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Transforming Growth Factor β1 release by human adipose tissue is enhanced in obesity

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Abstract

The present studies examined the effect of obesity in humans on the release of transforming growth factor β1 (TGF-β1) by human adipose tissue. The regulation of TGF-β1 release by adipose tissue as well as the question of whether its release is due to the adipocytes or the nonfat cells in adipose tissue was also examined. There was a statistically significant (r = 0.50) correlation between the body mass index of the fat donors and the subsequent release of TGF-β1 release by subcutaneous adipose tissue. There was also a positive correlation between total TGF-β1 release by adipose tissue explants and body fat content (r = 0.69). The question of whether tumor necrosis factor α (TNF-α) and/or interleukin 1β (IL-1β) regulate the release of TGF-β1 was investigated by incubation of adipose tissue explants with a soluble human TNF-α receptor (etanercept) and a neutralizing antihuman IL-1β antibody. The release of TGF-β1 over 48 hours by adipose tissue explants was significantly enhanced in the presence of both the inhibitor of TNF-α and of IL-1β. It is of interest, in view of the elevated circulating insulin in blood of morbidly obese women, that the release of TGF-β1 by adipose tissue was enhanced in the presence of insulin. The question of whether the release of TGF-β1 by human adipose tissue explants was primarily due to adipocytes, as is the case for leptin, or the nonfat cells present in human adipose tissue, as is the case for IL-8 and prostaglandin E_2, was examined. The release of TGF-β1 was primarily by the nonfat cells of human adipose tissue because release by adipocytes was less than 10% of that by the nonfat cells of adipose tissue.

1. Introduction

There is increasing evidence that transforming growth factor–β (TGF-β) has 2 faces in carcinogenesis, because it can function as either a tumor suppressor or oncogene, depending upon experimental condition [1]. A significant correlation has been reported between body mass index (BMI) of humans and circulating TGF-β1 [2] or adipose tissue TGF-β1 content [3]. In contrast, 2 reports found no correlation between BMI and circulating levels of TGF-β1 [4,5]. However, Samad et al [6] reported an elevated expression of TGF-β1 in obese mice. It is unclear why such different effects of obesity on TGF-β1 were seen, but it may reflect the complex roles of this growth factor.

Possibly, the enhanced expression of TGF-β1 in human obesity is linked to its ability to inhibit adipocyte differentiation [7]. This hypothesis is compatible with the findings of Rahimi et al [8] who reported that murine 3T3-L1 adipocytes released both hepatocyte growth factor, a potent mitogenic factor, and latent TGF-β1 that is able to inhibit DNA synthesis in murine SP1 cells. The latent form of TGF-β1 that is released by cells is unable to bind its receptors until the active form is released from the complex of the propeptide, the TGF-β1 binding protein and the active subunit [9].

The present studies were designed to determine whether release of TGF-β1 by human adipose tissue explants correlated with BMI and fat mass and how TGF-β1 release by fat is regulated. We also examined the question of whether the TGF-β1 released by adipose tissue is derived from the adipocytes or the nonfat cells in adipose tissue.

2. Materials and methods

Visceral or abdominal subcutaneous adipose tissue was obtained from 13 women undergoing abdominal surgery (abdominoplasty) with a total fat mass ranging from 16 to 62 kg approximately 1 year after gastric bypass surgery. Tissue was also obtained from 12 obese women with a total

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fat mass ranging from 41 to 75 kg undergoing laparoscopic gastric bypass with Roux-en-Y gastroenterostomy surgery for the treatment of morbid obesity. The average BMI and total fat content of the tissue donors for each set of experiments is shown in the legends for the figures. The average age of the women was 39 years, and 5 were African American whereas one was Hispanic. Each experimental replication involved tissue from a separate individual, and the fasting blood glucose value exceeded 120 mg/dL in one bypass patient (153 mg/dL). Body fat content was determined using bioelectrical impedance (Tanita TBF-310GS Body Composition Analyzer/Scale, Tanita Corp, Arlington Heights, Ill). The study had the approval of the local international review board, and all patients involved gave their informed consent. The patients were told to be on a 4184-kJ (1000-kcal) liquid diet (high protein) for 2 weeks before surgery.

Samples of adipose tissue were immediately transported to the laboratory. The handling of tissue and cells was done under aseptic conditions. The tissue was cut with scissors into small pieces (5-10 mg). All the studies used explants of adipose tissue that had been previously incubated in buffer [10] containing 1% albumin (3 mL/g of tissue) for approximately 5 to 30 minutes to reduce contamination of the tissue with blood cells and soluble factors. At the conclusion of this incubation, the tissue explants were centrifuged for 30 seconds at 400*g* to remove blood cells and pieces of tissue containing insufficient adipocytes to float. The explants (100 mg/mL) were incubated in duplicate for the indicated times in suspension culture under aseptic conditions.

Adipocytes were prepared by incubating 0.5 g of cut adipose tissue per milliliter of incubation medium containing 0.6 mg/mL of bacterial collagenase in a rotary water bath shaker (100 rpm) for 2 hours. The collagenase digest was then separated from undigested tissue matrix by filtration through 200-μm mesh fabric. Five milliliters of medium was then added back to the digestion tubes and used to wash the undigested matrix on the filter mesh. This wash solution was combined with the collagenase digest, and stromal-vascular (SV) cells were separated from adipocytes and medium by centrifugation in 15-mL tubes for 1 minute at 400*g*. The SV cells are defined as those cells isolated by collagenase digestion that do not float. The SV cells and adipocyte fractions were each suspended in 5 mL of fresh buffer and centrifuged for 10 seconds at 400*g*. The medium was removed, and the undigested tissue matrix on the nylon mesh, the SV cells, and the adipocytes were then incubated in a volume of 5 mL for the indicated periods. The values are uncorrected for loss of adipocytes or other fractions during separation and washing of the 3 fractions.

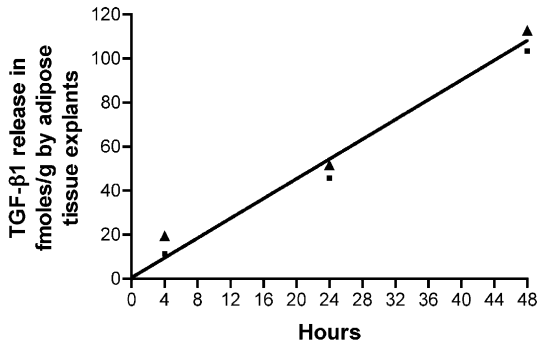

Fig. 1. Time course for TGF-β1 release by adipose tissue. Explants of subcutaneous adipose tissue (squares) from 13 women with an average BMI of 37 were incubated for 48 hours with samples of medium removed at 4, 24 and 48 hours for assay of TGF-β1. The values for visceral adipose tissue (triangles) are from 8 women with an average BMI of 41.

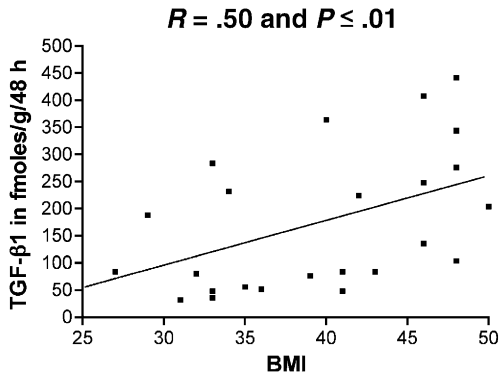

Fig. 2. Correlation between BMI and TGF-β1 release. Explants of subcutaneous adipose tissue were incubated for 48 hours from 25 women with BMI values as shown. The Pearson correlation coefficient was 0.5 and the $P < .01$.

The pH of the buffer was adjusted to 7.4 and then filtered through a 0.2-μm filter. Aliquots of the medium were stored at −20 °C for measurement of adipokine release to the medium. Leptin, interleukin 8 (IL-8), and TGF-β1 were determined by enzyme-linked immunosorbent assay using Duoset reagents from R&D Systems of Minneapolis, Minn. The TGF-β1 samples are activated by acid treatment before assay to convert the latent form of TGF-β1 to the immunoreactive form. The prostaglandin E_2 (PGE_2) content of the medium was determined by radioimmunoassay as previously described [10].

Bovine serum albumin powder (Bovuminar, containing <0.05 mol of fatty acid per mole of albumin) was obtained from Intergen, Purchase, NY. Bacterial collagenase *Clostridium histolyticum* (type 1) was obtained from Worthington Biochemical Corporation (lot CLS1-4197-MOB3773-B, 219 U/mg). Etanercept (Enbrel) was manufactured by Immunex Corp of Seattle, Wash. The monoclonal antihuman IL-1β was purified using protein A affinity chromatography and obtained from a mouse hybridoma (clone 8516.311). It was obtained from Sigma Chemical Co of St Louis, Mo.

3. Results

The release of TGF-β1 by visceral or subcutaneous adipose tissue explants from women was fairly linear over 48 hours (Fig. 1). This is unlike the situation with some

cytokines released by adipose tissue such as plasminogen activator inhibitor–1 whose release is up-regulated over 48 hours or tumor necrosis factor α (TNF-α) that is down-regulated [11]. There was a statistically significant ($r = 0.50$) Pearson correlation coefficient between the BMI of the fat donors and the subsequent release of TGF-β1 per gram of adipose tissue explants over a 48-hour incubation (Fig. 2).

The release of TGF-β1 by explants of visceral adipose tissue was 130% greater than that by subcutaneous adipose tissue from the 10 women undergoing abdominoplasty who had a mean BMI of 32.9 and a total fat mass of 34.8 kg (Fig. 3). However, no significant difference was seen between release by visceral and subcutaneous adipose tissue in the 12 women undergoing gastric bypass surgery who had a mean BMI of 45.4 and a total fat mass of 58.2 kg.

We also compared total TGF-β1 release by adipose tissue vs fat mass and found a highly significant Pearson correlation coefficient of 0.69 (Fig. 3B). Total TGF-β1 release was computed by averaging the values for release per kilogram of visceral and subcutaneous adipose tissue in each individual and multiplying by the total fat mass in that individual.

The question of whether the release of TGF-β1 by human adipose tissue explants was primarily due to adipocytes, as is the case for leptin, or the nonfat cells present in human adipose tissue, as is the case for IL-8 and PGE_2 [10,11], was examined in the studies shown in Fig. 3C. These data indicate that the release of TGF-β1 is primarily by the nonfat cells of human adipose tissue because release by adipocytes was 4% of that by the same amount of adipose tissue or 9% of that by the combined adipose tissue matrix and SV cells. The matrix is what remains after collagenase digestion of tissue and the SV cells, those cells that do not float after collagenase digestion. In contrast, adipocytes are those cells that contain enough lipid to float.

If the enhanced release of TGF-β1 by adipose tissue from morbidly obese women is involved in feedback regulation, then possibly, TNF-α and/or IL-1β inhibit the release of TGF-β1 as was observed in human osteoarthritic cartilage [12]. We investigated this by incubation of adipose tissue explants with a soluble human TNF-α receptor (etanercept) and a neutralizing antihuman IL-1β antibody. The release of TGF-β1 over 48 hours by adipose tissue explants was significantly enhanced by 36% because of the inhibitors of TNF-α and IL-1β in the presence of dexamethasone (Fig. 4). Dexamethasone itself had no significant effect on TGF-β1 release, but it reduced that of IL-8 and PGE_2, whereas enhancing that of leptin (Fig. 4). The inhibitors of TNF-α and IL-1β also increased the release of leptin by 46% in the absence of dexamethasone. However, dexamethasone enhanced leptin release to such an extent that no further increase was seen due the inhibitors of TNF-α and IL-1β. The effect of the combination of the inhibitors of TNF-α and IL-1β on IL-8 and PGE_2 release was the opposite of their effects on TGF-β1 release.

In view of the elevated circulating insulin in blood of morbidly obese women, it was of interest to see if the

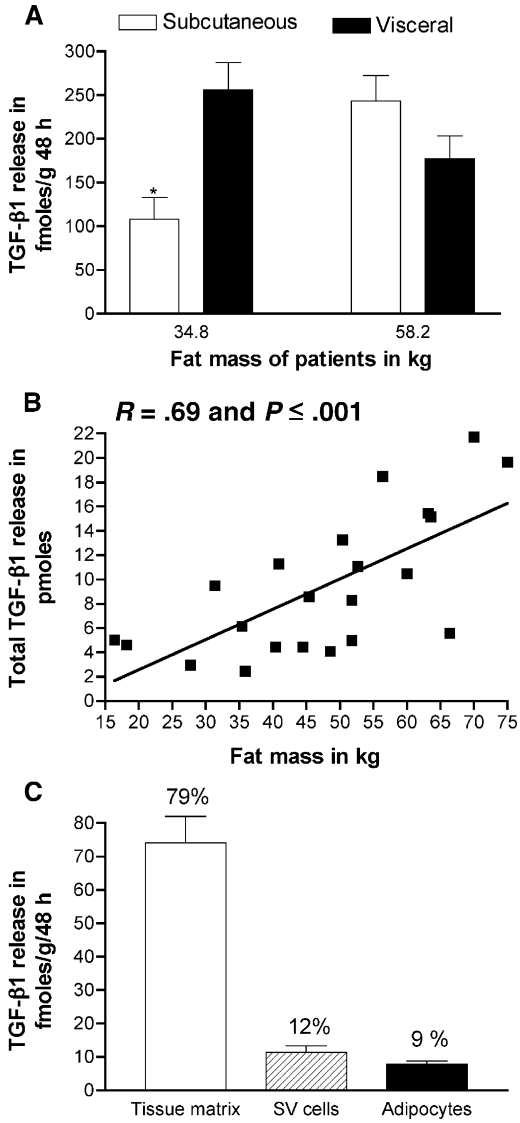

Fig. 3. TGF-β1 release by adipocytes, SV cells, the adipose tissue matrix and intact adipose tissue. A, Release of TGF-β1 by explants from the visceral and subcutaneous adipose tissue of 10 women with a body mass of 34.8 kg and an average BMI of 32.9 (abdominoplasty patients examined approximately 1 year after bariatric surgery) was compared with that by explants from 12 women with an average body fat content of 58.2 kg and a BMI of 45.4 (gastric bypass patients). The values are shown as the mean ± SEM. The difference between release by visceral vs subcutaneous fat at a body fat mass of 34.8 was significant with a $P < .005$. B, The total TGF-β1 release was obtained by averaging the 48-hour release values for visceral and subcutaneous fat from each individual and multiplying that by the total fat mass, which was then plotted against the fat mass for each woman. The values for all 22 individuals are shown, and the Pearson correlation coefficient was 0.69 with a $P < .001$. C, The release of TGF-β1 over 48 hours by the matrix, the SV cells, and the adipocytes from both subcutaneous and visceral adipose tissue explants from all 22 women is shown. The values for matrix, the SV cells, and the adipocytes are expressed per gram of adipose tissue digested to obtain the fractions and shown as the mean ± SEM. The percentage values above the bars represent the fractional share of total release by the 3 fractions.

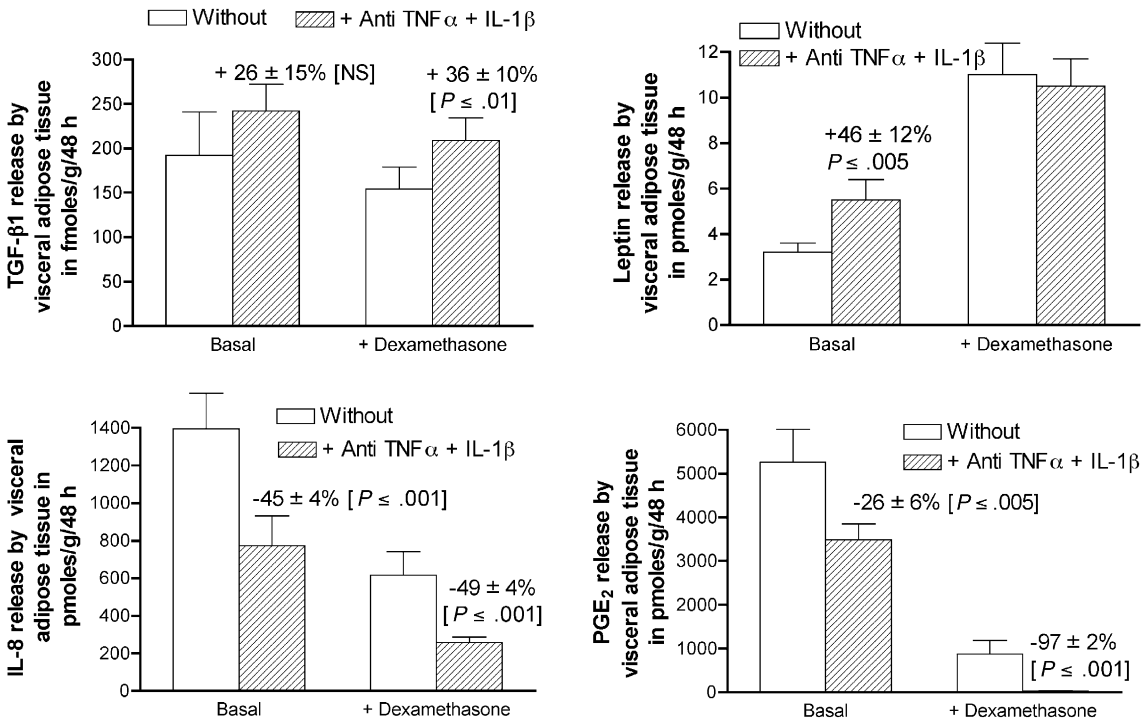

Fig. 4. Effect of inhibiting endogenous TNF-α and IL-1β on TGF-β1, IL-8, PGE_2, and leptin release by adipose tissue. Explants of visceral adipose tissue were incubated for 48 hours in the absence or presence of 2 μg/mL of etanercept, which is a soluble TNF-α receptor, plus 2 μg/mL of a blocking antihuman IL-1β antibody either in the absence or presence of 10 nmol/L concentration of dexamethasone. The values are shown as the mean ± standard error of 5 paired experiments from 5 individuals with a mean BMI of 46. The effect of the combination of the anti–TNF-α and IL-1β agents is also shown as the percentage ± standard error of the paired differences along with the *P* value for those differences.

release of TGF-β1 by adipose tissue was enhanced in the presence of insulin. Insulin (10 nmol/L) enhanced the release of TGF-β1 by explants of adipose tissue over 48 hours by 25% ± 6% as the mean ± standard error of 22 experiments ($P < .001$).

4. Discussion

In humans, there is a so-called Pro 10 variant of the TGF-β1 protein resulting from the substitution of leucine at codon 10 with proline that is present in about 39% of Swedish men [13]. This was associated with a 4% increase in BMI, 6% increase in waist circumference, and a 24% increase in fasting insulin. It is not known how the Pro 10 variant form of the TGF-β1 protein is linked to visceral adiposity and elevated levels of circulating insulin. However, the data do suggest that TGF-β1 might be involved in the insulin resistance seen in obesity.

Samad et al [6] originally reported enhanced amounts of TGF-β1 messenger RNA and protein in 2 strains of genetically obese mice (ob/ob and db/db) as compared with lean mice. Our findings confirm the conclusion that obesity in humans is associated with an enhanced expression and release of TGF-β1 [2,3]. The correlation coefficients reported by Alessi et al [3] for the relationship between the BMI and the adipose tissue content amount of TGF-β1 was 0.54 for subcutaneous and 0.57 for visceral adipose tissue. This was remarkably similar to what we found because the correlation coefficient was 0.50 for the relationship between TGF-β1 release over 48 hours by explants of the subcutaneous adipose tissue and the BMI. We also saw an even greater correlation between the total release of TGF-β1 and the fat mass of women.

Alessi et al [3] found that the amounts of TGF-β1 protein and the messenger RNA were equivalent in subcutaneous and visceral adipose tissue in humans with a mean BMI of 42. We found similar results for women with a BMI of 45, but in women with a BMI averaging 33, there was 130% greater release of TGF-β1 by visceral than by subcutaneous adipose tissue. Previously, in similar women with a BMI of 32, these are women from whom adipose tissue samples were obtained during abdominoplasty approximately 1 year after gastric bypass surgery, we reported that vascular endothelial growth factor release was 375% greater and IL-6 release of 213% greater by explants of visceral as compared with subcutaneous adipose tissue [11]. Because release of these factors by adipocytes is also less than 10% of that by the nonfat cells of adipose tissue [11], their release by adipose tissue, as well as that of TGF-β1, is primarily by the nonfat cells of visceral adipose tissue. The 40% reduction in total fat mass because of gastric bypass surgery may have resulted in a relative loss of adipocytes in visceral adipose tissue so that these differences reflect a greater amount of connective tissue, blood vessels, and fat cells

without enough fat to float in visceral as compared with subcutaneous adipose tissue.

An association between BMI and circulating TGF-β1 was not observed in 2 reports [4,5]. However, in the study of Bastelica et al [5], there was a positive correlation coefficient of 0.58 between the BMI and the content of TGF-β1 in human subcutaneous adipose tissue. In the study of Corica et al [4], the basal value for circulating TGF-β1 was 160 ng/mL. That is a far higher value than has been reported by others for total TGF-β1, and the assay they used may have measured factors other than TGF-β1. Generally, circulating levels of total TGF-β1 have been reported to be between 5 and 30 ng/mL [14-17]. Less than 1% of total TGF-β1 circulates as the active form [17].

Most human studies indicate that TGF-β1 release by adipose tissue is enhanced in obesity and associated with higher circulating levels of total TGF-β1. However, it should be noted that the most important factor in the ability of TGF-β1 to affect cells is the conversion of the latent form to the active form, which has not been examined in any studies to date. Because of the low level of TGF-β1 secretion by human adipose tissue, it is not feasible to assay for the active form of TGF-β1.

Because TGF-β1 inhibits differentiation of preadipocytes [7,8,18] and is an antiinflammatory cytokine, its enhanced release in obesity may act to block the effects of the inflammatory cytokines such as IL-8 whose release is enhanced in obesity. Thus, it may be turned on only under certain conditions. Although the name for this protein was derived from its ability to induce malignant behavior of normal fibroblasts, it is clear that in many cells such as epithelial and lymphoid cells, TGF-β1 has growth suppressive effects [1].

Previously, we found that neither the anti–TNF-α nor the IL-1β alone inhibited IL-6 or IL-8 release, whereas the combination of both antagonist reduced IL-6 release by 39% and IL-8 release by 46% [19]. The opposite effects of inhibiting TNF-α and IL-1β on TGF-β1 release as compared to IL-8 and PGE_2 release are compatible with the hypothesis that TGF-β1 is an antiinflammatory cytokine such as IL-10 [20]. It is has been shown that TNF-α inhibits expression of IL-10 and TGF-β1 in bone cartilage [12]. Furthermore, dexamethasone, which is a potent antiinflammatory agent, inhibited IL-8 and PGE_2 release whereas it had little effect on TGF-β1 release except to enhance the effects of the TNF-α and IL-1β antagonists. Interestingly, PGE_2 formation over 48 hours was almost completed blocked by the combination of dexamethasone and the anti–TNF-α and IL-1β, whereas that of leptin was maximally stimulated in the presence of dexamethasone.

The finding that blocking endogenous TNF-α and IL-1β enhanced leptin release suggests that these cytokines block leptin as well as TGF-β1 release by human adipose tissue. It is unclear why dexamethasone is such a potent stimulator of leptin release by human adipose tissue explants or adipocytes in primary culture [21]. Insulin has little stimulatory effect on leptin release in the absence of dexamethasone, but it potentiates the effect of dexamethasone in adipose tissue and is required for demonstration of a dexamethasone effect on human adipocytes [21]. Dexamethasone administration to humans elevates plasma insulin and leptin values [22,23], and elevated leptin values are seen in patients with Cushing's syndrome [23]. The present results suggest that some of the elevation in leptin release because of dexamethasone in human adipose tissue explants cultured in vitro could be due to blocking inhibitory effects of proinflammatory agents such as eicosanoids (PGE_2) and IL-8.

The release of TGF-β1 over 48 hours was fairly linear suggesting that there was little up- or down-regulation in adipose tissue explants over 48 hours of primary culture. The amount of TGF-β1 released by human visceral adipose tissue explants over 48 hours in our studies was less than 0.015% that of IL-8, 0.004% that of PGE_2 and about 6% of that for leptin. The release of TGF-β1 was elevated in the presence of insulin and this may explain the positive correlation between BMI and TGF-β1 release that we saw in subcutaneous adipose tissue and the correlation between total release of TGF-β1 and body fat content. It is known that circulating levels of insulin are elevated in obesity [4,5].

The present results indicate that the major source of the TGF-β1 released by human adipose tissue in primary culture is cells other than the adipocytes of adipose tissue. Transforming growth factor–β1 release by adipocytes was only 9% of that by the combined isolated SV cells and the undigested tissue matrix. This is comparable to what we have reported with regard to IL-6, IL-8 and PGE_2 [11]. Resistin release over 48 hours was less than 5% of that by the nonfat cells of human adipose tissue over 48 hours [24] and comparable to that of vascular endothelial growth factor, TNF-α, IL-1β, hepatocyte growth factor, and IL-10 [11]. In contrast, leptin release by adipocytes was 2080% of that by the nonfat cells over 48 hours clearly demonstrating that leptin is almost exclusively a product of mature adipocytes [11]. The release of all the cytokines, except for leptin as noted above, and TNF-α, which was released in greater amounts by the isolated SV cells [25], was predominantly by the cells of the adipose tissue matrix. In conclusion, the release of TGF-β1 is predominantly by the nonfat cells present in incubated human adipose tissue, greater in adipose tissue of obese individuals and enhanced in the presence of anti–TNF-α and IL-1β.

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