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Relationship between Adipocyte Size and Adipokine Expression and Secretion

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Context: Adipocytes are known to release a variety of factors that may contribute to the proinflammatory state characteristic for obesity. This secretory function is considered to provide the basis for obesity-related complications such as type 2 diabetes and atherosclerosis.

Objective: To get a better insight into possible underlying mechanisms, we investigated the effect of adipocyte size on adipokine production and secretion.

Design, Patients, and Main Outcome Measures: Protein secretion and mRNA expression in cultured adipocytes separated according to cell size from 30 individuals undergoing elective plastic surgery were investigated.

Results: The mean adipocyte volume of the four fractions ranged from 205 ± 146 to 1.077 ± 471 pl. There were strong linear correlations for the secretion of adipokines over time. Secretion of leptin, IL-6, IL-8, TNF-α, monocyte chemoattractant protein-1, interferon-γ-inducible protein 10, macrophage inflammatory protein-1β, granulocyte colony stimulating factor, IL-1ra, and adiponectin was positively correlated with cell size. After correction for cell surface, there was still a significant difference between fraction IV (very large) and fraction I (small cells), for leptin, IL-6, IL-8, monocyte chemoattractant protein-1, and granulocyte colony-stimulating factor. In contrast, antiinflammatory factors such as IL-1ra and adiponectin lost their association after correction for cell surface area comparing fraction I and IV. In addition, there was a decrease of IL-10 secretion with increasing cell size.

Conclusions: The results clearly suggest that adipocyte size is an important determinant of adipokine secretion. There seems to be a differential expression of pro- and antiinflammatory factors with increasing adipocyte size resulting in a shift toward dominance of proinflammatory adipokines largely as a result of a dysregulation of hypertrophic, very large cells. **(*J Clin Endocrinol Metab* 92: 1023–1033, 2007)**

OBESITY IS CHARACTERIZED by an elevated fat mass mainly resulting from enlarged adipocytes (1, 2). Previous studies have demonstrated that the enlargement of adipocytes is associated with substantial changes in metabolic functions, *e.g.* in lipid metabolism (3, 4). It has been hypothesized that such alterations may contribute to the health risks of obesity. Recently, adipocyte size in the sc abdominal depot was identified to be a significant predictor for the future development of diabetes mellitus type 2 (5).

Adipocytes are known to release a variety of factors, including cytokines, chemokines, and many other biologically active molecules, commonly called adipokines. These secreted products may be involved in the development of a chronic low-grade inflammatory state, which may represent the "common soil" for the pathogenesis of the metabolic and cardiovascular complications of obesity (6, 7).

Obese individuals are known to exhibit elevated circulating levels of inflammation markers such as C-reactive protein or of proinflammatory cytokines like IL-6 and TNF-α (8–11). Moreover, the adipocyte hormone leptin is a cytokine, which elicits a proinflammatory immune response (12). Its expression and release were shown to depend on adipocyte size in rodents and humans (13–15).

However, adipose tissue is also a rich source of antiinflammatory factors. Adiponectin, which is exclusively produced by adipocytes, is considered to protect from diabetes and atherosclerosis (16). In obese subjects, the circulating concentrations of adiponectin are reduced (17), and subsequently, hypoadiponectinemia could be associated with insulin resistance (18). IL-1 receptor antagonist (IL-1ra) is another product from adipose tissue (19). It antagonizes the proinflammatory activity of IL-1 (20, 21) and may improve insulin sensitivity in rodents (22). Recently, IL-10 was also identified to be released from adipose tissue (23). Clinical studies demonstrated a close association between reduced levels of IL-10 in obese women and the prevalence of the metabolic syndrome (24).

Although several groups associated the secretion of selected adipose tissue-derived factors to mean adipocyte size, there are no studies to our knowledge that separated the adipocyte fraction according to cell volume and studied the secretory pattern of pro- and antiinflammatory factors in parallel. For this purpose, a simple technique was established and validated to separate mature adipocytes according to density and to study the effect of adipocyte volume on adipokine secretion.

Patients and Methods

Materials

Collagenase was obtained from Biochrom (Berlin, Germany). Culture media were purchased from Life Technologies, Inc. (Berlin, Germany).

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Abbreviations: G-CSF, Granulocyte colony-stimulating factor; IL-1ra, IL-1 receptor antagonist; IP-10, interferon-γ-inducible protein 10; KRP, Krebs-Ringer-phosphate buffer; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein.

All other chemicals were either from Merck (Darmstadt, Germany) or Roth (Karlsruhe, Germany). Sterile plasticware for tissue culture was obtained from Falcon (Bedford, CT), TPP (Trasadingen, Switzerland), or Corning (Corning, NY).

Patients

Subcutaneous adipose tissue samples (50–100 g wet weight) were obtained by incision from 29 (seven male, 22 female) subjects undergoing elective plastic abdominal surgery. The mean age of the donors was 48.3 ± 16.1 yr, and their average body mass index 28.6 ± 10.4 kg/m^2. All subjects were healthy and did not take any medication. They were all of white origin and did not suffer from acute infections or consuming diseases. All participants gave their informed consent, and the protocol was approved by the ethical committee of the University of Düsseldorf.

Isolation and culture of adipocytes

Adipose tissue samples were immediately transported to the laboratory in DMEM containing 20 μg/ml gentamicin. Connective tissue and visible blood vessels were removed with scissors. For preparation of mature adipocytes, the adipose tissue was minced and digested in Krebs-Ringer-phosphate buffer (KRP; 154 mM NaCl, 100 mM NaH_2PO_4, 154 mM KCl, 154 mM $MgSO_4$, 110 mM $CaCl_2$, pH 7.4) containing 100 U/ml collagenase and 4% BSA for 60 min at 37 C in a shaking water bath. After this step, the undigested tissue was removed by filtration through a nylon mesh with a pore size of 250 μm (VWR, Darmstadt, Germany). The floating adipocytes were washed three times with KRP containing 0.1% BSA and were either used for direct incubation of the total fraction or to perform standardized separation into four fractions. Macrophage contamination was excluded by CD67$^+$ (BD, Franklin Lakes, NJ, kindly donated by Dr. Silvia Roser) staining of floating adipocytes and microscopic analysis.

Fractionation of adipocytes

Ten milliliters of the isolated adipocytes were separated into four fractions (small, medium, large, and very large) by flotation. Briefly, cells were transferred into a 100-ml separating funnel containing 50 ml KRP/0.1% BSA. Cells were gently mixed and allowed to float for 60 sec to obtain the first (small) cell fraction. After 60 sec, 35 ml of buffer containing the small cells were collected. Then, 35 ml of KRP was replaced and the suspension was mixed again. To obtain the second (medium) and third (large) fraction, cells were allowed to float for 45 sec and 30 sec, respectively, and again 35 ml of cell solution was collected and then refilled after each step. The remaining adipocytes were defined as the fourth (very large) fraction. Aliquots of the original sample (total fraction) and of each separate fraction were used to determine mean cell diameters. The assessment was based on the measurement of 100 cells from each fraction under the microscope. The cell volume was calculated from the diameter using the formula $4/3*d^3*\pi$ and subsequently 10^6 cells of each fraction were incubated in 5.4 ml DMEM/F-12 standard medium (catalog. no. 42400-010) containing 17.5 mM glucose without fetal bovine serum supplemented with 20 μg/ml gentamicin for 24 h.

After 24 h, cell viability was assessed using lactate dehydrogenase measurement. For further analysis, the conditioned media were immediately stored at −80 C. Adipocytes were collected in 350 μl cell lysis buffer for RNA isolation and were stored immediately at −80 C.

Measurement of adipokine release

The concentrations of adiponectin, leptin, and IL-1ra protein in the cell culture media were measured using highly sensitive sandwich ELISAs from R&D Systems (Wiesbaden, Germany). The detections limits for adiponectin, leptin, and IL-1ra were 3.9 ng/ml, 15.6 pg/ml, and 14 pg/ml, respectively. The concentrations of IL-6, IL-8, IL-10, macrophage infammatory protein-1β (MIP-1β), and granulocyte colony-stimulating factor (G-CSF) protein in cell culture infranatants were measured with a bead-based Luminex assay from Bio-Rad. Interferon-γ-inducible protein 10 (IP-10) concentrations were measured with a Luminex assay as described previously (25). The amount of protein in the medium from the fractionated incubations was either normalized for 10^6 cells or calculated per square micron by the formula $4\pi r^2$. Detection limits depended on the analyte and varied between 0.5 and 10 pg/ml. The inter- and intraassay coefficients of variation for all adipokines under investigation were less than 10%.

cDNA synthesis and real-time RT-PCR

Total RNA from 1×10^6 cells was isolated with the Aurum Total RNA kit from Bio-Rad (Munich, Germany). RNA was transcribed with the iScript Synthesis kit from Bio-Rad. Quantitative PCR was performed in 20 μl reactions using an equivalent of 50 ng mRNA. All PCR reagents were purchased from ABI (Applied Biosystems, Foster City, CA) and used according to the instructions of the manufacturer. 18S RNA was used to normalize for the starting amount of cDNA. Amplification was performed with the ABI 7700 sequence detection system using the following protocol: 40 cycles (30 sec at 95 C and 30 sec at 60 C) after an initial activation step for 10 min at 95 C. Control sequences provided by the manufacturer for amplified IL-6, leptin, and adiponectin are given in Table 1.

Statistical analysis

SAS version 9.1.3 (Heidelberg, Germany) or GraphPad PRISM Vers.4 (GraphPad Software Inc., San Diego, CA) was used for statistical analysis. Simple linear regression was applied for the time-dependent release of adipokines into the culture medium. For comparison of the fractionated adipocyte volumes and adipokine secretion, normality was tested by Shapiro-Welch's test; for testing homoscedasticity, Bartlett's test was used. As secretion data were not homogeneously distributed data were logarhythmically transformed for ANOVA. Differences between the fractions were tested in a single factor design with repeated measurements followed by Dunnett's *post hoc* test *vs.* fraction I as control. The threshold for significance was set at $P < 0.01$.

Results

Separation of adipocytes according to adipocyte volume

Differences in floating properties were used to generate four fractions of adipocytes from each donor. Analysis of the mean volumes of the adipocyte populations showed marked differences among the fractions. Fraction I contained the smallest cells (mean volume, 204 ± 151 pl) and fraction IV represented the fraction containing the largest cells (1.077 ± 471 pl) ($P < 0.01$ between fractions I and IV). This is also reflected by an increase of the mean adipocyte surface from fraction to fraction (Fig. 1, A–C).

Time course of the release of adipokines from adipocyte fractions

To exclude an artificial cytokine release related to the isolation procedure using collagenase, a repeated medium

TABLE 1. Reference sequences

Gene	Reference sequence	Catalog no.	Accession no.
IL-6	ATTCAATGAGGAGACTTGCCTGGTG	Hs00174131_m1	NM_000600
Adiponectin	TTCCCAGATGCCCCAGCAAGTGTAAC	Hs00605917_m1	NM_004797
Leptin	ATTTCACACACGCAGTCAGTCTCCT	Hs00174877_m1	NM_000230
18S	TGGAGGGCAAGTCTGGTGCCAGCAG	Hs99999901_s1	x03205

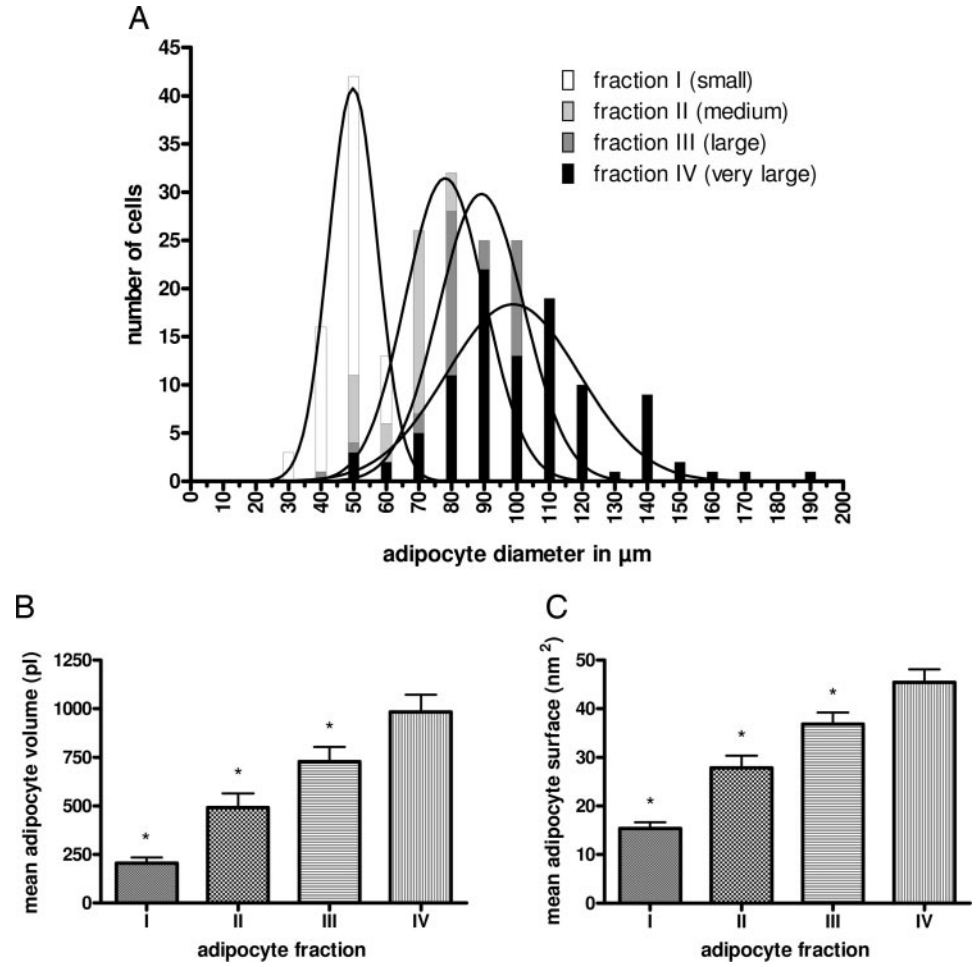

FIG. 1. Separation of adipocytes according to cell volume. Adipocytes were separated into four fractions (I, very small; II, small; III, large; and IV, very large) according to their floating properties as described under *Patients and Methods*. A, The histogram shows one representative experiment. B, The mean adipocyte volumes. C, The mean surface area per cell of the four fractions obtained from 29 donors. Results are given as mean values ± SEM. *, $P < 0.01$ *vs.* fraction IV.

collection at the time points 1, 4, 8, 16, and 24 h was performed. Measurement of IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1) and leptin revealed a constant secretion of these proinflammatory adipokines with time. This time-dependency of secretion could be observed either per 10^6 cells or calculated as release per square micron (Fig. 2). In addition, to assess cellular integrity, lactate dehydrogenase was measured at the end of the culture period to exclude significant cell damage. Lactate dehydrogenase activity was higher in the very large adipocytes compared with the small adipocyte fraction, but this difference was not significant (20.3 ± 4.2 *vs.* 47.8 ± 32.2 mU/ml, $P = 0.122$).

Adipokine release from fractionated adipocytes

Because leptin is known to be almost exclusively released from adipocytes in proportion to adipocyte volume, this cytokine was used to validate the system. Leptin release depended on adipocyte size. Fraction I released 205 ± 67 pg/ml; adipocytes from the fractions II, III, and IV, respectively, released 1310 ± 471, 2305 ± 516, and 2533 ± 492 pg/ml of leptin into the culture medium (*left panel*). When expressed per square micron surface, this significant association was still present with the highest release from fraction IV (fraction I 13.5 ± 3.9 *vs.* fraction IV 57.0 ± 13.5 pg/ml, $P < 0.01$; *right panel*) (Fig. 3A).

Figure 3, B–D, demonstrates the secretion of the proinflammatory adipokines IL-6, IL-8, and TNF-α from fractionated adipocytes kept in suspension culture for 24 h. Data are presented per 10^6 cells (*left panels*) and were also calculated per square micron adipocyte surface (*right panels*). IL-6 release per 10^6 cells was positively associated with adipocyte volume (117 ± 51 pg/ml in fraction I, 365 ± 158 pg/ml in fraction II, 414 ± 121 pg/ml in fraction III, and 1364 ± 410 pg/ml in fraction IV). Calculated per square micron surface, there was still an increased IL-6 release from the larger adipocytes (6.5 ± 2.7 pg/ml in fraction I compared with 28.9 ± 7.8 pg/ml in fraction IV; $P < 0.01$) (Fig. 3B). Similarly, IL-8 protein release was significantly associated with adipocyte volume either referred to 10^6 cells (180 ± 77 pg/ml in fraction I and 1720 ± 570 pg/ml in fraction IV; $P < 0.01$) or calculated per square micron surface (9.5 ± 4.0 in fraction I and 36.1 ± 12.3 in fraction IV; $P < 0.01$; Fig. 3C). In contrast, for TNF-α, there was only a significant association between fraction I and IV per 10^6 cells (1.7 ± 0.3 pg/ml in fraction I *vs.* 5.1 ± 1.8 pg/ml in fraction IV; $P < 0.01$). When TNF-α release was expressed per cell surface unit, there was no association between the fractions any more (Fig. 3D).

Figure 4, A–D, summarizes the secretion of selected chemokines and G-CSF from human adipocytes in suspension culture separated according to cell volume. Some of these

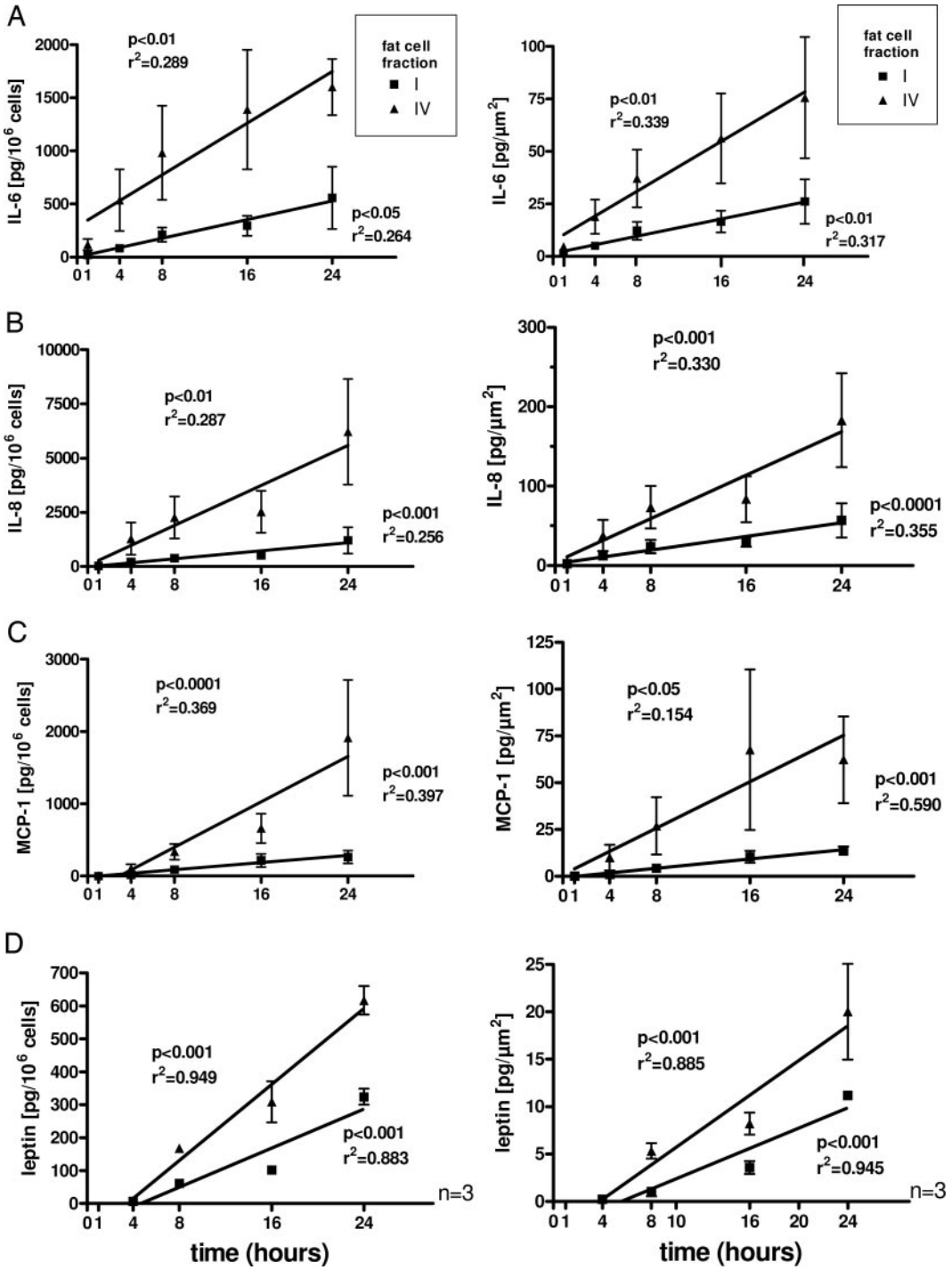

FIG. 2. Time-dependent release of proinflammatory cytokines into the culture medium. Culture medium from 10^6 adipocytes of the fractions I and IV was collected after the time points indicated to measure the concentrations of (A) IL-6, (B) IL-8, (C) MCP-1, and (D) leptin. Adipokine release was also calculated as release per square micron adipocyte surface (*right panels*) and a linear regression analysis was performed. Data are given as mean ± SEM of six independent experiments (for leptin n = 3).

factors were recently shown to be secreted by adipocytes (25–27) except G-CSF. Cultured adipocytes from fraction IV showed a significantly increased release of MCP-1 and IP-10 per 10^6 cells (149 ± 61 pg/ml in fraction I and 1208 ± 296 pg/ml in fraction IV; $P < 0.01$, and 31 ± 8.7 pg/ml in fraction I and 153 ± 46 pg/ml in fraction IV; $P < 0.01$, respectively). After calculation, the release per square micron surface, this association disappeared for IP-10 but was still present for MCP-1 (10 ± 3 pg/ml in fraction I *vs.* 23 ± 4 pg/ml in fraction IV; $P < 0.01$) (Fig. 4, A and B). Also, MIP-1β release per 10^6 cells was highest in fraction IV (41 ± 9 and 142 ± 22 pg/ml; $P < 0.01$), per square micron surface MIP-1β release declined significantly only in fraction III (3.2 ± 0.5 pg/ml in fraction I and 1.9 ± 0.5 pg/ml in fraction III), whereas it was no longer

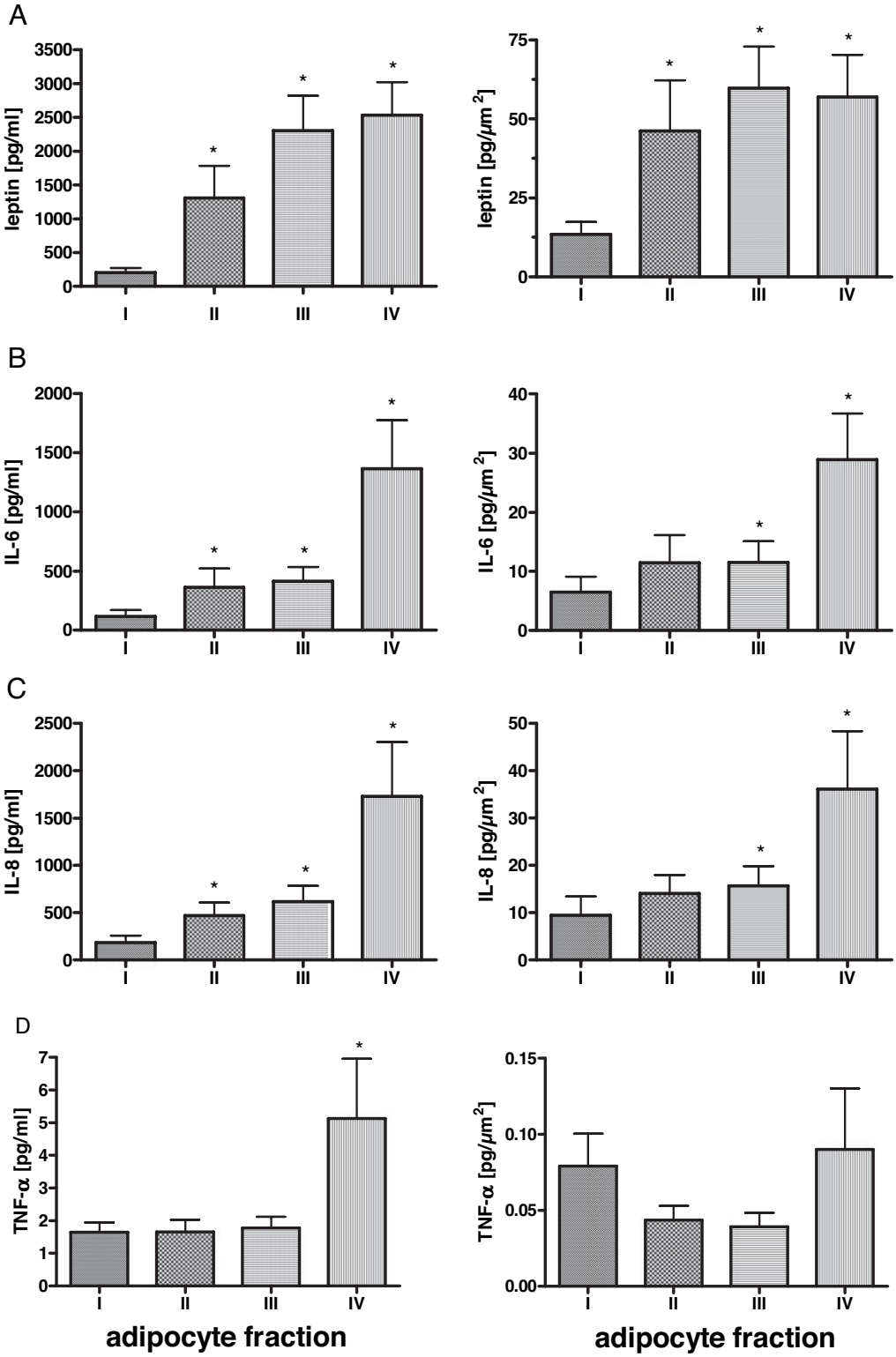

FIG. 3. Release of leptin and proinflammatory adipokines from freshly isolated adipocytes separated according to adipocyte volume. Culture medium from 10^6 cells of each fraction was collected after a 24-h culture period, and adipokine levels were determined using multiplex technique (*left panels*). Adipokine release was also calculated per square micron adipocyte surface (*right panels*). Protein levels for (A) leptin, (B) IL-6, (C) IL-8, and (D) TNF-α are given as mean ± SEM. *, $P < 0.01$ *vs.* fraction I calculated by an ANOVA test followed by Dunnett's *post hoc* test.

different comparing fraction I and IV (Fig. 4C). G-CSF release from fraction IV was significantly higher than secretion from the three smaller fractions both per 10^6 cells [9.8 ± 3.1 pg/ml (fraction I) and 135.4 ± 53.1 pg/ml (fraction IV); $P < 0.01$] and per square micron surface [0.7 ± 0.2 pg/ml (fraction I) *vs.* 3.0 ± 1.0 pg/ml (fraction IV); $P < 0.01$] (Fig. 4D).

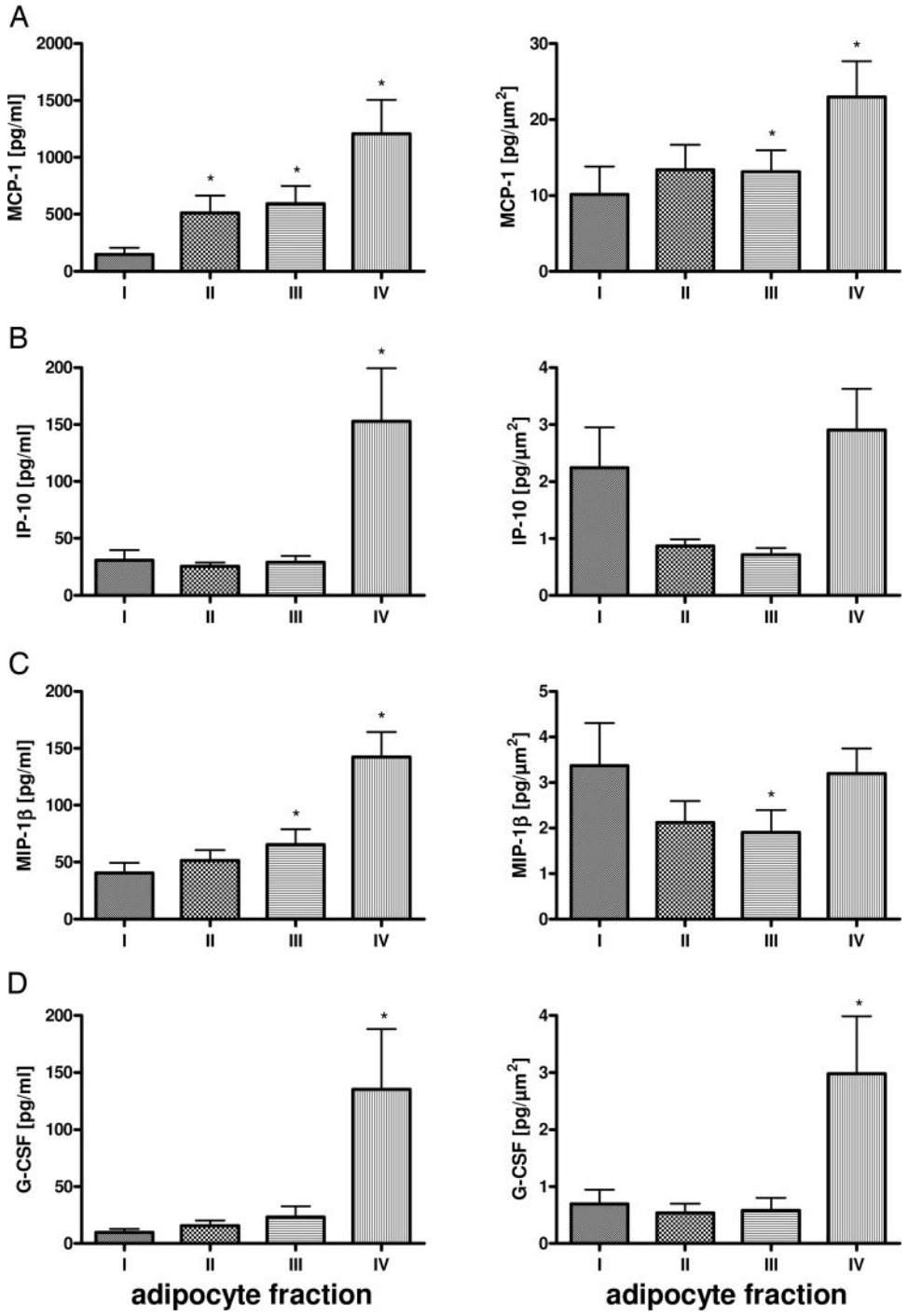

FIG. 4. Release of chemokines from freshly isolated adipocytes separated according to adipocyte volume. Culture medium from 10^6 cells of each fraction was collected after a 24-h culture period and adipokine levels were determined using multiplex technique (*left panels*). Protein release was also calculated per square micron adipocyte surface (*right panels*). Protein levels for (A) MCP-1, (B) IP-10, (C) MIP-1β, and (D) G-CSF are given as mean ± SEM. *, $P < 0.01$ *vs.* fraction I calculated by an ANOVA test followed by Dunnett's *post hoc* test.

Human adipocytes are also known to release factors that exhibit distinct antiinflammatory actions. Therefore, we also assessed the secretory pattern of adiponectin, IL-1ra, and IL-10. Adiponectin protein release from 10^6 cells was positively associated with increasing adipocyte volume (1.810 ± 513 ng/ml in fraction I *vs.* 12.418 ± 3780 ng/ml in fraction IV; $P < 0.01$). After correction per cell surface unit, this association was no longer significant. A positive association was only found between fraction I and III (Fig. 5A). Likewise, IL-1ra release from 10^6 adipocytes showed significant differences between the fractions I (27.9 ± 8.2 pg/ml) and fraction IV (154 ± 54 pg/ml; $P < 0.001$). However, after calculation per square micron surface, this difference was no longer significant (Fig. 5B). In contrast, the release of IL-10 showed no dependency on adipocyte size; after correction per square micron surface, there was a tendency to an inverse relationship between cell size and protein release with the lowest levels in the largest cells (Fig. 5C).

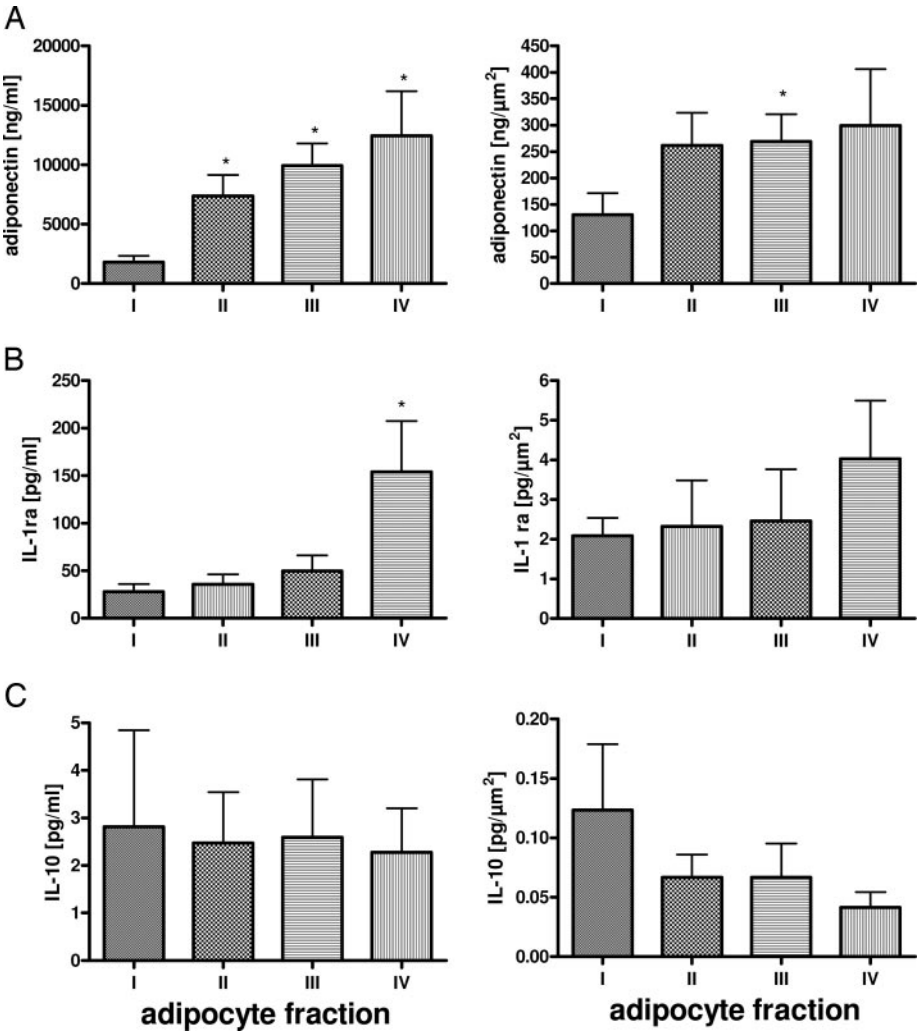

FIG. 5. Release of adipokines with antiinflammatory properties from freshly isolated adipocytes separated according to adipocyte volume. Culture medium from 10^6 cells of each fraction was collected after a 24-h culture period and adipokine levels were determined using multiplex technique (*left panels*). Adipokine release was also calculated per square micron adipocyte surface (*right panels*). Protein levels for (A) adiponectin, (B) IL-1ra, and (C) IL-10 are given as mean ± SEM. *, $P < 0.01$ *vs.* fraction I calculated by an ANOVA test followed by Dunnett's *post hoc* test.

mRNA expression of selected adipokines in cultured human adipocytes

To examine possible alterations of adipokine production, we further determined mRNA levels of leptin, IL-6, and adiponectin in the four adipocyte fractions. Total RNA was obtained from fractionated sc adipocytes of five adult individuals. Related to 10^6 cells, leptin mRNA levels showed a clearly significant continuous increase from fraction I to fraction IV (Fig. 6A). Likewise, similar differences in mRNA levels were detected for IL-6 (Fig. 6B) but not for adiponectin, which only showed a modest and nonsignificant increase (Fig. 6C).

Discussion

Previous studies have shown that adipose tissue cellularity in obesity is characterized by adipocyte hypertrophy (1, 2). Obesity is the predominant risk factor for the development of metabolic diseases like type 2 diabetes mellitus. A recent clinical investigation demonstrated a highly predictive value of mean adipocyte size in sc abdominal depots for type 2 diabetes mellitus (5). A very recent *in vitro* study identified a variety of factors overexpressed in enlarged adipocytes, which are potential candidates to link hypertrophic obesity to insulin resistance (28). In turn, weight loss results in a hypercellular state with reduced adipocyte size and subsequent alterations in the secretory activity, *e.g.* for leptin (29). In this study, adipocyte volume, but not percent body fat, was directly proportional to leptin secretion and serum leptin concentrations. Because obesity represents a chronic subclinical inflammatory process, the important question may arise how adipocyte size may contribute to this phenomenon. The hypothesis that cell size influences adipocyte biology is supported by findings from another group, in which adipocytes from fat-specific insulin receptor knockout mice showed a substantial difference in protein expression between small and large cells affecting lipid and energy metabolism (30).

The results of our study clearly indicate that adipocyte size is an important determinant for the secretion of several adipokines. In particular, the secretion of proinflammatory adipokines is significantly elevated in very large adipocytes compared with small or medium-sized adipocytes, even after correcting for cell volume and surface.

As expected and already known, leptin protein release was positively associated with adipocyte size (13, 15) and was, therefore, used as a control adipokine for the validity of the

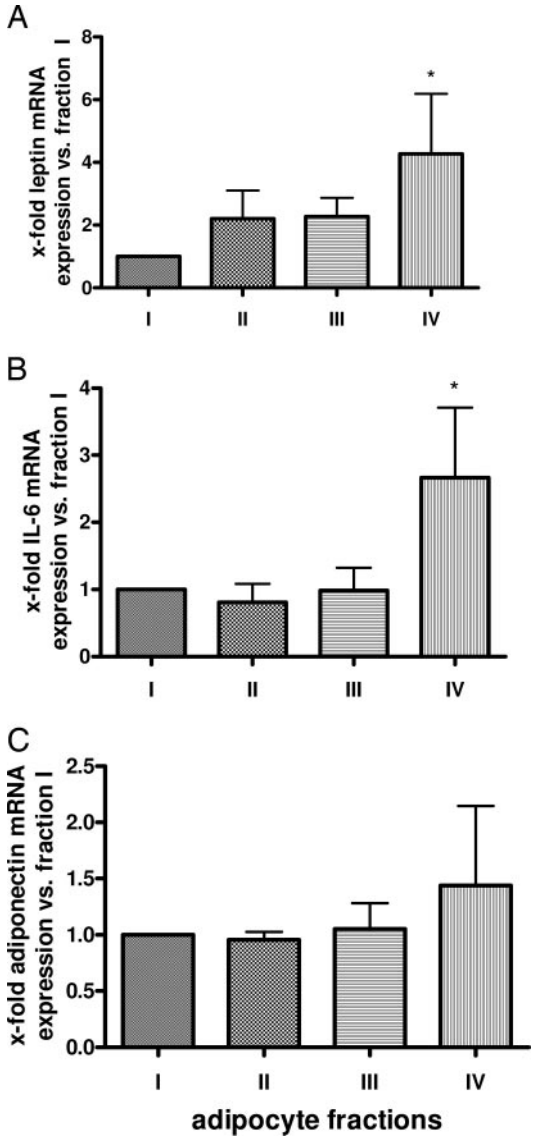

FIG. 6. mRNA expression of leptin, IL-6, and adiponectin in the four adipocyte fractions. mRNA was obtained from fractionated adipocytes of five subjects incubated for 24 h in culture medium and measured by quantitative RT-PCR. 18S RNA was used to normalize for the starting amount of cDNA. mRNA level of fraction I was defined as 1. mRNA levels for (A) leptin, (B) IL-6, and (C) adiponectin compared with fraction I are presented. All data represent mean ± SEM from five independent experiments.

model. Recent clinical trials revealed a strong correlation between adipocyte size and circulating leptin levels (14, 31, 32). Whereas in our study, a similar effect of cell size on leptin mRNA and protein levels was seen, there is also a report that could not detect an association between leptin secretion rates and mRNA levels (33). Nevertheless, these close correlations support leptin's physiological role as a signal of fat mass and may contribute to the elevated circulating levels found in obese subjects (34).

It is well established that adipose tissue from obese subjects has a higher capacity to produce cytokines such as TNF-α, IL-6, IL-8, and others (35–38). Because TNF-α, IL-6, and IL-8 were demonstrated to be increased in plasma samples of obese subjects (39–41), it was suggested that adipose tissue substantially contributes to the circulating levels. From these factors, a positive association between adipocyte size and serum adipokine exist at least for TNF-α (41), although there are also conflicting results on circulating levels of TNF-α in the obese state (39, 42). The present observation that TNF-α release does not depend on adipocyte size supports these studies, which did not show an elevation of circulating TNF-α in obese subjects (39, 42). Our results show that the production and release of both IL-6 and IL-8 are significantly increased only in the largest adipocytes, thereby providing another link between adipocyte size and inflammation in obesity. IL-6 was reported to be an independent risk marker for cardiovascular disease and IL-8 was also suggested to be involved in the development of atherosclerosis (43, 44).

Recent experimental work suggested that human obesity is associated with an accumulation of macrophages and other cells of the immune system in adipose tissue (45–48). We and others recently reported adipose secretion of factors that are known chemoattractants for monocyte/macrophage infiltration (25, 26, 49). We now studied the effect of cell size on IP-10, MIP-1β, MCP-1, and G-CSF from human adipocytes. Whereas all factors showed a continuous increase from the smallest to the largest cell fraction per 10^6 cells, G-CSF and MCP-1 remained highly significant after correction per surface area. At least elevated MCP-1 levels were associated with incident type 2 diabetes (50). Moreover, it has been demonstrated to have clear implications for the pathogenesis of atherosclerosis by recruiting monocytes to the vessel walls (51). Recent data suggest that MCP-1 $-/-$ mice and mice with genetic deficiency of its higher affinity receptor CCR2 show less macrophage infiltration and inflammation as well as improved insulin sensitivity (52, 53).

IP-10 is suspected to have similar proinflammatory functions and may play a role in the development of cardiovascular disease (54, 55). It was reported that IP-10 levels are elevated in patients with type 2 diabetes (56) but are unlikely to represent an independent risk factor for type 2 diabetes (57). We also found an increasing release of G-CSF with increasing adipocyte size. This factor may also be involved in leukocyte accumulation in adipose tissue by mediating cell attachment to endothelial cells (58). In our study, G-CSF was clearly detectable in most culture media with highest concentrations in media from the very large adipocyte fraction. This is in contrast to a recent work of Juge-Aubry *et al.* (23) in which a cytokine array revealed no signal for G-CSF. The reason for this discrepancy is currently unclear and remains to be addressed in future studies.

It is also well established that adipose tissue is a source of antiinflammatory adipokines. In fact, adipose tissue has a high capacity to produce and release IL-1ra (19) whose serum levels are elevated severalfold in obese compared with lean subjects (59). IL-1ra has antiinflammatory properties by antagonizing IL-1 activities (20) but was also reported to antagonize the anorexigenic properties of leptin in hypothalamic receptors (60). According to our data, the effect of adipocyte size on IL-1ra release is smaller than that on leptin release. The physiological significance of this shifted balance is currently unknown.

Adiponectin is another important adipocyte-specific adi-

pokine with antiinflammatory properties. Low adiponectin was shown to be predictive for the development of type 2 diabetes (61). Plasma levels of adiponectin are generally reduced in obesity (17) and, again, the reduction is more pronounced if metabolic disturbances are present (62). *In vitro* data investigating mRNA levels of adiponectin suggest that the reduction in serum adiponectin levels in obese humans may not be the result of reduced synthesis and release from adipose tissue (63, 64). Interestingly, although adiponectin liberation increased with increasing cell volume, after calculating the secretion per surface area, there was no significant difference between the smallest and the largest cell fraction. However, it is noteworthy that a reduced adiponectin expression *in vivo* may be the result of cytokines, *e.g.* TNF-α, IL-6, or IL-8 (64, 65), which are released from surrounding cells, particularly preadipocytes and macrophages. This down-regulation may be reduced or absent in a pure adipocyte suspension culture as shown in our study.

The antiinflammatory IL-10 is a pleiotropic cytokine, which attenuates immune responses by counteracting proinflammatory activities of IL-1, IL-6, and others (66). In a recent study by Fain and co-workers (67), IL-10 was found to be released by cultured adipose tissue pieces, but the secretion of IL-10 was largely attributed to the nonadipocyte fraction of adipose tissue. In contrast, in another very recent study, adipocytes were assumed to account for the majority of IL-10 release from fat tissue (23). In our study, there was a tendency to reduced IL-10 release with increasing adipocyte size indicating a direct contribution of adipocytes to low IL-10 serum concentrations in obesity. This may be physiologically relevant, because a recent clinical study related low IL-10 levels to the development of the metabolic syndrome (24).

In contrast to the published studies, which demonstrated associations between average adipocyte size and serum levels or secretion, our study is unique because it investigated the secretory capacity of adipocyte fractions from the same individual separated by cell size. The results obtained by the technique clearly suggest that only the very large adipocytes are dysregulated. Adipocyte hypertrophy appears to cause a differentially impaired secretion between pro- and antiinflammatory adipokines shifting the immunological balance toward the expression of proinflammatory proteins. This abnormal function of adipocytes may play an important role in the development of a chronic low-grade proinflammatory state in obesity, which is considered to build the common soil for the development of insulin resistance, type 2 diabetes, and atherosclerosis (5, 68). It is currently completely unknown which molecular mechanisms underlie this dysregulation in the enlarged adipocytes. One possible explanation is that shear stress during the cell isolation procedure could have led to some cell activation and thus increased adipokine release. However, any isolation procedure for adipocyte fraction with different cell size would include a certain degree of shear stress to the cells so that this aspect required thorough investigation.

In conclusion, the results of this study clearly indicate that adipocytes *per se* are an important production site for many adipokines, although the relative contribution to the overall secretion from adipose tissue remains to be elucidated. The fractionation of the adipocytes according to cell size also revealed that the hypertrophic, very large adipocytes show a markedly impaired adipokine secretion, which promotes inflammation in human adipose tissue. Therefore, it appears desirable to prevent adipocyte hypertrophy and/or to strive for a rapid removal of enlarged adipocytes.

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