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Release in vitro of adipsin, vascular cell adhesion molecule 1, angiotensin 1–converting enzyme, and soluble tumor necrosis factor receptor 2 by human omental adipose tissue as well as by the nonfat cells and adipocytes

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Abstract

The relative release in vitro of adipsin, vascular cell adhesion molecule (VCAM) 1, angiotensin 1–converting enzyme (ACE), and soluble tumor necrosis factor α receptor 2 (sTNFR2) by explants of human omental and subcutaneous adipose tissue as well as the nonfat cell fractions and adipocytes from morbidly obese gastric bypass women was compared with that by tissue from obese abdominoplasty patients. Release of VCAM-1 and ACE by omental adipose tissue explants was 220% and 80% greater, respectively, over 48 hours of incubation than that by subcutaneous adipose tissue explants. However, this difference was not seen when release by adipocytes derived from omental fat was compared with that by adipocytes from subcutaneous fat. The release of adipsin and sTNFR2 by omental adipose tissue explants did not differ from that by subcutaneous tissue adipose tissue. The release of adipsin by tissue explants over 48 hours was 100-fold greater than that of VCAM-1, ACE, or sTNFR2. Most of the release of all 4 adipokines was due to the nonfat cells because adipsin release by adipocytes was only 13% of that by the nonfat cells derived from the same amount of adipose tissue, whereas ACE release by adipocytes was 7% and release of VCAM-1 as well as sTNFR2 by adipocytes was 4% or less of that by nonfat cells. The total release in vitro of ACE and sTNFR2, but not that of adipsin or VCAM-1, was enhanced in adipose tissue explants from morbidly obese women as compared with those by explants derived from obese women. We conclude that although human adipose tissue explants release appreciable amounts of adipsin and far smaller amounts of VCAM-1, ACE, and sTNFR2 in vitro, more than 87% of the release is due to the nonfat cells present in adipose tissue. Furthermore, the enhanced release of VCAM-1 and ACE seen in omental adipose tissue explants as compared with explants of subcutaneous adipose tissue is due to release by nonfat cells.

1. Introduction

Leptin is the only adipokine found to date that is released exclusively by freshly isolated human adipocytes [1,2]. In marked contrast, more than 90% of the release of resistin [3], tumor necrosis factor (TNF) α [4], monocyte chemoattractant protein 1 [5], transforming growth factor β1 [6], interleukin (IL) 8 [2], IL-6 [2], vascular endothelial growth factor [2], plasminogen activator inhibitor (PAI) 1 [2], IL-10 [2], hepatocyte growth factor [2], IL-1β [2], IL-1Ra [7], and IL-18 [7] is by the nonfat cells present in human adipose tissue. The release by adipocytes of adiponectin was 64% of that by the nonfat cells from a gram of tissue [1], and a similar finding was seen with haptoglobin [8] and nerve growth factor [7]. The present studies were designed to determine whether adipsin, vascular cell adhesion molecule (VCAM) 1, angiotensin 1–converting enzyme (ACE), and soluble TNF-α receptor 2 (sTNFR2) are released by the adipocytes or the nonfat cells present in human adipose tissue. These adipokines were chosen because of their putative role in obesity.

Adipsin was isolated from adipocytes in 1986 and reported to be a serine protease [9]. Subsequently, it was reported that adipsin expression is severely impaired in both

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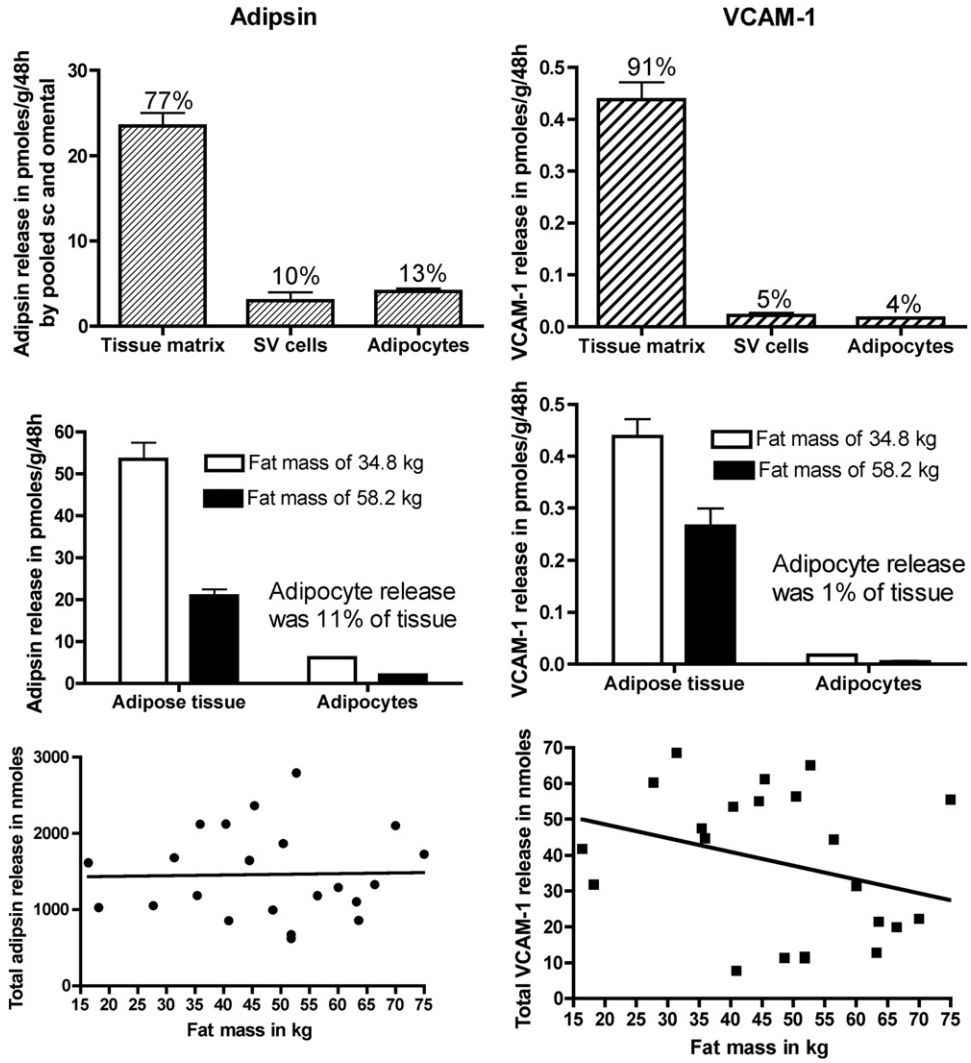

Fig. 1. Release of adipsin as well as VCAM-1 by explants as well as adipocytes, adipose tissue matrix, and SV cells. The values are shown in picomoles per gram per 48 hours as the mean ± SEM of explants or adipocytes from 12 morbidly obese women with a total fat mass of 58.2 kg or 10 abdominoplasty patients with a total fat mass of 34.8. The data are the pooled values for subcutaneous and visceral adipose tissue from each woman. Release is shown per gram of tissue that was taken for digestion and uncorrected for losses during collagenase digestion or separation of the fractions. The percentage of distribution of total release by the tissue matrix, SV cells, and adipocytes over 48 hours is shown above the bars. Total release was obtained by averaging release per kilogram for omental and subcutaneous explants from each individual and multiplying that value by the kilogram of body fat. There was no statistically significant correlation between total release of VCAM-1 and fat mass because although the Pearson correlation coefficient was −0.3, the *P* value was .2.

genetic and acquired obesity in rodents [10]. Later, it was realized that adipsin is the complement factor D involved in the alternative pathway of complement action; and adipsin was claimed to be an immune-related defect in obesity [11]. However, Napolitano et al [12] found that circulating levels of adipsin are actually elevated in obese humans. Furthermore, in adipsin-deficient mice, development is normal as is body weight; but the mice have a defective antibacterial host defense early after infection [13]. An adipsin/factor D deficiency has been found in a family with a history of meningococcal disease [14].

Vascular cell adhesion molecule 1 is involved in the modulation of leukocyte recruitment and platelet adhesion during inflammation [15]. It has been reported that circulating levels of VCAM-1 correlated positively (r = 0.3-0.6) with obesity in men [16] and women [17,18]. Furthermore, a reduction over a year in body mass index (BMI) of women from 37.2 to 32.5 was accompanied by a 17% reduction in circulating VCAM-1 [17].

Circulating levels of ACE have been reported to positively correlate with BMI and after bariatric surgery dropped by 50% [19]. Jonsson et al [20] first demonstrated ACE messenger RNA (mRNA) in human adipose tissue; and subsequently, ACE mRNA was found in human preadipocytes [21]. The renin-angiotensin system is present in adipocytes as well as the nonfat cells of human adipose tissue [22,23].

Two different inhibitors of TNF-α are shed by cells and are soluble fragments of a TNF receptor molecule [24]. Subsequently, it was found that TNFR2 mRNA content of

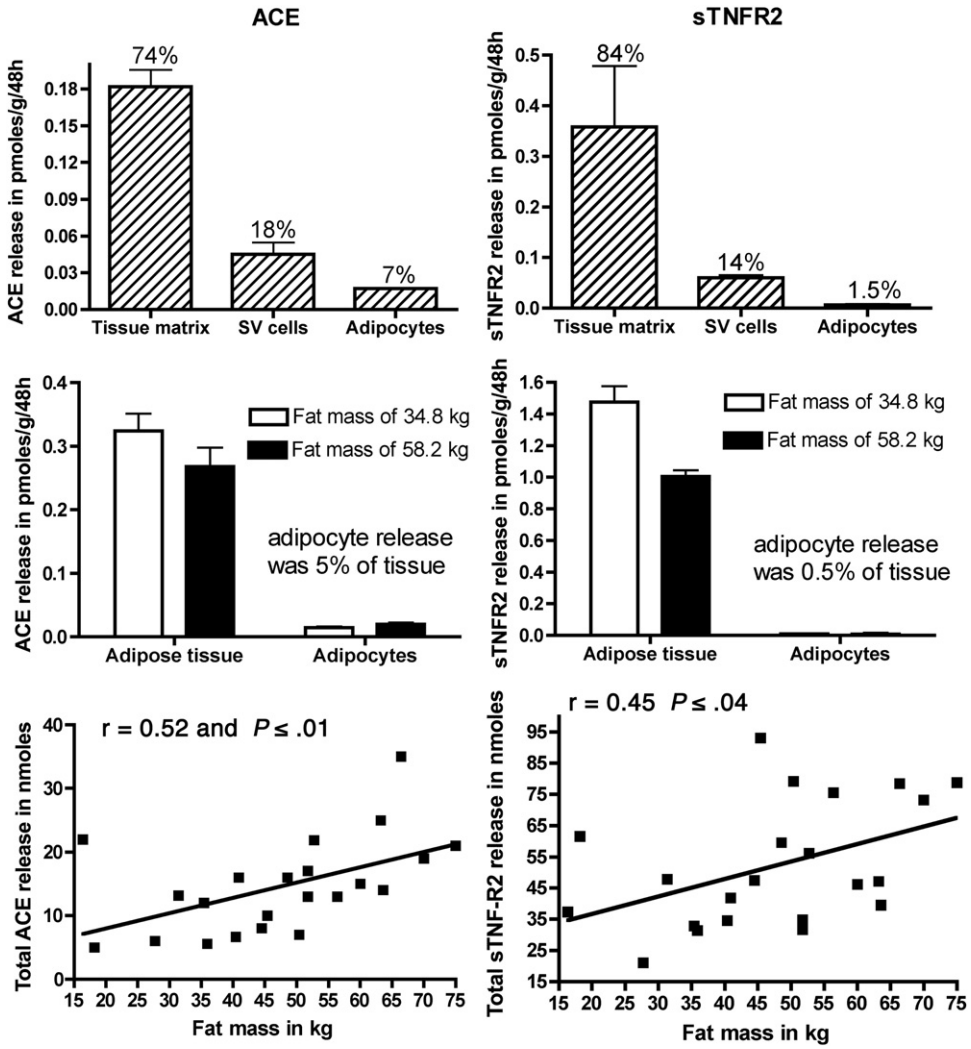

Fig. 2. Release of VCAM-1 and sTNFR2. The experimental conditions are outlined in Fig. 1, as the data are from the same experiments. The Pearson correlation coefficients for both total release of ACE (0.52) and sTNFR2 (0.45) as compared with total fat mass were statistically significant.

adipose tissue correlated with BMI [25] and that circulating levels of sTNFR2 are elevated in obesity [25,26].

2. Materials and methods

Abdominal subcutaneous and visceral omental adipose tissues were obtained from 10 obese women who were undergoing open abdominal surgery (abdominoplasty) or 12 morbidly obese women who were undergoing laparoscopic gastric bypass with Roux-en-Y gastroenterostomy surgery for the treatment of morbid obesity for the studies shown in Fig. 1, Fig. 2, and Fig. 3. The mean BMI of the morbidly obese women was 46 and their average total body fat mass was 58.2 kg, whereas the BMI of the abdominoplasty patients was 32.9 and their total fat mass was 34.8 kg. Fifty-nine percent of the women were 40 years or younger, 18% were 40 to 49 years old, and 23% were 50 years or

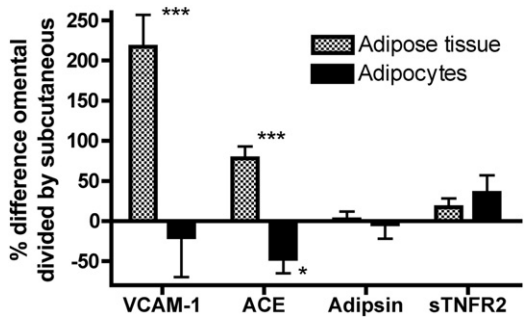

Fig. 3. Release of adipokines by adipose tissue and adipocytes from the subcutaneous as compared with omental adipose tissue. The release of VCAM-1, ACE, adipsin, and sTNFR2 by the adipocytes and adipose tissue explants derived from omental adipose tissue from each of the 22 individuals was compared with that by adipocytes or tissue derived from subcutaneous adipose tissue. The values are the means ± SEM of the paired differences for each person. Significant differences are indicated as follows: $*P < .05$ and $***P < .001$.

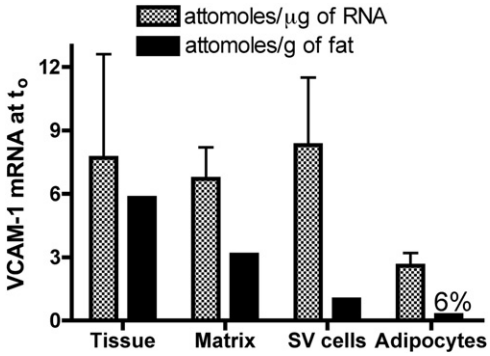

Fig. 4. Distribution of mRNA for VCAM-1 between omental adipose tissue, adipose tissue matrix, SV cells, and adipocytes. The mRNA values are the specific activity in attomoles of VCAM-1 mRNA per microgram of total RNA and in attomoles per gram of omental adipose tissue from which the mRNA was derived. The data are the means ± SEM from 5 experiments from as many different individuals. Per gram of adipose tissue, the percentage of recovery of total RNA was 62% in the matrix, 16% in the SV fraction, and 13% in the adipocytes, for a total recovery of 91%. An average of 0.75 μg of RNA was extracted per gram of adipose tissue.

older, with the average age being 39 years. Fasting blood glucose values exceeded 125 mg/dL in only one woman (a gastric bypass patient whose value was 153 mg/dL). The studies shown in Fig. 4 were done using omental adipose tissue obtained from 5 morbidly obese women with an average BMI of 48.8 and a fat mass of 70 kg. The studies on plasma adipsin content were done in a third group of 44 women undergoing abdominoplasty or gastric bypass surgery. Body fat content was determined using bioelectrical impedance (Tanita TBF-310, Tanita Corp, Arlington Heights, IL). Each experimental replication involved tissue from a separate individual. The study had the approval of the local institutional review board, and all patients involved gave their informed consent.

The buffer for incubation of adipose tissue and adipocytes was the Dulbecco modified Eagle medium/Ham F12 (1:1, no. 2906 from Sigma Chemical of St Louis, MO) containing 17.5 mmol/L glucose, 121 mmol/L NaCl, 4 mmol/L KCl, 1 mmol/L $CaCl_2$, 25 mmol/L 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), 2.4 mmol/L sodium bicarbonate, 10 mg/mL bovine serum albumin, 5 μg/mL ethanolamine, 0.1 ng/mL sodium selenite, 90 μg/mL penicillin G, 150 μg/mL streptomycin sulfate, 50 μg/mL gentamicin, and 55 μmol/L ascorbic acid. The pH of the buffer was adjusted to 7.4 and then filtered through a 0.2-μm filter.

The omental adipose tissue was transported to the laboratory within 15 to 30 minutes of its removal from the donor. 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 incubated in medium (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 then 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 [27]. 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 stromovascular (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 adipocytes 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. Adipsin, VCAM-1, ACE, and sTNFR2 were determined using enzyme-linked immunosorbent assays with Duoset reagents from R&D Systems of Minneapolis, MN.

For studies involving mRNA isolation, the matrix, cells, or tissues were separated from the medium; and RNA was extracted from 0.5 g of tissue or the fractions derived from 1 g of tissue by Polytron (Brinkman Inst, Westbury, NJ) homogenization using 5 mL of a monophasic solution of phenol and guanidine isothiocyanate (TRIzol reagent from Invitrogen of Carlsbad, CA, or Tri reagent from Sigma Chemical). The extracts were then spun at 12 000*g* for 10 minutes at 2°C to 8°C, and the fat layer was removed. Total RNA was obtained from the cleared homogenate by the procedure of Chomczynski and Sacchi [28]. The quantization of mRNA was based on hybridization of samples using gene-specific biotin-labeled capture oligonucleotide probes and digoxigenin-labeled detection probes in a microplate. The hybridization solution was then transferred to a streptavidin-coated microplate, and the amount of the digoxigen-labeled probe bound to the streptavidin plate was detected by use of an antidigoxigen alkaline phosphatase complex (Quantikine mRNA kits from R&D Systems). The sensitivity of this procedure is comparable with that of Northern blots.

Statistical analyses were carried out with Student *t* test. The Pearson correlation coefficients were determined using the GraphPad Prism program (San Diego, CA), assuming a Gaussian population and a 2-tailed *P* value.

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 (Lakewood, NJ; lot CLS1-4197-MOB3773-B, 219 U/mg).

3. Results

3.1. Comparison of adipsin, VCAM-1, ACE, and sTNFR2 release by nonfat cells vs omental adipocytes

In these studies, either cut pieces of omental or subcutaneous adipose tissue or the tissue, matrix, SV cell fraction, or the adipocytes obtained by collagenase digestion of adipose tissue were incubated for 48 hours. Two groups of women were compared, with one being morbidly obese individuals with an average fat mass of 58.2 kg (group A) and the other women 1 year after bariatric surgery with a fat mass of 34.8 kg (group B). The data are expressed both as picomoles per gram of adipose tissue and also as nanomoles per total fat mass for each woman.

The release of adipsin by adipose tissue explants on a per gram basis from the women in group B was approximately twice that by explants from women in group A (Fig. 1). However, the calculated total release was similar because the women in group A had 67% more fat than the leaner women. If total release of adipsin for each woman was plotted against her fat mass, there was no correlation, indicating that as fat mass increases, the release per gram decreases so that total release is maintained (Fig. 1). Most release of adipsin was due to the nonfat cells because release by adipocytes was only 11% that of tissue or 13% of release by the tissue matrix, SV cells, and adipocytes obtained by collagenase digestion (Fig. 1).

The failure to see an increased adipsin release by adipose tissue in morbidly obese as compared with obese women prompted us to see if we could confirm the report that circulating adipsin levels are elevated in obesity [13]. A positive Pearson correlation coefficient of 0.45 has been reported between BMI and plasma adipsin [13]. We found a similar correlation coefficient of 0.42 that was significant with a $P < .004$ when measuring circulating adipsin in 44 women with BMI values between 27 and 65.

Soluble VCAM-1 was also released by human adipose tissue explants, but VCAM-1 release was less than 1% of adipsin release (Fig. 1). Less release per gram of adipose tissue of VCAM-1 was seen in tissue from women in group A as compared with tissue from women in group B. However, total release of VCAM-1 did not correlate with the fat mass of each woman (Fig. 1). The release of VCAM-1 by adipocytes was less than 1% of that by intact tissue and 4% of that by the fractions obtained after collagenase digestion (Fig. 1). These data agree with the current view that VCAM-1 is primarily released by endothelial cells of blood vessels that are found in the adipose tissue matrix fraction [29,30].

Angiotensin 1–converting enzyme release by adipocytes was comparable with that of VCAM-1 in both the amount released and the finding that most of the net release was accounted for by the nonfat cells of omental adipose tissue (Fig. 2). There was no difference in the amount of ACE released per gram of adipose tissue; but when total release was plotted against fat mass for each woman, a highly significant positive Pearson correlation coefficient of 0.52 was seen (Fig. 2).

Soluble TNF-α receptor 2 release by human omental adipose tissue explants was comparable with that of ACE and VCAM-1 (Fig. 2). However, the release of sTNFR2 by adipocytes was so small as to be near the limits of sensitivity of the assay, indicating that more than 99% of its release is due to the nonfat cells in adipose tissue (Fig. 2). Just as was seen with ACE, the total release of sTNFR2 by adipose tissue explants positively correlated ($r = 0.45$) with the fat mass of each woman (Fig. 2).

3.2. Comparison of adipokine release by adipocytes and tissue explants from omental and subcutaneous adipose tissue

The release by the omental adipose tissue explants and adipocytes was compared with that by the subcutaneous adipose tissue and adipocytes from all 22 women (Fig. 3). The release of VCAM-1 was 117% greater and ACE 78% greater by omental adipose tissue explants as compared with subcutaneous explants. However, this difference was restricted to the intact tissue and was not seen in adipocytes. In fact, the release of ACE by adipocytes from omental adipose tissue was actually 47% less than that by subcutaneous adipocytes. Interestingly, when adipsin and sTNFR2 release was examined from the same individuals, no significant differences were seen between omental and subcutaneous adipose tissue explants (Fig. 3).

3.3. VCAM-1 mRNA distribution in nonfat cells vs adipocytes

The VCAM-1 mRNA distribution between the matrix, SV cells, and adipocytes was compared with that in intact omental tissue in a separate set of experiments using zero time samples (Fig. 4). The specific activity of VCAM-1 mRNA was comparable in both the matrix and SV factions with that in the tissue and was far higher than that in adipocytes. The total amount of VCAM-1 mRNA in adipocytes was 6% of that in the combined matrix, SV, and adipocyte fractions obtained after collagenase digestion and was comparable with the finding that VCAM-1 release by adipocytes was 4% of that by the matrix plus SV fractions over 48 hours (Fig. 4 vs Fig. 1). We also examined the VCAM-1 mRNA specific activity in adipose tissue explants after a 48-hour incubation and found that it was 53% of that seen at the start of the incubation (data not shown).

4. Discussion

Adipose tissue is a specialized connective tissue that contains large numbers of adipocytes that account for its appearance. Adipocytes are essentially specialized connective tissue cells that contain a large internal fat droplet. Although adipose tissue has often been considered as a mesh

bag that contains adipocytes, it is actually loose or areolar connective tissue that contains blood vessels, connective tissue, and cells involved in immunologic defense. There are large numbers of fibroblasts, mast cells, macrophages, leukocytes, and endothelial as well as smooth muscle and endothelial cells. Collagenase digestion of rodent fat results in the liberation of nearly all the nonfat cells as well as the adipocytes because everything passes through a 200-μm mesh filter. However, with human adipose tissue, even after digestion with collagenase for 2 hours, most of the recovered cells are in the undigested tissue matrix containing blood vessels and connective tissue that does not pass through the filter. Approximately 44% of the recovered DNA was found in the tissue matrix, whereas 23% was found in the nonfat cells that are released during collagenase digestion and that do not float and are referred to as the *SV cells* [1-8]. Only 31% of the DNA is recovered in adipocytes, but there is a preferential loss of large adipocytes amounting to 21%; so the corrected figure for adipocytes is 38% of total cells [7]. However, even after this correction, most of the cells are nonfat cells in human omental adipose tissue. The main point is that the matrix fraction contains twice as many cells as are found in the SV fraction and that release of adipsin by matrix was 7.7-fold greater than that by the SV cells. The release of sTNFR2 by matrix was 6-fold greater whereas that of ACE was 4-fold greater than the release by the SV cells. The finding that VCAM-1 release by the matrix fraction was 18-fold greater than was released by SV cells is hardly unexpected because VCAM-1 is considered as an endothelial cell marker [29,30]. Our data indicate that most (87%-98%) of the in vitro release of adipsin, VCAM-1, ACE, and sTNFR2 is by the nonfat cells of adipose tissue and that most of that is by the cells remaining in the adipose tissue matrix after collagenase digestion. The data on distribution of VCAM-1 mRNA at the beginning of the incubation between adipocytes, SV cells, and matrix cells are in agreement with that on VCAM-1 release over 48 hours, indicating that the nonfat cells of adipose tissue account for most of the mRNA as well as the release of VCAM-1.

The possibility that under the conditions of these experiments, namely, 48 hours of incubation in vitro, the small release of sTNFR2 (1.5%) by adipocytes is due to contamination of the fat cells by nonfat cells cannot be excluded. However, if this is the case and this value is subtracted from the amount of adipsin, VCAM-1, and ACE released by adipocytes, the release of these adipokines is, respectively, 11.5%, 2.5%, and 5.5% of the release by the nonfat cells plus adipocytes. In either case, release by nonfat cells accounts for most of their release over 48 hours of incubation.

Although circulating levels of adipsin, VCAM-1, ACE, and sTNFR2 have been reported to be elevated in obesity, the present data suggest that only ACE and sTNFR2 are released in vitro by adipose tissue of morbidly obese humans in enhanced amounts. Although some reports [16-18] found elevations in circulating VCAM-1 in obese individuals, others have not [31-33]. Vazquez et al [32] and Hanusch-Enserer et al [33] looked at morbidly obese humans before and either 4 or 6 months after bariatric surgery. Six months after gastric banding, the BMI went from 47 to 37; circulating CRP was down by 35% and PAI-1 by 32% without any change in circulating VCAM-1 [33]. Vazquez et al [32] found a decrease in BMI of 46 to 37 by 4 months after bariatric surgery; but circulating VCAM-1 actually was up by 25%, whereas ACE activity was reduced by 13% and PAI-1 by 40%. Clearly, there is no consistent effect of obesity on circulating levels of VCAM-1.

The release of VCAM-1 by omental adipose tissue in vitro is twice that by subcutaneous tissue explants and is due to release by nonfat cells. The cells involved are probably the endothelial cells of the blood vessels, and the greater release may well reflect enhanced activation of VCAM-1 expression in endothelial blood vessels of omental fat. Vascular cell adhesion molecule 1 is induced by cytokines and is thought to be involved in the recruitment of mononuclear leukocytes into inflammatory sites [29,30]. The combined endogenous release of TNF-α and IL-1β during a 48-hour incubation of omental adipose tissue explants accounts for about half of the up-regulation of IL-8 release [34]. These cytokines are also potent up-regulators of sTNFR2 formation [35]. Because the release of sTNFR2 by visceral fat was the same as that by subcutaneous fat [2], it is unlikely that TNF-α and IL-1β are responsible for the enhanced release of VCAM-1 by omental adipose tissue. Omental adipose tissue from morbidly obese bariatric surgery patients has been found to have twice as many macrophages as subcutaneous fat from the same individuals [36]. Thus, the enhanced release of VCAM-1 and ACE by nonfat cells in omental adipose tissue may well be linked to the greater accumulation of macrophages.

The release of adipsin by the nonfat cells as well as by the adipocytes over 48 hours was 100-fold greater than that of VCAM-1, ACE, or sTNFR2 and was comparable with that of adiponectin [1,2]. Over a 48-hour incubation, the net release of only IL-8, IL-6, PAI-1, and monocyte chemoattractant protein 1 was greater than that of adipsin [1]. The only known function of adipsin is its protease activity resulting in the activation of another protease involved in the alternative pathway of complement activation [13,14]. The role of adipsin in adipose tissue metabolism is unclear. The same amount is released by omental as subcutaneous adipose tissue, and circulating levels are elevated in obesity. What is now clear is that defective adipsin release is not characteristic of obesity as once claimed [10,11]. On a per gram basis, the release of adipsin was reduced by at least 50% in both adipose tissue and adipocytes from morbidly obese women with a fat mass of 58.2 as compared with women with a fat mass of 34.8. This appears to be an adaptive response to keep total adipsin release by fat roughly comparable. The positive correlation between circulating adipsin and obesity we as well as others [12] have seen may well reflect release by nonadipose tissue sites in response to the generalized low-level inflammation seen in obesity [37].

The enhanced total net release of ACE by adipose tissue in obesity could be due to either enhanced formation or decreased breakdown because human adipose tissue is able to inactivate ACE [21]. Central obesity is linked to elevation in ACE but not circulating renin, and ACE levels in morbidly obese women drop in half after bariatric surgery [19]. There is evidence suggestive of a role for the local adipose tissue renin-angiotensin in the development of insulin resistance and hypertension in obesity, but a definitive role remains elusive [22].

The incubation of human adipose tissue explants as well as the tissue matrix, SV cells, and adipocyte fractions obtained by collagenase digestion in primary culture for 48 hours was used to evaluate release of adipokines. This procedure permits an approximation of release in vivo, but the major problem is that this results in an inflammatory response that is not due to the effects of collagenase digestion because it is seen in adipose tissue explants [34]. The level of IL-8 and IL-6 mRNA is markedly elevated after only 3 hours of incubation, and the rate of IL-8 release is 15-fold greater over 3 to 51 hours of culture than it is during the first 3 hours [34]. There are clearly changes in tissue metabolism over 48 hours of incubation, and it is impossible to maintain completely normal function at the present time. We assume that these changes affect primarily the true rates of release, and the rates reported should be considered as approximations of the in vivo situation. However, we found that the level of VCAM-1 mRNA in adipose tissue explants after 48 hours of incubation was 53% of that at the start of the incubation. It should also be noted that we are comparing the release of adipose tissue from morbidly obese to merely obese women and have no nonobese controls.

The novel features of this study are primarily that if there is enhanced release in vivo of adipsin, VCAM-1, ACE, and sTNFR2 by adipose tissue in obesity, it is probably due to the nonfat cells in human adipose tissue rather than the adipocytes. These 4 adipokines were chosen because their circulating levels are positively correlated with BMI in humans, but the present results suggest that adipose tissue could contribute to the elevation of ACE and sTNFR2 but probably not that of adipsin and VCAM-1. An unanswered question is why the release of adipsin is so high by human adipose tissue and what, if any, is its role in adipose tissue physiology.

In conclusion, the present results suggest the following: (1) Adipsin, VCAM-1, ACE, and sTNFR2 are primarily released by the nonfat cells of adipose tissue. (2) Only the release of VCAM-1 and ACE is elevated in omental as compared with subcutaneous adipose tissue from obese women. (3) The total release of ACE and sTNFR2 but not that of adipsin or VCAM-1 by adipose tissue explants incubated in vitro for 48 hours is greater in tissue from morbidly obese women with a total fat mass of 58 kg as compared with the release by tissue from obese women with a fat mass of 35 kg.

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