocytes isolated from the stromal vascular fraction of adipose tissue that can be kept under defined conditions for a relatively long duration. The current study was designed to test whether regional variations in adipocyte function are primary events and are therefore observed in preadipocytes at different stages of adipocyte differentiation. We compared lipolysis and secretion of leptin and TNF-α in preadipocytes derived from omental and subcutaneous adipose tissue depots from the same individual that were incubated in either a minimal differentiation medium or a medium supplemented with the thiazolidinedione (TZD) rosiglitazone. Rosiglitazone is a potent enhancer of preadipocyte differentiation, which acts by stimulating the peroxisome proliferator–activator receptor (PPAR)-γ (20,21).

RESEARCH DESIGN AND METHODS

Subjects and adipose tissue biopsies. The study group consisted of 24 obese patients who were undergoing a gastric restrictive procedure (vertical banded gastroplasty). Except for obesity, they were all healthy and not taking any medication. No selection was made for age (range 22–65 years, mean ± SE 40 ± 3 years) or sex (3 men, 21 women). The BMI was measured on the day before surgery and after an overnight fast. Values ranged from 31 to 60 kg/m^2 (mean ± SE 46 ± 2 kg/m^2). The study was approved by the Ethics Committee at Huddinge Hospital. All subjects gave their informed consent. On the day of surgery, general anesthesia was induced at 9.00 A.M., as previously described (22). Adipose tissue specimens from the subcutaneous and omental adipose tissue regions were obtained within 30–45 min after the onset of surgery. In general, ~10–15 g of adipose tissue was obtained from each region, which yielded one 24-well plate of preadipocytes per depot.

Isolation and culture of adipocyte precursor cells. The isolation and differentiation of preadipocytes was performed according to the method described by Hauner et al. (23), with some modification described below.

Tissue fragments were incubated with 0.5 g/l of collagenase (Sigma, St. Louis, MO) in Krebs Ringer Phosphate buffer (pH 7.4) with 40 g/l of dialyzed BSA (fraction V; Sigma) for 1 h at 37°C. The desegregated adipose tissue was filtered through a nylon mesh with a pore size of 250 μm, and the cell suspension was centrifuged at 200*g* for 10 min at room temperature; the pellet was incubated in 10 ml erythrocyte lysis buffer consisting of 0.154 mol/l NH_4Cl, 5.7 mmol/l K_2HPO_4, and 0.1 mmol/l EDTA (pH 7.3) for 10 min at room temperature. The preadipocyte fraction was centrifuged as above, resuspended in 10 ml of DMEM/NUT.MIX.F12 medium (Life Technologies, Paisley, Scotland), and filtered through a nylon filter with a pore size of 70 μm. After an additional centrifugation step, the fraction was resuspended in DMEM/NUT.MIX.F12 medium and supplemented with 10% fetal calf serum, 100 μg/ml penicillin, and 2.5 μg/ml amphotericin B. Cells were inoculated into 24-well plates at a density of 50,000 cells/cm^2 and kept at 37°C in 5%CO_2 for 20 h. The cells were then washed and incubated in serum free medium, which consisted of DMEM/NUT.MIX.F12 medium with 100 nmol/l cortisol, 66 nmol/l insulin, 15 mmol/l HEPES, 1 nmol/l triiodothyronine, 33 μmol/l biotin, 17 μmol/l patothenate, 10 μg/ml transferrin, 100 μg/ml penicillin-streptomycin, and 2.5 μg/ml amphotericin B with or without 10 μmol/l rosiglitazone (BRL 49653) (kindly provided by Smith Kline Beecham Pharmaceuticals, Harlow, Essex, U.K.) until day 16. For three subjects, a time-course experiment was performed for leptin and TNF-α secretion. In these experiments, aliquots of the medium with and without rosiglitazone were kept during differentiation, every time the cells were refed and subsequently used for preadipocyte leptin and TNF-α measurement. In six experiments, half of the cells were differentiated with rosiglitazone for 16 days, compared with 14 days for the other half. Then the latter cells were kept without rosiglitazone until day 16. In three experiments, cells were also differentiated with other TZDs (pioglitazone 1 μmol/l or troglitazone 1 μmol/l). Plates containing >5% of endothelial cells were discarded in all experiments.

Assessment of preadipocyte differentiation and biochemical measurements. All of the measurements were performed on medium or cells from wells that contained no or 10 μmol/l rosiglitazone, 16 days after starting the differentiation process. For all subjects, preadipocyte medium (300 μl) was removed from 12 wells for subsequent leptin and TNF-α secretion analysis. Medium proteins were precipitated overnight at −20°C with three volumes absolute ethanol. After centrifugation at 1,500*g* for 30 min at 4°C, the pellets were dissolved in 300 μl of distilled water, and the leptin and TNF-α levels determined with a human leptin enzyme linked immunoassay and a high sensitivity human TNF-α enzyme linked immunoassay, respectively (RD systems, Abingdon, U.K.). Leptin and TNF-α standards were precipitated as above and added to the respective enzyme-linked immunosorbent assays (ELISAs). Under these conditions, the detection limit of leptin and TNF-α in the incubation medium was 0.7 and 0.02 pg/ml, respectively. It was shown that there was a constant recovery of ~50% during the concentration procedure. Moreover, ~5% vol/vol ethanol was added to (unprecipitated) standards, and it was shown that ethanol per se did not affect the ability of the ELISAs to detect TNF-α or leptin, respectively. TNF-α is generally thought to be more stable in the presence of BSA. However, secretion here was assessed in BSA-free medium, since the presence of BSA increases markedly the risk of bacterial/fungoid contamination. However, in methodological experiments, the effect of BSA on the measurement of leptin or TNF-α was investigated. Subcutaneous preadipocytes were incubated in the presence or absence of 20 g/l BSA, the last time the cells were refed and standards were dissolved in the presence or absence of BSA. It was possible to obtain three experiments that were free of infection. TNF-α secretion from cells incubated in the absence or presence of BSA was almost identical: 0.107 ± 0.05 and 0.103 ± 0.04 ng/(l × 24 h), respectively. There was also no effect of BSA on the secretion of leptin (values not shown).

In nine experiments, total RNA was prepared from cells in three wells using the RNeasy mini kit (Qiagen, Hilden, Germany). This total RNA was used as an index of total cell amount per well and to quantify leptin mRNA by a solution hybridization assay, as described previously (12), as well as TNF-α mRNA expression with a TNF-α Quantikine mRNA kit (RD systems). In 12 experiments, proteins were extracted from cells in three wells using a protein lysis buffer that contained 1% Triton X-100, Tris HCl (pH 7.6), and 150 mmol/l NaCl supplemented with protease inhibitors (Complete; Boehringer Mannheim, Indianapolis, IN). These total proteins were also used as an index of total cell amount per well.

In 13 experiments, cells in 12 wells were washed with DMEM/NUT.MIX.F12 medium and then incubated for 3 h with DMEM/NUT.MIX.F12 medium containing 20 g/l BSA without (six wells) or with 10^{-9}, 10^{-7} and 10^{-5} mol/l norepinephrine (two wells each). The medium was removed and kept for measuring glycerol concentration (an index of lipolysis) using a bioluminescence method. Methodological experiments revealed that during this period glycerol release was linear with time.

In seven experiments, preadipocyte differentiation was assessed using Oil Red O staining. Cells in three wells were fixed with 7% formaldehyde in phosphate buffer saline, and their triglyceride content was stained with 1% Oil Red O in 60% isopropanol. After repeatedly washing the cells with water, their Oil Red O content was dissolved in 100% isopropanol and the optical density of this solution was measured at 500 nm. An empty well was treated the same way and used as a blank. In 11 experiments, differentiation of preadipocytes was also estimated by direct counting of differentiated cells under the microscope. Six-well diameters were counted from left to right within <30 min. Cells were regarded as differentiated when they had a round shape and their cytoplasm was completely filled with multiple fat droplets. This is referred to as relative cell number. Additionally, differentiation was measured in 12 experiments by quantifying glycerol-3-phosphate dehydrogenase (GPDH) activity, as described previously (23). Cells from three wells were washed with PBS (pH 7.4) and harvested in prechilled 25 mmol/l Tris-HCl buffer containing 1 mmol/l EDTA (pH 7.5). After sonification, aliquots of the cell extracts were added to an assay mixture containing 100 mmol/l triethanolamine HCl buffer (pH 7.5), 2.5 mmol/l EDTA, 0.12 mmol/l NADH, and 0.1 mmol/l mercaptoethanol, and GPDH activity was measured spectrophotometrically, at 340 nm. The reactions were started by adding 0.2 mmol/l dihydroxyacetonphosphate.

Release of glycerol, leptin, and TNF-α were expressed in a number of ways, including as the absolute concentration in the medium, the concentration per amount of total protein or total RNA or the concentration per GPDH actvity. GPDH activity was expressed as mU/mg total protein. Values were expressed per amount of total RNA rather than per total DNA, because the cells in the secretion experiments were used for RNA extraction and subsequent mRNA analysis. There were not enough preadipocytes to do both DNA and RNA extraction, and there are no reasons to believe that the amount of total RNA per cell does differ between omental and subcutaneous preadipocytes. In

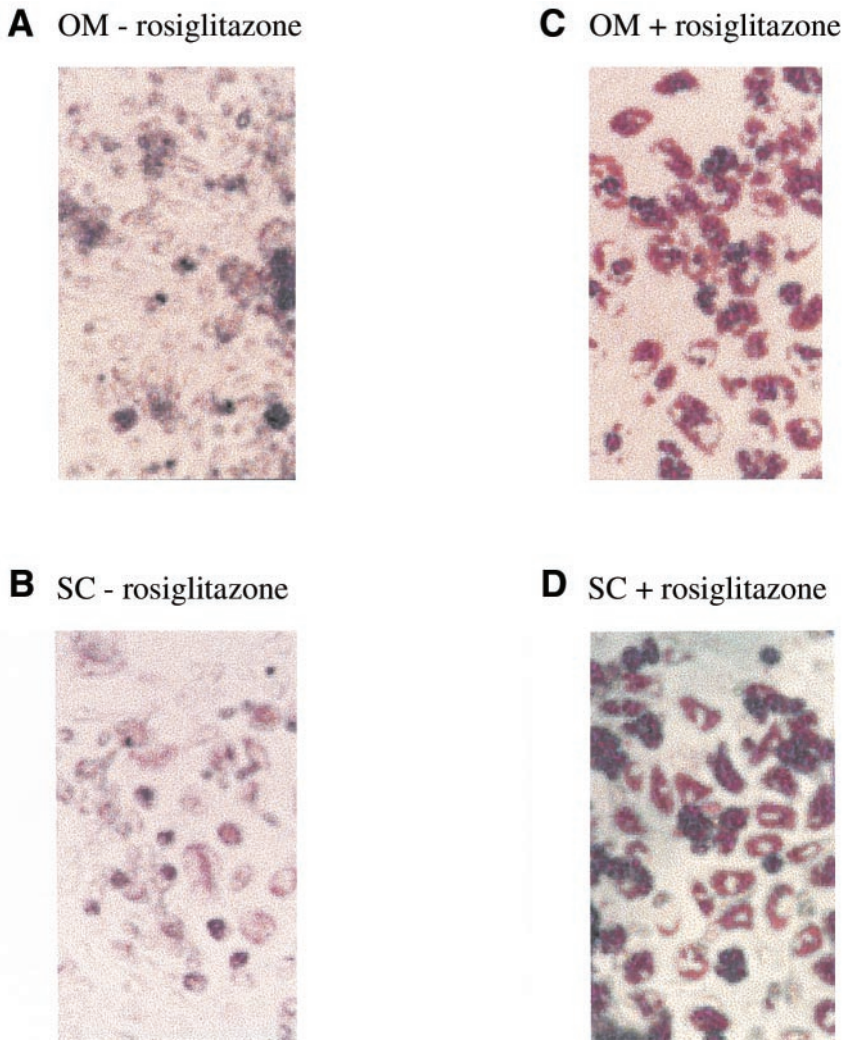

FIG. 1. Rosiglitazone induces differentiation of both omental and subcutaneous preadipocytes. Cells from the stromal vascular fraction of adipose tissue of either depot were grown in differentiation medium with (+ rosiglitazone; *C* and *D*) or without 10 μmol/l rosiglitazone (− rosiglitazone; *A* and *B*). After 16 days of culture, the cells were fixed and stained with Oil Red O. Cells from one representative subject are shown. OM, omental preadipocytes; SC, subcutaneous preadipocytes.

addition, there were not enough preadipocytes to isolate both total RNA and total proteins in the same experiment. Total RNA was not isolated in the experiments in which lipolysis was measured.

Statistical analysis. Values are given as means ± SE. Repeated-measures ANOVA, nonparametric Wilcoxon's signed-rank test, and linear regression analysis were used for statistical comparisons. $P < 0.05$ (two-sided) was regarded as statistically significant.

RESULTS

Differentiation. Preadipocytes were differentiated with or without rosiglitazone for 16 days, and their triglyceride content was stained with Oil Red O. Figure 1 shows the stimulatory effect of rosiglitazone on lipid accumulation in omental and subcutaneous preadipocytes. Differentiation was also quantified as shown in Table 1. Three different methods were used to quantify differentiation, i.e., Oil Red O staining, direct counting of differentiated cells, and measuring GPDH activity. These three methods gave the same information: rosiglitazone markedly increased differentiation of both omental and subcutaneous preadipocytes ($P < 0.05$). Furthermore, the degree of differentiation did not differ between omental and subcutaneous preadipocytes. However, it should be noted that in most of the cases, GPDH activity (six of nine cases) was below the detection limit when the cells were differentiated without rosiglitazone, whereas in cells differentiated with rosiglitazone, GPDH activity was always detectable. In three experiments, preadipocytes were also differentiated for 16 days with TZDs other than rosiglitazone (i.e., pioglitazone and troglitazone). In all three experiments, in both omental and subcutaneous preadipocytes, GPDH activity was increased with pioglitazone, troglitazone, and rosiglitazone (Fig. 2). GPDH values in Fig. 2 and Table 1 differ somewhat because they represent nine experiment in the table and three experiments in the figure.

Lipolysis. Figure 3 shows the effect of increasing concentrations of norepinephrine on preadipocyte lipolysis. Norepinephrine stimulated lipolysis in a concentration-dependent fashion in preadipocytes from both regions, when they were differentiated either with or without rosiglitazone. Both with and without rosiglitazone, omental preadipocytes showed a significantly higher norepinephrine-induced lipolysis than subcutaneous preadipocytes (repeated-measures ANOVA, without rosiglitazone, $F = 16$,

TABLE 1
The effect of rosiglitazone on omental and subcutaneous preadipocyte differentiation

	n	Without rosiglitazone	With rosiglitazone	*P* (with vs. without rosiglitazone)
Oil Red O staining (OD 500 nm)				
OM	9	0.03 ± 0.01	0.09 ± 0.02	<0.01
SC		0.02 ± 0.01	0.10 ± 0.02	<0.01
P (OM vs. SC)		NS	NS	
Relative number of differentiated cells				
OM	11	323 ± 132	3,700 ± 854	<0.01
SC		274 ± 123	2,828 ± 560	<0.01
P (OM vs. SC)		NS	NS	
GDDH (mU/mg total protein)				
OM	9	130 ± 100	700 ± 140	<0.05
SC		80 ± 50	600 ± 140	<0.05
P (OM vs. SC)		NS	NS	

Data are means ± SE. Differentiation of preadipocytes was determined by Oil Red O (ORO) staining, by direct counting under the microscope of differentiated cells in six well diameters, and by measuring GPDH activity. Differentiation was compared between omental (OM) and subcutaneous (SC) preadipocytes and between cells differentiated for 16 days with or without rosiglitazone. NS, nonsignificant. For more information see Fig. 1 and RESEARCH DESIGN AND METHODS. *n*, number of experiments.

$P < 0.005$; with rosiglitazone, $F = 6$, $P < 0.05$). Table 2 shows glycerol release expressed in several ways, i.e., as the absolute values released in the medium, the values corrected for total protein, as well as the values corrected for preadipocyte differentiation level (i.e., GPDH activity). With either way of expression, basal lipolysis levels did not differ between cells from the two regions, whereas omental preadipocytes showed a significantly higher norepinephrine-induced lipolysis both with and without rosiglitazone. Rosiglitazone slightly increased the absolute values for basal lipolysis, whereas it did not affect basal lipolysis expressed as glycerol per total protein. Rosiglitazone markedly and significantly increased norepinephrine-induced lipolysis in both omental and subcutaneous preadipocytes, both when glycerol release was expressed as absolute values and as values corrected for total amount of protein (Table 2).

Leptin. Figure 4*A* shows that during the differentiation of the preadipocytes, there was a time-dependent and significant increase in leptin secretion, both in the presence and absence of rosiglitazone and for preadipocytes from both adipose tissue regions ($P < 0.01$). Leptin over time was higher in the presence than in the absence of rosiglitazone. Data with leptin secretion at day 16 of differentiation are shown in Table 3. Based on leptin concentrations in the medium, it was found that subcutaneous preadipocytes secreted more leptin than omental preadipocytes, both in the absence or presence of rosiglitazone ($P < 0.05$). Subcutaneous preadipocytes also secreted more leptin than omental preadipocytes ($P < 0.01$), when the absolute leptin values were corrected for differences in total RNA and GPDH activity. Depending on the way data were expressed, leptin secretion was about two- to eightfold higher in subcutaneous than in omental cells.

The omental or subcutaneous cell preparations that

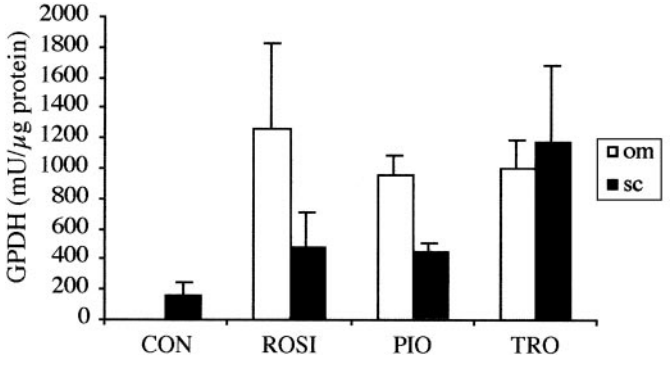

FIG. 2. The effect of the TZDs rosiglitazone (ROSI 10 μmol/l), pioglitazone (PIO 1 μmol/l), and troglitazone (TRO 1 μmol/l) on differentiation of subcutaneous (sc) and omental (om) preadipocytes measured as GPDH activity. Number of experiments = 3. CON, without TZDs.

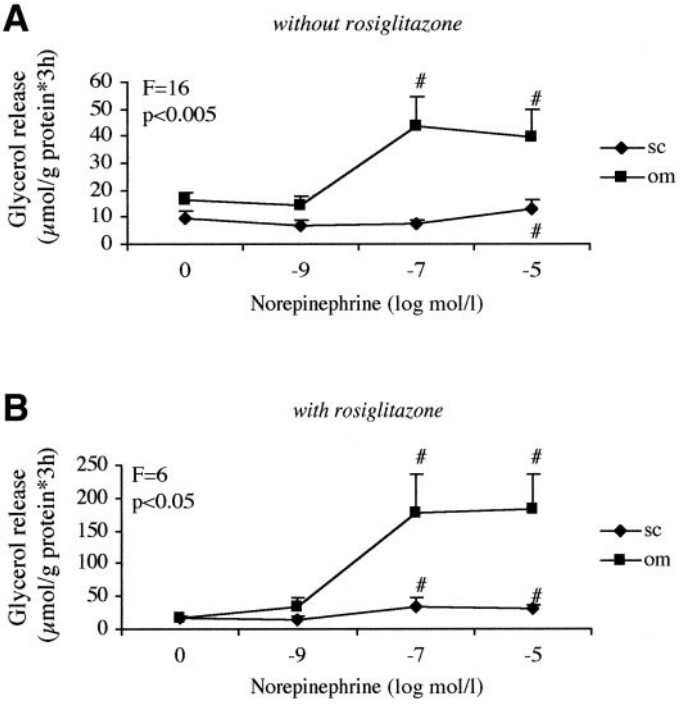

FIG. 3. Lipolysis during stimulation of omental (om) and subcutaneous (sc) preadipocytes with norepinephrine for 3 h. Cells were grown in the absence (*A*) or presence (*B*) of 10 μmol/l rosiglitazone. Glycerol release was expressed per total protein. The effect of norepinephrine on lipolysis was statistically analyzed (#$P < 0.05$). Number of experiments = 7. Norepinephrine-induced lipolysis was compared between omental and subcutaneous preadipocytes by repeated-measures ANOVA (*F*).

TABLE 2
Comparison of basal and norepinephrine-induced lipolysis between omental and subcutaneous preadipocytes differentiated for 16 days in the presence or absence of rosiglitazone

	−Rosiglitazone			+Rosiglitazone		
Glycerol release	OM	SC	*P* (OM vs. SC)	OM	SC	*P* (OM vs. SC)
A) μmol/(1 × 3 h)						
Basal	1.35 ± 0.26	0.87 ± 0.19	NS	1.98 ± 0.42*	1.54 ± 0.23*	NS
NE 10^{-7} mol/l	3.75 ± 0.98	0.73 ± 0.14	<0.05	22.97 ± 8.37*	2.90 ± 0.78*	<0.05
NE 10^{-5} mol/l	3.17 ± 0.60	1.20 ± 0.22	<0.05	23.16 ± 7.23*	2.91 ± 0.31*	<0.05
B) μmol/(g protein × 3 h)						
Basal	16.09 ± 3.32	9.63 ± 2.07	NS	15.51 ± 14.60	16.49 ± 3.24	NS
NE 10^{-7} mol/l	43.62 ± 10.71	7.52 ± 1.18	<0.05	177.52 ± 59.60*	34.14 ± 14.04*	<0.05
NE 10^{-5} mol/l	39.75 ± 9.97	12.80 ± 3.01	<0.05	181.24 ± 54.13*	31.35 ± 4.77*	<0.05
C) nmol/(mU GPDH × μg protein × 3 h)						
Basal	ND	ND	—	14.23 ± 5.78	9.00 ± 1.15	NS
NE 10^{-7} mol/l	ND	ND	—	114.32 ± 34.33	17.62 ± 3.53	<0.05
NE 10^{-5} mol/l	ND	ND	—	104.88 ± 24.36	18.95 ± 3.39	<0.05

Data are means ± SE. Glycerol release is expressed as *A*) absolute concentration released into the medium, *B*) *A* corrected for differences in total cell amount expressed as amount of total protein, and *C*) *A* corrected for preadipocyte differentiation expressed as GPDH activity. Glycerol release was statistically compared between omental (OM) and subcutaneous (SC) preadipocytes and between cells differentiated with (+rosiglitazone) or without rosiglitazone (−rosiglitazone) (*$P < 0.05$). NS, nonsignificant. ND, GPDH activity was below the detection limit. Number of experiments = 7.

were differentiated in the presence of rosiglitazone secreted significantly more leptin than those in the absence of rosiglitazone ($P < 0.05$). There was also a tendency to a difference due to rosiglitazone when absolute values were corrected for total RNA, but this difference was not significant ($P = 0.10$).

When preadipocytes were differentiated in the absence of rosiglitazone, the subcutaneous preadipocytes showed a fourfold higher leptin mRNA expression than omental preadipocytes (Table 3, $P < 0.05$). There was no regional difference in leptin mRNA expression when cells were cultured in the presence of rosiglitazone.

Subcutaneous but not omental preadipocytes that were differentiated with rosiglitazone had significantly lower leptin mRNA expression levels than preadipocytes from the same region that were differentiated without rosiglitazone.

TNF-α. Figure 4*B* shows that during differentiation of preadipocytes, TNF-α secretion showed a time-related tendency to decrease, but this did not reach a statistical level, except for subcutaneous preadipocytes differentiated in the presence of rosiglitazone ($P < 0.05$). TNF-α levels over time were lower in the presence than in the absence of rosiglitazone. Data with TNF-α secretion at day 16 of differentiation are shown in Table 4. TNF-α secretion did not differ significantly between omental and subcutaneous preadipocytes, differentiated in the presence of rosiglitazone. This was true when the absolute values were used or when the values were corrected for differences in total RNA or level of differentiation (GPDH). In the absence of rosiglitazone, omental preadipocytes showed a significant higher absolute secretion of TNF-α but this difference disappeared when the values were corrected for differences in total RNA. Both subcutaneous and omental preadipocytes differentiated with rosiglitazone showed a significantly decreased TNF-α release as compared with cells differentiated without rosiglitazone, when the absolute values were corrected for total RNA ($P < 0.05$).

In Table 4, it is shown that there was a 10% lower level of TNF-α gene expression in subcutaneous preadipocytes as compared with omental preadipocytes in the absence of rosiglitazone. No significant regional differences in TNF-α gene expression were found for cells differentiated in the presence of rosiglitazone. Omental but not subcutaneous preadipocytes that were differentiated with rosiglitazone had significantly lower TNF-α mRNA expression than preadipocytes from the same region that were differentiated without rosiglitazone ($P < 0.05$)

The effect of removing rosiglitazone from the differentiation medium at day 14 of the differentiation process. In the above-mentioned experiments, preadipocytes were differentiated in the absence or presence of rosiglitazone for 16 days. In six experiments, norepinephrine-induced lipolysis, leptin release, or TNF-α release were compared between preadipocytes that were differentiated for 16 days with rosiglitazone and preadipocytes that were differentiated for 16 days but from which rosiglitazone was removed at day 14. It was observed that removal of rosiglitazone for 2 days had no effect on lipolysis and leptin and TNF-α secretion (figure not shown).

Age and BMI. The values for lipolysis and leptin or TNF-α secretion were analyzed for correlations to BMI and age. No statistically significant correlations were found.

DISCUSSION

To our knowledge, this is the first comparison of lipolysis and leptin and TNF-α secretion between human preadipocytes obtained from the omental and subcutaneous adipose tissue regions. The aim of the study was to elucidate whether previously demonstrated regional differences in metabolic and endocrine functions of human mature adipocytes are due to intrinsic or environmental factors (i.e., factors from the circulation or the central nervous system).

Norepinephrine-induced lipolysis differed between the omental and subcutaneous preadipocytes when the cells were differentiated in either the presence or absence of

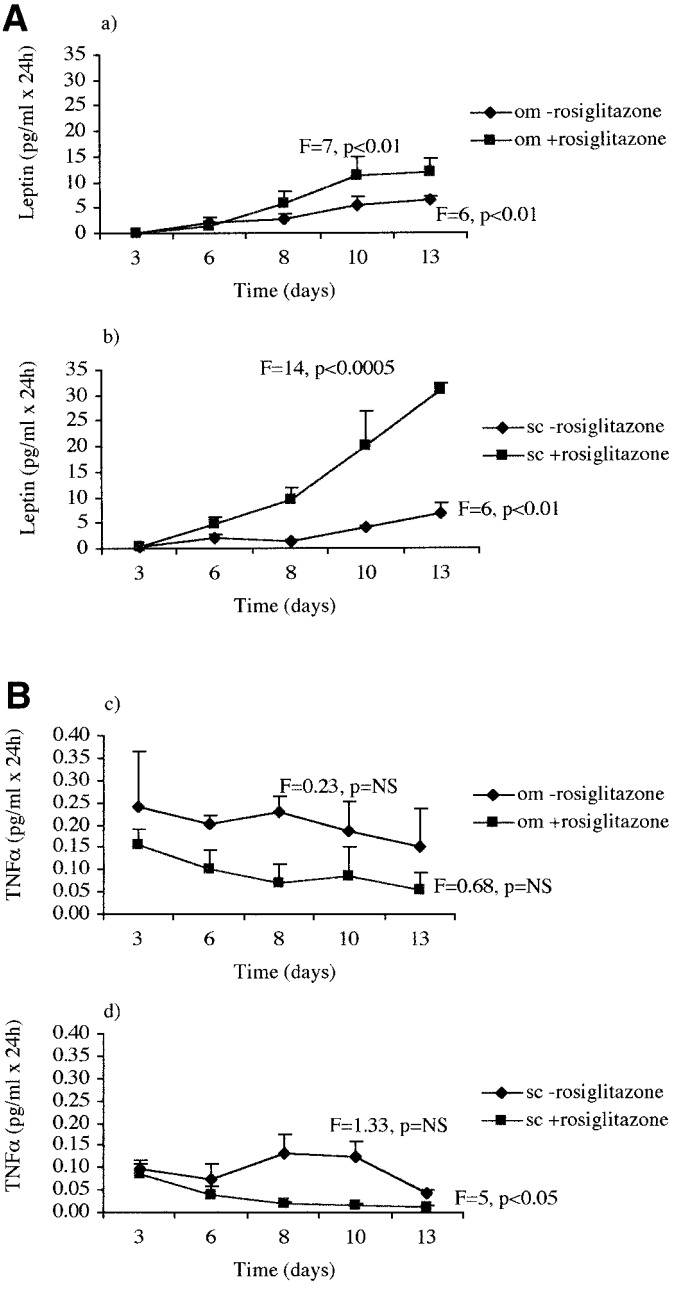

FIG. 4. Leptin (*A*) and TNF-α (*B*) secretion by human omental (*a* and *c*) and subcutaneous (*b* and *d*) preadipocytes during differentiation in the absence and presence of 10 μmol/l rosiglitazone. Leptin and TNF-α were measured every 2–3 days in the aspirated medium that was renewed thereafter. Number of experiments = 3. Secretion during differentiation was statistically analyzed using ANOVA (*F*).

rosiglitazone. Irrespective of how lipolysis was expressed, the norepinephrine effect was much more apparent in omental than in subcutaneous preadipocytes. These regional differences in preadipocyte lipolysis are in agreement with former studies performed on mature adipocytes. Numerous studies have demonstrated that catecholamine-induced lipolysis is higher in omental than in subcutaneous adipocytes (3–5). Thus, when the current and previous results are put together, it is apparent that intrinsic factors and not external factors from the circulation are responsible for the regional variations in catecholamine-induced lipolysis seen in mature adipocytes. This in turn suggests that omental and subcutaneous adipocytes might originate from precursor cells that are genetically different or that are programmed differentially by the surrounding tissue before their differentiation.

On the other hand, basal lipolysis did not differ between omental and subcutaneous preadipocytes. This was seen when cells were differentiated both with and without rosiglitazone. Thus no regional differences in basal lipolysis were observed either at low or high levels of preadipocyte differentiation. Studies on mature adipocytes have shown that subcutaneous adipocytes have a higher basal lipolysis than omental adipocytes (6–8). Thus, the regional difference in basal rate of lipolysis in mature cells seems to be a secondary phenomenon, maybe related to circulatory factors, nervous system, or environmental factors derived from the stroma of adipose tissue.

Leptin secretion was much higher in the subcutaneous than in the omental preadipocytes and was independent of the presence or absence of rosiglitazone or the mode of expression of leptin secretion. Leptin mRNA might be one factor underlying the regional difference in preadipocyte leptin secretion since leptin mRNA was significantly and four times higher in the subcutaneous as compared with the omental preadipocytes, when the cells were differentiated in the absence of rosiglitazone. However, other, post-translational factors should not be excluded since there was no significant regional difference in leptin mRNA in the presence of rosiglitazone. On the other hand, in the presence of rosiglitazone, leptin mRNA levels might have been too low to detect statistically significant regional differences, although a sensitive mRNA assay was used. Studies on mature adipocytes and adipose tissue have shown that the subcutaneous adipose depot has a higher leptin secretion rate and a higher leptin gene expression than the omental adipose depot (12–17), so our findings are in agreement with these studies. Our findings therefore suggest that regional differences in adipocyte leptin secretion are intrinsically determined and are specific for leptin.

Preadipocyte TNF-α secretion and gene expression did not show any statistical significant difference between the two regions in the presence of rosiglitazone. In the absence of rosiglitazone, TNF-α secretion and gene expression were marginally but significantly higher in the omental than in the subcutaneous preadipocytes, although the regional difference disappeared when secretion was expressed per total RNA. There might be small regional differences in TNF-α release from preadipocytes that could not be detected in the current study due to the statistical power. However, the putative differences of such small (and theoretical) differences seem to be minor and therefore of less biological and pathophysiological significance than the regional difference in preadipocyte leptin release and lipolysis. Previous observations have shown no regional differences in TNF-α secretion from the two adipose tissue regions (18) or higher TNF-α mRNA expression in subcutaneous versus omental adipose tissue samples (19). Therefore, at present it is unclear whether TNF-α production differs between subcutaneous and omental adipocytes.

The present rates of leptin and TNF-α secretion as well as mRNA levels in the two depots differ in magnitude from pervious values reported for native omental and subcutaneous adipocytes (12,18,19). This discrepancy could be

TABLE 3
Comparison of leptin secretion and mRNA expression between omental and subcutaneous preadipocytes differentiated for 16 days in the presence or absence of rosiglitazone

		−Rosiglitazone			+Rosiglitazone		
	n	OM	SC	*P* (OM vs. SC)	OM	SC	*P* (OM vs. SC)
Leptin secretion							
A) ng/(1 × 24 h)	22	14 ± 2	27 ± 7	<0.05	23 ± 4*	58 ± 13*	<0.001
B) μg/(g total RNA × 24 h)	9	8 ± 2	67 ± 25	<0.01	16 ± 4	104 ± 35	<0.01
C) pg/(mU GPDH × μg protein × 24 h)	7	ND	ND	—	23 ± 6	39 ± 9	<0.05
Leptin mRNA expression							
D) pmol mRNA/g total RNA	9	18 ± 9	69 ± 30	<0.05	15 ± 6	23 ± 7*	NS

Data are means ± SE. Leptin secretion is expressed as *A*) absolute concentration released into the medium, *B*) *A* corrected for differences in total cell amount expressed as amount of total RNA, *C*) *A* corrected for differentiation expressed as GPDH activity, and *D*) leptin mRNA expression was expressed as pmol mRNA per gram of total RNA. Leptin secretion and mRNA expression were statistically compared between omental (OM) and subcutaneous (SC) preadipocytes. NS, nonsignificant. Leptin secretion and mRNA expression were compared between cells differentiated with (+ rosiglitazone) or without rosiglitazone (− rosiglitazone): *$P < 0.05$. *n*, number of experiments. ND, GPDH activity was below the detection limit.

due to a number of factors. First, preadipocytes may behave quite differently from native cells in their turnover of protein and mRNA. Second, the earlier studies were conducted on intact pieces of adipose tissue floating in the medium under acute conditions. The present studies were performed under long-term culture conditions with cells adhered to the bottom of a culture plate.

Rosiglitazone is a potent stimulator of adipocyte differentiation (20,21). We found that rosiglitazone enhanced the differentiation of preadipocytes that were cultured in differentiation medium containing cortisol, insulin, and T3. After being cultured for 16 days in either the presence or absence of rosiglitazone, the omental and subcutaneous preadipocytes were seen to have differentiated to the same extent. These data are in contrast to findings of Adams et al. (20) who showed that rosiglitazone promotes lipid accumulation in human subcutaneous but not in omental preadipocytes. The discrepancy is likely due to methodological differences. In contrast to primary cultures used in our study, the preadipocytes used in the study of Adams et al. (20) were harvested and subcultured two to three times. It has previously been shown that the differentiation capacity of primary human preadipocytes is almost completely lost when they are subcultured (23). In addition we were able to stimulate differentiation of both subcutaneous and omental preadipocytes with TZDs other than rosiglitazone, i.e., troglitazone and pioglitazone.

Norepinephrine-induced lipolysis for both adipose tissue regions increased markedly in preadipocytes differentiated in the presence of rosiglitazone. This is in concordance with nonhuman adipose cell lines, which demonstrated an increased catecholamine-induced lipolysis in troglitazone stimulated C3H10T1/2 preadipocytes (24).

Rosiglitazone has been found to repress leptin gene expression in earlier studies (25–28). Rosiglitazone also diminished leptin mRNA expression of the subcutaneous preadipocytes in the current study. In the omental preadipocytes, however, leptin mRNA levels were not affected by rosiglitazone. This could be due to the already low levels of leptin mRNA in omental preadipocytes in the absence of rosiglitazone. On the other hand, leptin secretion was stimulated significantly by rosiglitazone, when measured as absolute medium concentrations. This probably reflected the stimulatory effect of the compound on preadipocyte fat accumulation, since it was also demonstrated that leptin secretion from both omental and subcutaneous preadipocytes, in both the presence and absence of rosiglitazone, increased significantly during the differentiation process.

TNF-α secretion and gene expression were inhibited in preadipocytes differentiated in the presence of rosiglitazone. A previous study has shown that rosiglitazone had no effect on adipose TNF-α release (29). However, this earlier study was performed on mature adipocytes and adipose tissue from nonobese subjects. Moreover, pioglitazone was shown to decrease TNF-α expression in adipocytes from obese animals (30).

The effects of rosiglitazone on lipolysis, leptin, and TNF-α secretion seemed to be chronic, since removing rosiglitazone from the differentiation medium did not change any of these

TABLE 4
Comparison of TNF-α secretion and mRNA expression between omental and subcutaneous preadipocytes differentiated for 16 days in the presence or absence of rosiglitazone

		−Rosiglitazone			+Rosiglitazone		
	n	OM	SC	*P* (OM vs. SC)	OM	SC	*P* OM vs. SC
TNF-α secretion							
A) ng/(1 × 24 h)	22	0.25 ± 0.07	0.16 ± 0.04	<0.05	0.16 ± 0.03*	0.13 ± 0.04	NS
B) ng/(g total RNA × 24 h)	9	33 ± 8	35 ± 14	NS	4 ± 2*	8 ± 4*	NS
C) pg/(mU GPDH × μg protein × 24 h)	7	ND	ND	—	0.31 ± 0.08	0.26 ± 0.05	NS
TNF-α mRNa expression							
D) pmol mRNA/g total RNA	9	0.61 ± 0.006	0.56 ± 0.011	<0.05	0.54 ± 0.005*	0.54 ± 0.008	NS

For more information see Table 3.

parameters as compared with when rosiglitazone was present continuously during the differentiation process.

In conclusion, possible differences in TNF-α secretion and basal lipolysis in adipocytes seem to be secondary to external factors. On the other hand, the difference in norepinephrine-induced lipolysis and leptin release, between omental and subcutaneous preadipocytes, is in agreement with the literature on mature adipocytes and adipose tissue. Thus, regional differences in adipocyte leptin secretion and catecholamine-induced lipolysis seem to be determined by intrinsic factors, which could be genetic. That leptin secretion is higher and norepinephrine-induced lipolysis is lower in subcutaneous than in omental preadipocytes, irrespective of the degree of preadipocyte differentiation, suggests that the two types of adipocytes either originate from different precursor cells with different intrinsic programs or that they, in preadipocyte form, receive different signals from the surrounding tissue, which in turn induce different endocrine/metabolic programming during differentiation.

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