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Most of the interleukin 1 receptor antagonist, cathepsin S, macrophage migration inhibitory factor, nerve growth factor, and interleukin 18 release by explants of human adipose tissue is by the non–fat cells, not by the adipocytes

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Abstract

The present studies were designed to compare the relative release of interleukin 1 receptor antagonist (IL-1Ra), cathepsin S, macrophage migration inhibitory factor (MIF), nerve growth factor (NGF), and interleukin 18 (IL-18) by adipocytes as compared with the non–fat cells present in subcutaneous and omental adipose tissue from morbidly obese gastric bypass patients as compared with obese abdominoplasty patients. The release of IL-1Ra, cathepsin S, and MIF by explants of human adipose tissue incubated for 48 hours averaged 6, 9, and 19 pmol/g, respectively, and was far greater than the release of NGF (0.05 pmol/g) or IL-18 (0.006 pmol/g). The release by human adipocytes of IL-1Ra, cathepsin S, and MIF was 0.13, 0.32, and 2.6 pmol/g, respectively, over 48 hours, whereas NGF release was 0.003 and IL-18 0.001 pmol/g. Only the total release of MIF by human adipose tissue explants was enhanced, whereas that of IL-18 was significantly reduced in explants from morbidly obese women. Most of (55%-73%) the release of IL-1Ra, cathepsin S, MIF, NGF, and IL-18 was by the adipose tissue matrix, whereas release by stromal-vascular (SV) cells was 3% to 28% of total release over 48 hours by the adipose tissue matrix, SV cells, and adipocytes. The release of NGF by adipocytes was 42%, that of MIF was 27%, and for the other factors 15% or less of release over 48 hours by the adipose tissue matrix, SV cells, and adipocytes. Our results suggest that the non–fat cells in human adipose tissue contribute to most of the release of NGF, IL-18, IL-1Ra, cathepsin S, and MIF seen during primary culture of adipose tissue explants from obese women.

1. Introduction

Previously, we reported that resistin [1], monocyte chemoattractant protein 1 [2], transforming growth factor β1 [3], interleukin 8 (IL-8) [4], vascular endothelial growth factor [4], interleukin 6 (IL-6) [4], prostaglandin E_2 [4], tumor necrosis factor α [4], hepatocyte growth factor [4], interleukin 1β (IL-1β) [4], and interleukin 10 (IL-10) [4] were released by human adipocytes at levels of 11% or less of that by the adipocytes plus non–fat cells of human adipose tissue. The present studies were designed to extend the studies examining interleukin 1 receptor antagonist (IL-1Ra) [5], cathepsin S [6], macrophage migration inhibitory factor (MIF) [7], and interleukin 18 (IL-18) [8] release by human adipose tissue because of recent reports that they are inflammatory response proteins whose circulating levels are elevated in obesity. Nerve growth factor (NGF) was also examined because it is secreted by murine 3T3-L1 adipocytes [9]. The focus of these studies was the relative contribution of adipocytes as contrasted to the non–fat cells present in human adipose tissue to the in vitro release of IL-1Ra, cathepsin S, MIF, NGF, and IL-18.

Interleukin 1 receptor antagonist levels are elevated in human obesity [5,10]. This protein is a physiologic antagonist of IL-1α and IL-1β because it competes with them for binding to their receptors. The IL-1Ra protein is induced by many of the same stimuli that enhance release of IL-1β in cells and by interferon β. The reason that IL-1Ra levels are elevated in obesity is still unclear, but could possibly involve feedback termination of the IL-1β–induced inflammatory response in adipose tissue.

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Cathepsin S is an elastolytic cysteine protease secreted by mononuclear cells [11] that is active at neutral pH [12,13]. Circulating levels of cathepsin S have a positive correlation with body mass index (BMI) [6], and the level of cathepsin messenger RNA (mRNA) expression is elevated in subcutaneous adipose tissue from obese subjects [14]. The elevated levels of cathepsin S in human obesity have recently been postulated to be a link between obesity and atherosclerosis [14]. The greater expression of cathepsin S message in the adipose tissue of obese individuals may reflect elevated levels of macrophages, mast cells, and other cells involved in the immune response [15,16].

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine whose serum concentration is elevated in obesity [17]. Uniquely, the circulating concentration of MIF is at least 1000-fold greater than that of IL-6, IL-8, IL-10, or IL-1β. Church et al [7] found that in obese individuals with elevated MIF values, participation in physical activity and a dietary-focused weight management resulted in reductions in both weight and MIF. The presence of MIF in the adipocytes of rodent adipose tissue was originally reported by Hirokawa et al [18]. More recently, Skurk et al [19] reported that both human preadipocytes and adipocytes release MIF.

Esposito et al [8] reported that the mean IL-18 serum value was 14 pmol/L in obese women (BMI of 34), which was higher than the value of 7 pmol/L seen in nonobese women (BMI of 24). The AtheroGene investigators suggested that serum IL-18 level is a predictor of death from cardiovascular causes in patients with coronary artery disease [20,21]. These data suggest that IL-18 could be a link between obesity and increased risk of both diabetes and cardiovascular disease. Skurk et al [22] have even postulated that the increase in serum IL-18 seen in obese humans is due to elevated release by adipocytes.

2. Materials and methods

Abdominal subcutaneous and visceral omental adipose tissue were obtained from 10 women who were undergoing open abdominal surgery (abdominoplasty) and 12 women who were undergoing laparoscopic gastric bypass with Roux-en-Y gastroenterostomy surgery for the treatment of morbid obesity. Body fat content was determined using bioelectrical impedance (Tanita TBF-310, Tanita Corp, Arlington Heights, IL). The study had the approval of the local institutional review board, and all patients involved gave their informed consent. Approximately 59% of the women were 40 years or younger, 18% were 40 to 49 years old, and 23% were 50 years or older. The average age was 38.8 years. Fasting blood glucose values exceeded 100 mg/dL in 20% of the abdominoplasty patients and in 50% of the gastric bypass patients, whereas only one individual (a gastric bypass patient) had a blood glucose of more than 125 mg/dL (153 mg/dL).

Samples of omental and abdominal subcutaneous 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 (5-15 mg). All the studies used explants of adipose tissue that had been incubated in buffer containing 1% bovine albumin (3 mL/g of tissue) for approximately 30 minutes to reduce contamination of the tissue with blood cells and soluble factors. At the conclusion of the 30-minute incubation, the tissue explants were centrifuged for 30 seconds at 400*g* to remove blood cells and pieces of tissues containing insufficient adipocytes to float. The explants were separated from the medium plus the sedimented cells and resuspended in fresh buffer. The explants (500 mg/5 mL) were then incubated in duplicate for 48 hours in suspension culture under aseptic conditions.

One gram of cut tissue, again in duplicate, was incubated in 2 mL of incubation medium containing 1.2 mg 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. The matrix fraction on the mesh was then incubated for 48 hours. The stromal-vascular (SV) cells were separated from adipocytes by centrifugation of the filtered collagenase digest in 15-mL tubes for 1 minute at 400*g*. The SV cells are defined as those cells isolated by collagenase digestion that deposit at the bottom of the tube after centrifugation, whereas the adipocytes are those cells that float. The SV cell 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. DNA and RNA were isolated according to Chomczynski [23] using TRIzol reagent (Invitrogen, Carlsbad, CA), which is a monophasic solution of phenol and guanidine isothiocyanate. Lipid recovery in tissue and fat cells was determined gravimetrically on the oil layer obtained after homogenization in TRIzol.

The serum-free buffer for incubation of adipose tissue and adipocytes was as previously described [24]. The pH of the buffer was adjusted to 7.4 and filtered through a 0.2-μm filter. Aliquots of the medium were stored at -20°C for measurement of adipokine release to the medium. Total cathepsin S, IL-1Ra, MIF, NGF, and IL-18 were determined by enzyme-linked immunosorbent assay using Duoset reagents from R&D Systems (Minneapolis, MN).

Bovine serum albumin powder (Bovuminar, containing <0.05 mol fatty acid/mol albumin) was obtained from Intergen (Purchase, NY). Bacterial collagenase *Clostridium histolyticum* (type 1) was obtained from Worthington Biochemical Corporation, Lakewood, NJ (lot CLS1-4197-MOB3773-B, 219 U/mg).

Statistical analyses were carried out with Student *t* test except for Pearson correlation coefficients that were determined using the GraphPad Prism program (GraphPad

Software, San Diego, CA), assuming a gaussian population and a 2-tailed P value. P values of less than .05 were considered statistically significant.

3. Results

The release of IL-1Ra, cathepsin S, MIF, NGF, and IL-18 was examined using subcutaneous and omental adipose tissue explants as well as the tissue matrix, SV cells, and adipocytes obtained by collagenase digestion of tissue from 12 gastric bypass patients and 10 abdominoplasty patients. The data from the subcutaneous and omental adipose tissue explants and adipocytes were pooled because there were significant differences ($P < .05$) only with respect to release of MIF and IL-18, which were higher in omental than in subcutaneous adipose tissue explants from abdominoplasty patients. The average BMI of the gastric bypass patients was 46.3 and the total fat mass was 58.2 kg, whereas for the abdominoplasty patients the BMI was 32.9 and the total fat mass was 34.8 kg. The total release of each cytokine/factor was also plotted vs the fat mass of each woman. Total release was calculated by averaging the data for omental and subcutaneous fat and multiplying the average release per kilogram of adipose tissue by the fat content of each individual.

The recovery of DNA, RNA, and lipid in the 3 fractions (undigested matrix, SV cells, and adipocytes) obtained after digestion of 1 g of adipose tissue with collagenase was 83% ± 8%, 76% ± 9%, and 64% ± 3% (mean ± SEM), respectively, of that in a gram of omental adipose tissue from 10 morbidly obese individuals. The distribution of DNA in the 3 non–fat cell fractions was 44% ± 7%, 23% ± 5%, and 31% ± 2% (mean ± SEM, n = 6) for the matrix, SV, and fat cell fractions, respectively, indicating that twice as many non–fat cells as fat cells are recovered per gram of human adipose tissue from morbidly obese humans. The percentage of distribution of RNA in the 3 fractions was 61% ± 9%, 20% ± 11%, and 19% ± 9% (mean ± SEM, n = 6), indicating that recovery of total RNA in the non–fat cell fractions was 3-fold greater than in fat cells. No detectable lipid could be found in the matrix and SV cell fractions. The recovery of 64% of the lipid in the adipocytes as contrasted to 83% recovery of DNA indicates a preferential loss of approximately 23% more of the relatively large fat cells than of the non–fat cells during collagenase digestion.

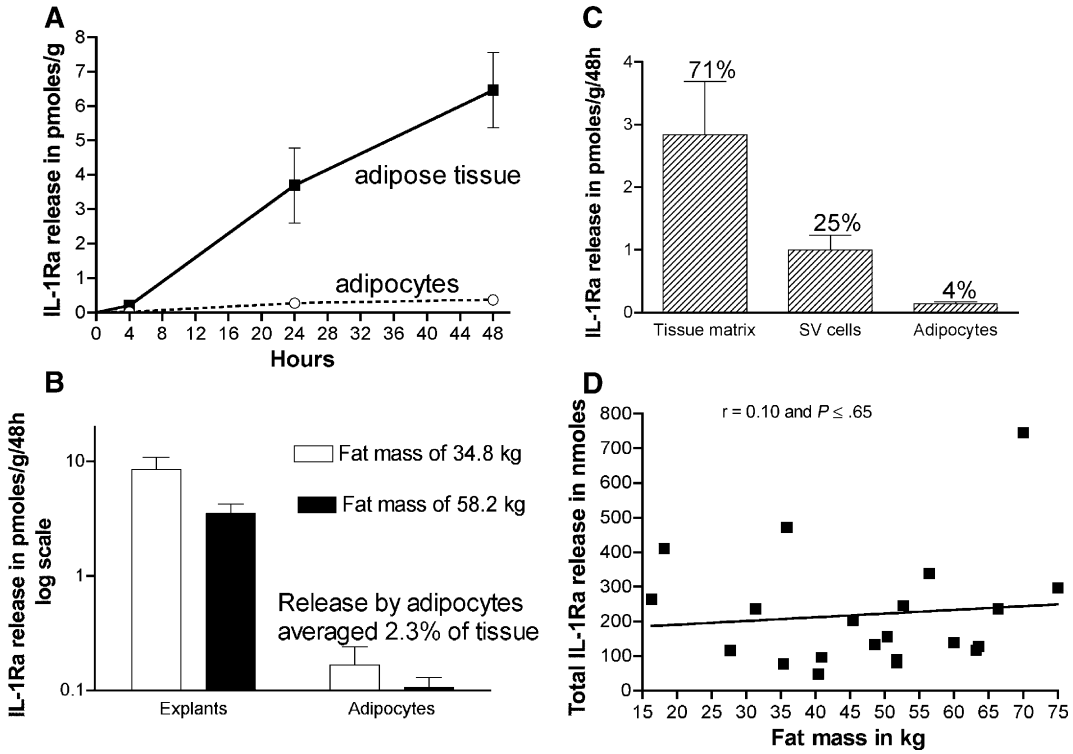

Fig. 1. Release of IL-1Ra by adipose tissue explants as well as the adipose tissue matrix, SV cells, and adipocytes derived from human adipose tissue. Visceral omental and subcutaneous adipose tissue was obtained from 12 gastric bypass patients with a mean BMI of 46.3 (total fat mass of 58.2 kg) and 10 abdominoplasty patients with a mean BMI of 32.9 (total fat mass of 34.8 kg). The values in A are shown (in pmol/g) as the mean ± SEM of explants of adipose tissue or adipocytes. The values are the increase in release at 4, 24, or 48 hours after subtraction of the zero-time value for tissue or cells plus medium. The amount of IL-1Ra present in the medium for the zero-time samples that included tissue was 26% of the 4-hour value. B, The data represent the pooled values for IL-1Ra release by explants and adipocytes isolated from omental and subcutaneous adipose tissue over a 48-hour incubation. The data in C are the pooled values for subcutaneous and omental adipose tissue from all 22 patients for the tissue matrix, SV cells, and adipocytes. The data are shown as release 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 the label released by the tissue matrix, SV cells, and adipocytes over 48 hours is shown above the bars. D, The data are shown as total release of IL-1Ra by adipose tissue and plotted against BMI for each woman This was obtained by averaging release per kilogram for omental and subcutaneous explants and multiplying that value by the kilogram of body fat for that individual. The correlation coefficient and the P value are shown above the values.

The time course for release of IL-1Ra by adipose tissue and adipocytes is shown in Fig. 1A. After an almost 4-hour lag period, there was appreciable release of IL-1Ra by explants that was much larger than the release by adipocytes. Most of the IL-1Ra released by adipose tissue over a 48-hour incubation was due to non-fat cells in the tissue because adipocyte release of IL-1Ra was less than 3% of that by tissue (Fig. 1B). This was obtained by averaging the release by omental and subcutaneous adipocytes and tissue from both gastric bypass and abdominoplasty patients. Another way of comparing release by the fat vs the non–fat cells of adipose tissue is to compare release by the undigested tissue matrix and SV cells with that by adipocytes to correct for effects of collagenase digestion. The data in Fig. 1C show that adipocytes accounted for 4% of the total release by the tissue matrix, SV cells, and adipocytes. The total release of IL-1Ra was not significantly different over the range of fat mass values from 15 to 75 kg of the 22 abdominoplasty and gastric bypass patients (Fig 1D).

There was appreciable release of cathepsin S, after an almost 4-hour lag period, over the next 44 hours by explants (Fig. 2A) that was comparable to that seen for IL-1Ra in Fig. 1A. There was also a much larger release of cathepsin S by tissue explants than by adipocytes (Fig. 2B). On a per-gram basis, the release of cathepsin S was significantly lower in adipose tissue explants or adipocytes from the bypass patients as compared with the abdominoplasty patients (Fig. 2B). Adipocytes accounted for 6% of total cathepsin S release by the tissue matrix, SV cells, and adipocytes (Fig. 2C). However, the total release of cathepsin S was not significantly different over the range of fat mass values from 15 to 75 kg (Fig. 2D).

The release of MIF by adipose tissue explants or adipocytes (Fig. 3) was greater than that of IL-1Ra and cathepsin S over an 48-hour incubation. Furthermore, the time course for release by tissue explants was quite different because most of the MIF release occurred during the first 4 hours as compared with IL-1RA and cathepsin where most of the release was seen during the next 44 hours (Fig. 3A). There was a much larger release of MIF by tissue explants than by adipocytes (Fig. 3B). There was a positive and significant Pearson correlation coefficient of 0.51 between total release of MIF and the total fat mass of the 22 individuals (Fig. 3D). This distinguishes the release of MIF from that of IL-1Ra and cathepsin S where there was no correlation between total release and fat mass.

Most of the MIF released by adipose tissue over a 48-hour incubation was due to the non–fat cells in the tissue because adipocyte release of MIF was only 14% of that by tissue. However, adipocytes accounted for 27% of the total release by the tissue matrix SV cells and adipocytes, suggesting that collagenase digestion reduced the release

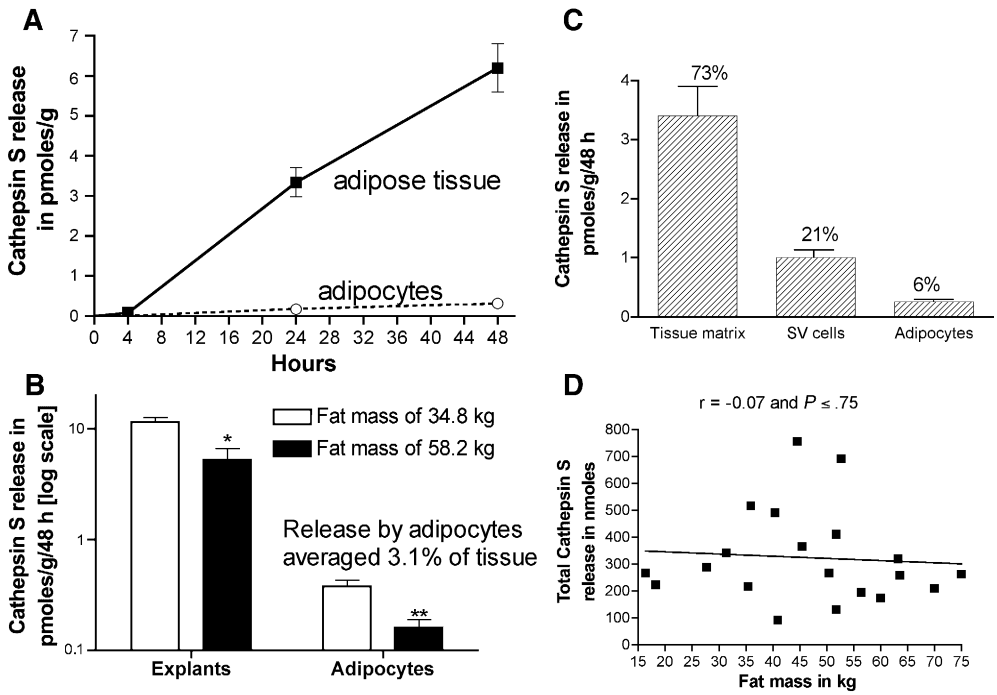

Fig. 2. Release of cathepsin S by adipose tissue explants as well as the adipose tissue matrix, SV cells and adipocytes derived from human adipose tissue. The experimental conditions are outlined in Fig. 1 as the data are from the same experiments. The values in [A] are shown in pmoles/g as the mean ± SEM for explants of adipose tissue or adipocytes. The amount of cathepsin S present in the medium of the zero time samples that included tissue was 57% of the 4-h value. The data in B represent the pooled values for IL-1Ra release by explants and adipocytes isolated from omental and subcutaneous adipose tissue. Significant differences between release by explants of tissue or adipocytes between the gastric bypass (total fat mass of 58.2 kg) and the abdominoplasty patients (total fat mass of 34.5 kg) are indicated as follows: * = $P < .005$ and ** = $P < .001$. The data in C are the pooled values for the tissue matrix, SV cells and adipocytes from subcutaneous and omental adipose tissue of all 22 patients. D, the data are shown as total release of IL-1Ra by adipose tissue and plotted against BMI for each woman. The correlation coefficient and the P value are shown above the values.

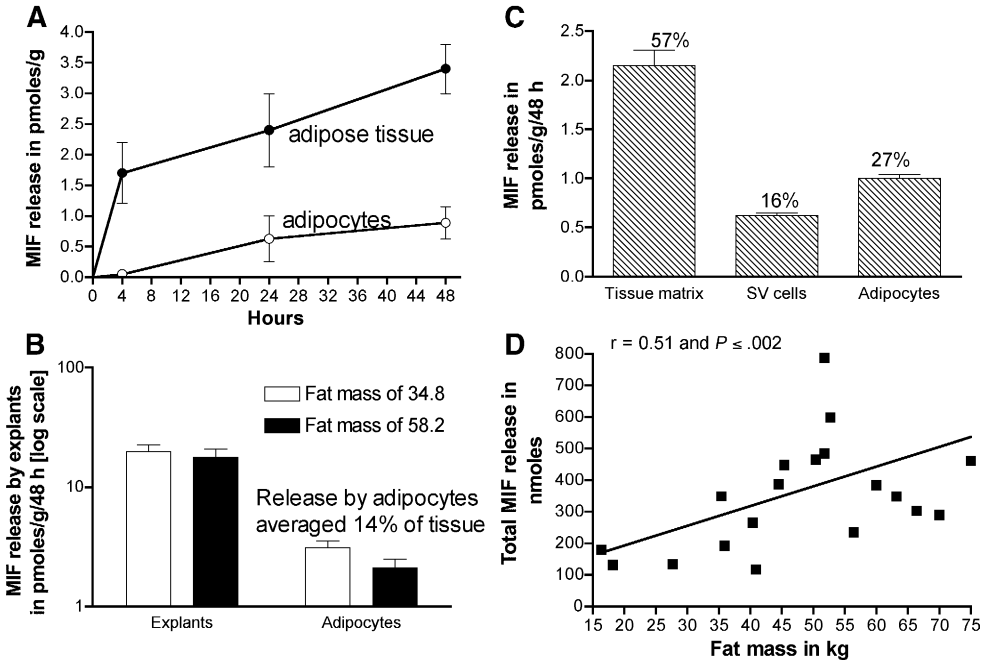

Fig. 3. Release of MIF by adipose tissue explants as well as the adipose tissue matrix, SV cells and adipocytes derived from human adipose tissue. The experimental conditions are outlined in Fig. 1 as the data are from the same experiments. The values in [A] are shown in pmoles/g as the mean ± SEM for explants of adipose tissue or adipocytes. The amount of MIF present in the medium of the zero time samples that included tissue was 55% of the 4-h value. The data in B represent the pooled values for IL-1Ra release by explants and adipocytes isolated from omental and subcutaneous adipose tissue. The data in C are the pooled values for the tissue matrix, SV cells and adipocytes from subcutaneous and omental adipose tissue of all 22 patients. D, the data are shown as total release of IL-1Ra by adipose tissue and plotted against BMI for each woman. The correlation coefficient and the *P* value are shown above the values.

of MIF by the tissue matrix and SV cells (Fig. 3D). This was also the case with NGF where release by adipocytes averaged 6% of that by explants, but NGF release accounted for 42% of that by the matrix, SV cells, and adipocytes obtained after collagenase digestion (Fig. 4D).

The average release of NGF by human adipose tissue explants over 48 hours was 0.05 pmol/g (Fig. 4), whereas that of IL-18 was 0.006 pmol/g (Fig. 5). This was far less than the release over 48 hours of IL-1Ra, cathepsin S, or MIF that was 6, 9, and 19 pmol/g (Figs. 1–3). Because of the low rate of NGF and IL-18 release by adipocytes it was not possible to examine their time course for release by adipocytes.

After an almost 4-hour lag, NGF was released to the medium by explants of adipose tissue (Fig. 4A), but NGF release was less than 1% of that seen for IL-1Ra (Fig. 1A). The release of NGF by adipocytes averaged 6% of that by tissue (Fig. 4B), but 42% of that by the non–fat cells of adipose tissue (Fig. 4C). The total release of NGF by tissue explants was not significantly different over the range of fat mass values from 15 to 75 kg (data not shown). However, in adipocytes there was a negative correlation, −0.58 or −0.54, between NGF release per gram of adipocytes over 48 hours and total fat mass (Fig. 4D).

The average release of IL-18 release by adipose tissue explants was 12% of that for NGF over 48 hours, but the lag periods were comparable (Fig. 5A vs Fig. 4A). On a per-gram basis, the release of IL-18 was significantly lower in adipose tissue explants from the bypass patients as compared with the abdominoplasty patients (Fig. 5B). The total release of IL-18 by tissue explants was negatively correlated ($r = -0.74$) with total fat content (Fig. 5D). Most of the IL-18 released by adipose tissue over 48-hour incubation was due to the non–fat cells in the tissue. Adipocyte release of IL-18 was 15% of total release by the tissue matrix, SV cells, and adipocytes (Fig. 5C).

4. Discussion

Adipose tissue has traditionally been considered a loose connective tissue in which some fibroblasts become transformed into adipocytes [25]. Currently, it is thought that mesenchymal stem cells of mesodermal origin differentiate into fibroblasts and preadipocytes, but it is difficult to distinguish between these cells [25,26]. Adipocytes (white fat cells) are specialized cells that contain a single large internal fat droplet with only a thin rim of cytoplasm between the lipid droplet and the plasma membrane. The precursor cells for adipocytes reside in the vascular stroma of loose connective tissue. However, adipose tissue also contains blood vessels, fibroblasts, and the so-called free cells of the mononuclear phagocytic system, especially monocytes and macrophages that have emigrated from the blood [25,26]. This is especially true in humans where there is a substantial amount of intercellular matrix consisting of collagen and elastic fibers. Operationally, we define the non–fat cells as those that after collagenase digestion do not

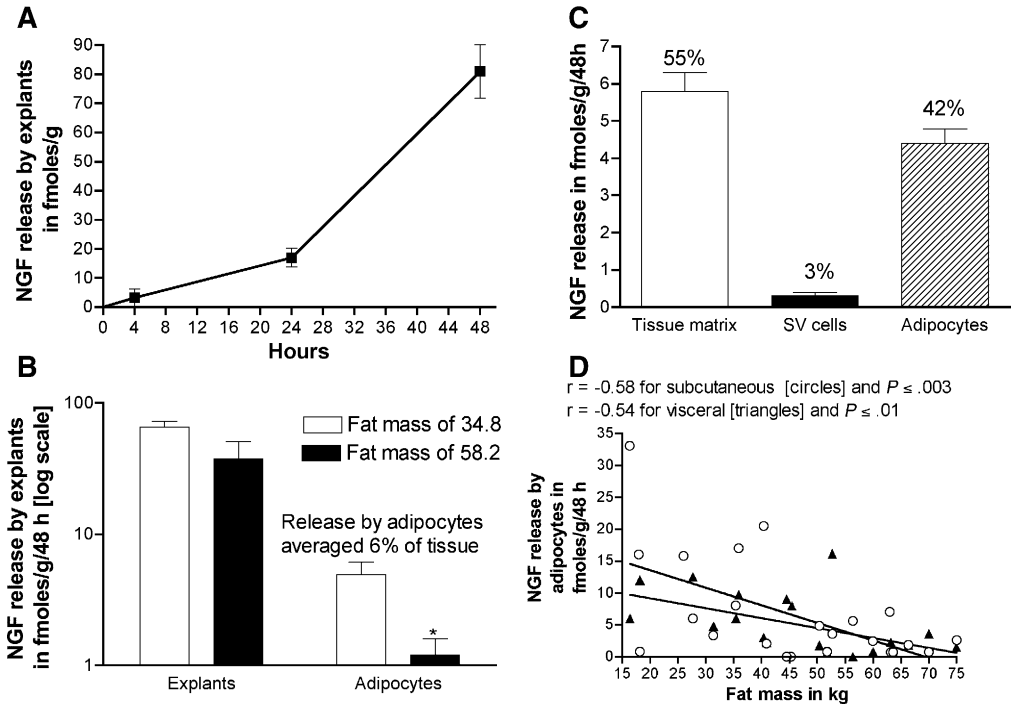

Fig. 4. Release of NGF by adipose tissue explants as well as the adipose tissue matrix, SV cells and adipocytes derived from human adipose tissue. The experimental conditions are outlined in Fig. 1 as the data are from the same experiments. The values in [A] are shown in fmoles/g as the mean ± SEM of adipose tissue explants. The amount of NGF present in the medium of the zero time samples was 79% of the 4-h value. The data in B represent the pooled values for IL-1Ra release by explants and adipocytes isolated from omental and subcutaneous adipose tissue. Significant differences between release by explants of tissue or adipocytes between the gastric bypass (total fat mass of 58.2 kg) and the abdominoplasty patients (total fat mass of 34.5 kg) are indicated as follows: * = $P < .01$. The data in C are the pooled values for the tissue matrix, SV cells and adipocytes from subcutaneous and omental adipose tissue of all 22 patients. Since there was no significant correlation between total NGF release and fat mass for each individuals these data are not shown. Instead in D, the release of NGF by subcutaneous or visceral adipocytes is plotted against the fat mass of each individual and the correlation coefficients are also shown along with the P values.

have enough lipid to float or are retained on a 200-μm mesh filter. This undigested matrix does not contain appreciable amounts of lipid and is unique to humans.

Rodbell [27,28] developed the collagenase procedure for digestion of adipose tissue to release adipocytes and recovered 67% of the tissue DNA in the adipocytes and SV (non-fat) cells, whereas we recovered 83% of the DNA in the matrix, SV, and adipocyte fractions. The total recovery of DNA was 54% greater in the non–fat cells than in adipocytes of rodents [28], whereas it was 116% greater in the matrix and SV fractions than in adipocytes of adipose tissue from morbidly obese humans. If we correct the data for 23% greater loss of adipocytes than of non–fat cells during collagenase digestion the number of cells in the non–fat cell fractions is still 76% higher than in adipocytes. These data indicate that obese humans have far more non-fat than fat cells in their adipose tissue. Furthermore, in human adipose tissue, the number of cells in the tissue matrix was 90% greater than that in the isolated SV cells. However, in rodent adipose tissue, there is no detectable undigested matrix, and the non–fat cell fraction after collagenase digestion contained mast cells, macrophages, connective tissue cells, and intact blood vessels which Rodbell [27] referred to as SV cells.

The present studies indicate that over a 48-hour incubation the release of cathepsin S and IL-1Ra by adipocytes is less than 6%, whereas that of IL-18 was only 15% of total release by the adipocytes plus the non–fat cells (tissue matrix plus SV cells) derived from the same amount of human adipose tissue. Previously, we reported that resistin [1], monocyte chemoattractant protein 1 [2], transforming growth factor β1 [3], IL-8 [4], vascular endothelial growth factor [4], IL-6 [4], prostaglandin E_2 [4], tumor necrosis factor α [4], hepatocyte growth factor [4], IL-1β [4], and IL-10 [4] were released by human adipocytes at levels 11% or less of that by the adipocytes plus non–fat cells of human adipose tissue. Thus, IL-1Ra, cathepsin S, and IL-18 join these factors as proteins whose release by adipocytes is minimal.

A second group of factors is released by adipocytes in greater amounts in relationship to release by the non–fat cells. The release of plasminogen activator inhibitor 1 by adipocytes was 20% of that by adipocytes plus non–fat cells [4], that of haptoglobin was 38% [29], and both were comparable to MIF at 27%. Nerve growth factor release by adipocytes was 42% of the total by adipocytes plus non–fat cells, which is rather similar to adiponectin at 39% [4]. Because the

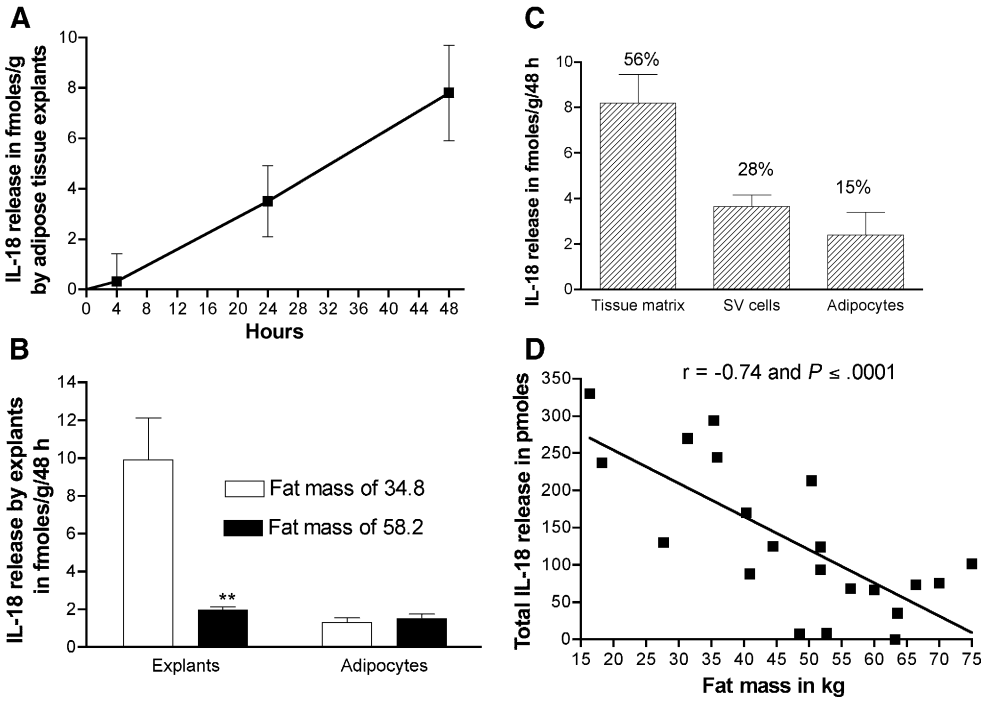

Fig. 5. Release of IL-18 by adipose tissue explants as well as the adipose tissue matrix, SV cells and adipocytes derived from human adipose tissue. The experimental conditions are outlined in Fig. 1 as the data are from the same experiments. The values in [A] are shown in fmoles/g as the mean ± SEM of adipose tissue explants. The amount of IL-18 present in the medium of the zero time samples was subtracted from the values and was 50% of the 4-h value. The data in B represent the pooled values for IL-1Ra release by explants and adipocytes isolated from omental and subcutaneous adipose tissue. Significant differences between release by explants of tissue or adipocytes between the gastric bypass (total fat mass of 58.2 kg) and the abdominoplasty patients (total fat mass of 34.5 kg) are indicated as follows: * = $P < .005$. The data in C are the pooled values for the tissue matrix, SV cells and adipocytes from subcutaneous and omental adipose tissue of all 22 patients. The data in A and C are for pooled omental and subcutaneous adipose tissue explants from the abdominoplasty patients. D, the data are shown as total release of IL-1Ra by adipose tissue and plotted against BMI for each women. The correlation coefficient and the P value are shown above the values.

adipocytes contain 32% of the DNA and 24% of the RNA recovered in the non–fat cells plus adipocytes, it appears that these factors are probably made, on a per-cell basis, in equal amounts by the non-fat and fat cells of human adipose tissue. In contrast, only leptin has been found to be released exclusively by the adipocytes of human adipose tissue [4].

Meier et al [5] reported that IL-1Ra levels are elevated 7-fold in morbid obesity and reduced after bariatric surgery. Subsequently, the same group demonstrated that IL-1Ra protein and IL-1Ra mRNA are elevated in adipose tissue from obese humans [11]. The IL-1Ra protein competes with the IL-1 receptors for binding of the IL-1 cytokines and thus functions as an anti-inflammatory cytokine [30]. In IL-1Ra–null mice, the body weight of females is reduced by 18% and that of males by 29% [31]. This is probably accounted for by the finding that, in the absence of IL-1Ra, the ability of endogenous IL-1 to suppress appetite is enhanced in mice [30]. Interleukin 1 receptor antagonist is an acute-phase protein secreted by the liver [32], and its elevation in the circulation of patients with a variety of infectious, immune, or traumatic conditions could possibly be involved in the anorexia often seen in such patients. We postulate that the enhanced circulating levels of IL-1Ra seen in obesity are more probably due to release by the liver or other tissues as was the case with haptoglobin, which is also an acute-phase protein secreted by the liver [29]. Furthermore, IL-1Ra release by human adipose tissue could be attributed to the non–fat cells rather than to the adipocytes.

Our interest in cathepsins originally derived from the report that cathepsin K is a novel marker of adiposity in white adipose tissue of mice [33]. Cathepsins S and K are found in greatest abundance in macrophages where they are thought to degrade elastin [34]. In rheumatoid and osteoarthritic synovium, the cathepsin S protein colocalized with the macrophage marker CD68 and degraded proteoglycans, but not collagen [35]. Cathepsin S is both stable and active at neutral pH, whereas cathepsin K appears to be active only in the acidic environment of lysosomes within cells [35]. We focused our attention on cathepsin S because it is secreted by macrophages both as the inactive procathepsin S and active cathepsin S [36]. Taleb et al [14] recently suggested that cathepsin S is a novel marker for obesity because the amount of its mRNA was elevated by a factor of 2 in samples of subcutaneous adipose tissue from obese as compared with nonobese individuals. However, the same authors found that synaptopodin 2 and hemoglobin β mRNA transcripts were overexpressed by a factor of 5 in subcutaneous adipose

tissue from obese individuals. It is unlikely that the almost 5-fold elevations in the genes for synaptopodin and hemoglobin in adipose tissue reflect what is present in adipocytes. We postulate that as the adipocytes enlarge in morbid obesity there is enhanced accumulation of connective tissue cells, especially those involved in the immune response as well as blood vessels plus reticulocytes. These cells probably account for the marked increases in expression of many genes, including cathepsin S, that have been reported in the adipose tissue of obese as compared with nonobese humans.

Although there was no significant correlation between total release of IL-1Ra, cathepsin S, or NGF by adipose tissue explants and fat mass, there was for total MIF release by adipose tissue explants in our studies. However, that is probably due to increased accumulation of non–fat cells in adipose tissue of massively obese humans because MIF release by adipocytes averaged 12% of that by tissue explants over 48 hours. The human MIF protein is a highly conserved protein containing only 115 amino acids with no sequence homology to any known protein [37]. Monocytes and macrophages constitutively express large amounts of MIF, and it circulates in humans at relatively high concentrations [37]. Obesity results in an elevation of circulating MIF levels [7], but we suggest that this is not the result of enhanced release by adipocytes.

Nerve growth factor is stored and released by immune cells such as mast cells, lymphocytes and eosinophils [38], human synovial fibroblasts [39], and human dermal endothelial cells [40], as well as by murine 3T3-L1 adipocytes [9,41]. The secretion of NGF was actually greater in undifferentiated 3T3-L1 cells than it was in the same cells differentiated into adipocytes [41]. This suggests that NGF is primarily secreted by macrophage-like cells and is supported by our finding that, in human adipose tissue explants, more than 90% of NGF release was due to cells other than adipocytes. However, after collagenase digestion, the formation of NGF by adipocytes was fairly similar to that by the non–fat cells whose release of NGF was reduced to a greater extent than that by adipocytes.

The release of IL-18 by human adipocytes was even lower than that of NGF, and total release by human adipose tissue explants was negatively correlated with fat mass. These data indicate that adipose tissue probably does not contribute to the increases in circulating concentration of IL-18 that have been reported in obesity [22]. The miniscule secretion of Il-18 by human adipocytes confirms the report by Wood et al [42].

The primary conclusions from the present report are as follows. No evidence could be found to support the hypothesis that secretion by human adipocytes of IL-1Ra, cathepsin S, MIF, or IL-18 contributes to the elevated levels of these factors that have been reported in obese humans. The release of IL-1Ra, cathepsin S, and MIF by explants of human adipose tissue incubated in vitro is far greater than the release of NGF or IL-18. Only the total release of MIF by human adipose tissue was enhanced, whereas that of IL-18 was significantly reduced in morbid obesity. Most of (55%-73%) the release of NGF, IL-18, IL-1Ra, cathepsin S, and MIF was by the cells present in the adipose tissue matrix, whereas release by the SV cells during collagenase digestion ranged from 3% to 28%. Release of NGF by adipocytes was 42%, that of MIF was 27%, and for the other factors 15% or less of total release by matrix, SV cells, and adipocytes of human adipose tissue. Clearly, the non–fat cells in human adipose tissue contribute most of the release of NGF, IL-18, IL-1Ra, cathepsin S, and MIF seen in vitro.

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