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Comparison of messenger RNA distribution for 60 proteins in fat cells vs the nonfat cells of human omental adipose tissue

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Abstract

The messenger RNA (mRNA) distribution of 60 proteins was examined in the 3 fractions obtained by collagenase digestion (fat cells and the nonfat cells comprising the tissue remaining after collagenase digestion [matrix] and the stromovascular cells) of omental adipose tissue obtained from morbidly obese women undergoing bariatric surgery. Fat cells were enriched by at least 3-fold as compared with nonfat cells in the mRNAs for retinol binding protein 4, angiotensinogen, adipsin, glutathione peroxidase 3, uncoupling protein 2, peroxisome proliferator–activated receptor γ, cell death–inducing DFFA-like effector A, fat-specific protein 27, 11β-hydroxysteroid dehydrogenase 1, glycerol channel aquaporin 7, NADPH:quinone oxidoreductase 1, cyclic adenosine monophosphate phosphodiesterase 3B, glyceraldehyde-3-phosphate dehydrogenase, insulin receptor, and amyloid A1. Fat cells were also enriched by at least 26-fold in the mRNAs for proteins involved in lipolysis such as hormone-sensitive lipase, lipoprotein lipase, adipose tissue triglyceride lipase, and FAT/CD36. The relative distribution of mRNAs in cultured preadipocytes was also compared with that of in vitro differentiated adipocytes derived from human omental adipose tissue. Cultured preadipocytes had far lower levels of the mRNAs for inflammatory proteins than the nonfat cells of omental adipose tissue. The nonfat cells were enriched by at least 5-fold in the mRNAs for proteins involved in the inflammatory response such as tumor necrosis factor α, interleukin lβ, cyclooxygenase 2, interleukin 24, interleukin 6, and monocyte chemoattractant protein 1 plus the mRNAs for osteopontin, vaspin, endothelin, angiotensin II receptor 1, butyrylcholinesterase, lipocalin 2, and plasminogen activator inhibitor 1. The cells in the adipose tissue matrix were enriched at least 3-fold as compared with the isolated stromovascular cells in the mRNAs for proteins related to the inflammatory response, as well as osteopontin and endothelial nitric oxide synthase. We conclude that the mRNAs for inflammatory proteins are primarily present in the nonfat cells of human omental adipose tissue.

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 inflammatory mediators such as resistin [3], tumor necrosis factor α (TNF-α) [4], monocyte chemoattractant protein 1 (MCP-1) [5], transforming growth factor β1 (TGF-β1) [6], interleukin (IL) 8 [2], IL-6 [2], plasminogen activator inhibitor 1 (PAI-1) [2], IL-10 [2], IL-1β [2], IL-1 receptor antagonist [7], IL-18 [7], adipsin [8], vascular cell adhesion molecule 1 [8], angiotensin-1–converting enzyme (ACE) [8], and sTNFr2 [8] is by the nonfat cells present in human adipose tissue. The level of many inflammatory proteins in freshly isolated human adipose tissue is very low and difficult to measure. However, the sensitivity of the reverse transcriptase–polymerase chain reaction (RT-PCR) procedure permits measurement of the messenger RNAs (mRNAs) for many proteins in adipose tissue samples.

The present studies were designed to compare the distribution of the mRNAs for 60 adipose tissue–expressed proteins between freshly isolated human omental adipose tissue fat cells (the cells that contain enough lipid to float) and nonfat cells. The nonfat cells in adipose tissue are those cells released during collagenase digestion of the tissue that

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do not float (stromovascular [SV] cells) plus the matrix fraction that remains after collagenase digestion and does not pass through a 200-μm mesh filter [1,2]. Studies were also done with cultured preadipocytes and in vitro differentiated adipocytes derived from the omental adipose tissue of the morbidly obese women similar to those from whom omental adipose tissue was obtained.

The 60 proteins with identified functions whose mRNAs were chosen fit into one of 3 categories: The first category was proteins known or suspected of having functions unique to adipocytes. The second was proteins known to be involved in signal transduction in many cell types. The third was proteins thought to be involved in the inflammatory response and/or proteins whose expression has been reported to be altered by obesity.

2. Methods

2.1. Subjects

Omental adipose tissue was obtained from morbidly obese, but otherwise healthy, women undergoing either laparoscopic adjustable gastric banding surgery or laparoscopic gastric bypass with Roux-en-Y gastroenterostomy surgery for the treatment of morbid obesity. The mean body mass index (BMI) of the morbidly obese women was 46, and their average total body fat mass was 58 kg. The average age of the women was 40 years, and approximately 12% had fasting blood glucose values more than 7 mmol/L. Approximately half of the women were being treated for hypertension. Exclusion criteria included any history of HIV and/or viral hepatitis positive, chronic coexistent inflammatory disease such as lupus or rheumatoid arthritis, smokers, and obvious cardiovascular disease. The study had the approval of the local institutional review board, and all patients involved gave their informed consent.

2.2. Isolation of fat cells and nonfat cells

The omental adipose tissue was transported to the laboratory within 5 to 15 minutes of its removal from the donor. Fat cells were prepared by incubating 0.5 g of cut adipose tissue per milliliter of incubation medium containing 0.6 mg/mL of bacterial collagenase in a rotary water bath shaker (100 rpm) for 2 hours. The composition of the serum-free buffer was as previously described [8,9]. The collagenase digest was then separated from undigested tissue 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 the SV cells were separated from fat cells 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 fat cells were each suspended in 5 mL of fresh buffer and centrifuged for 10 seconds at 400*g*.

2.3. Isolation of preadipocytes and in vitro differentiated adipocytes

Human preadipocytes were derived from the SV cell fraction obtained after collagenase digestion of omental adipose tissue from women with the same average BMI as used for isolation of nonfat cells and fat cells as previously described [10]. The SV cell pellet was suspended in preadipocyte medium (Dulbecco modified Eagle medium/F12, 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES) pH 7.4, fetal bovine serum, biotin, pantothenate, insulin, dexamethasone, and antibiotics) and plated. The cells that adhered were then subcultured before reaching confluence and plated at a density of 6300 cells per square centimeters for further expansion in preadipocyte media containing growth factors. Preadipocytes were used after incubation for 7 days. In vitro differentiated adipocytes were obtained by plating preadipocytes at a density of 40 000 cells per square centimeters in differentiation medium containing insulin, dexamethasone, isobutyl methyl xanthine, and a thiazolidinedione (AM-1; Zen-Bio, Research Triangle Park, NC) for an additional 7 days [10]. During this period, the cells developed lipid droplets indicative of the adipocyte phenotype.

2.4. Isolation and assay of mRNA

Isolation of total RNA from preadipocytes and differentiated adipocytes was performed using an RNAeasy Mini Kit according to the manufacturer's protocol (Qiagen, Valencia, CA). For studies involving mRNA isolation from omental adipose tissue, approximately 0.5 g of tissue or the fractions derived from 1 g of tissue (the matrix, SV cells, or adipocytes) were homogenized using a Polytron homogenizer (Brinkman Instruments, Westbury, NJ) with 5 mL of a monophasic solution of phenol and guanidine isothiocyanate (TRIzol reagent from Invitrogen, Carlsbad, CA). The extracts were then spun at 12 000*g* for 10 minutes at 2°C to 8°C, and the fat layer on top of the extract was discarded after removal using a Pasteur pipette.

The assay of mRNA involved real-time quantitative PCR. The complementary DNA (cDNA) was prepared using the Transcriptor First Strand cDNA Synthesis Kit from Roche Diagnostics (Indianapolis, IN). The quantification of mRNA was accomplished using the Roche Lightcycler 480 Real-time RT-PCR system and their Universal Probe Library of short hydrolysis locked nucleic acid probes in combination with the primers suggested by the Web-based assay design center (www.universalprobelibrary.com). Integrated DNA Technologies of Coralville, IA, synthesized the primers. The primers for peroxisome proliferator–activated receptor (PPAR) γ did not distinguish between the different isoforms. Approximately 70 ng per tube of total RNA was used, and the ratio of the right to left primers was 1 for each assay. The data are obtained as crossing point values (Cp) obtained by the second derivative maximum procedure. Samples with higher copy

Table 1
Comparison of 60 mRNAs in fat cells as compared with the nonfat cells derived from human omental adipose tissue

	Ratio	ΔΔCp ± SEM	*P* value	Cp	n
mRNAs significantly enriched in fat cells/adipocytes					
HSL	104	−6.7 ± 1.0	.001	32.4	8
LPL	79	−6.3 ± 0.9	.001	26.5	7
RBP-4	42	−5.4 ± 0.6	.001	29.4	10
ATGL	37	−5.2 ± 1.2	.005	35.4	9
Amyloid protein A1	34	−5.1 ± 0.5	.001	27.3	11
Leptin	28	−4.8 ± 0.7	.001	29.2	7
FAT/CD36	26	−4.7 ± 0.7	.001	25.8	7
11βHSD-1	18	−4.2 ± 0.7	.001	30.8	11
PPARγ	15	−3.9 ± 0.6	.001	30.4	15
UCP-2	14	−3.8 ± 0.3	.001	28.9	16
Fat-specific protein 27/CIDEC	13	−3.7 ± 0.7	.05	26.0	4
GPX-3	9	−3.2 ± 0.5	.001	27.0	11
Adipsin/complement D	9	−3.2 ± 0.5	.001	27.9	12
CIDEA	8	−3.0 ± 1.1	.05	27.1	4
Cyclic AMP phosphodiesterase 3B	7	−2.8 ± 0.4	.01	27.6	4
Angiotensinogen	5	−2.5 ± 0.8	.01	34.1	14
AQP7	4	−2.3 ± 0.5	.025	27.4	4
GAPDH	3	−1.6 ± 0.3	.001	26.7	21
NQO1	3	−1.7 ± 0.4	.005	27.2	7
Insulin receptor	3	−1.6 ± 0.4	.025	27.5	4
mRNAs present in both nonfat cells and fat cells					
Giα2 guanine nucleotide binding protein	2.1	−1.1 ± 0.6		32.0	12
OPG	1.9	−0.9 ± 0.8		31.0	4
Thrombospondin 1	1.8	−0.9 ± 0.9		24.5	8
AMPK α2 catalytic subunit	1.5	−0.6 ± 0.3		32.9	11
Akt/PKB	1.5	−0.6 ± 0.2		27.4	4
Lipin	1.5	−0.6 ± 0.3		26.5	4
Cyclophilin	1.4	−0.5 ± 0.4		29.0	18
RIP 140	1.3	−0.4 ± 0.8		24.6	4
Haptoglobin	1.0	0.0 ± 0.6		31.2	10
HIF-1α	0.7	0.5 ± 0.4		27.4	13
NFκB p50 subunit	0.7	0.3 ± 0.3		33.0	16
CD68	0.7	0.5 ± 0.4		24.0	17
Apelin	0.6	0.8 ± 1.6		33.1	4
Renin receptor	0.5	0.9 ± 1.7`		25.8	4
mRNAs enriched in nonfat cells					
eNOS	0.4	1.2 ± 0.4	.025	30.2	11
Map4k4/NIK	0.4	1.2 ± 0.7		30.2	4
Cathepsin S	0.4	1.4 ± 0.4	.025	31.4	10
NFκB p65 subunit	0.4	1.5 ± 0.5	.025	29.2	8
TNF-α receptor 2	0.4	1.5 ± 1.1		36.2	9
NGF	0.3	1.8 ± 0.6	.025	31.5	11
IL-10	0.3	2.0 ± 0.6	.025	30.0	7
TNF-α	0.2	2.0 ± 0.4	.005	30.5	9
IL-8	0.2	2.3 ± 0.5	.025	23.6	4
Complement C3	0.2	2.4 ± 0.6	.005	25.9	4
IL-1β	0.2	2.4 ± 0.3	.001	25.7	7
TRB3	0.2	2.5 ± 0.4	.001	30.6	10
PAI-1	0.2	2.5 ± 0.5	.005	27.3	8
COX-2	0.2	2.7 ± 0.3	.001	27.4	8
RANTES	0.2	2.7 ± 0.5	.025	30.0	4
ACE	0.2	2.7 ± 0.4	.01	31.0	4
IL-24	0.2	2.7 ± 0.6	.005	33.2	7
IL-6	0.1	2.8 ± 0.4	.01	25.0	4
TGF-β1	0.1	3.0 ± 1.1	.05	27.3	4
Lipocalin 2	0.1	3.1 ± 0.8	.025	33.5	5
mRNAs enriched in nonfat cells					
Butyrylcholinesterase	0.1	3.2 ± 0.7	.005	33.2	8
AIIR1	0.1	3.3 ± 1.0	.025	25.7	4
MCP-1	0.1	3.8 ± 0.5	.001	23.2	7
Endothelin-1	0.1	4.0 ± 0.1	.001	27.8	4
Vaspin	0.1	4.1 ± 0.2	.001	28.1	4
OPN	0.1	4.2 ± 0.8	.005	33.5	7

The ratio is calculated as the $\log_2\Delta$Cp of the ΔCp for each mRNA, except cyclophilin, of the nonfat cells (pooled undigested tissue + SV fractions) obtained by collagenase digestion of human omental adipose tissue subtracted from the ΔCp values of fat cells (adipocytes isolated from the same tissue). The ratio for cyclophilin is based on $\log_2$ of the ΔCp values for cyclophilin. A ratio greater than 1 means that the specific activity of the mRNA is greater in the adipocytes than in the nonfat cell fractions. The values are shown as the means ± SEM of the number of paired experiments indicated under n, and the values in the nonfat cells were compared with those in the fat cells derived from the same individual. The Cp values are the number of cycles based on the second derivative maximum procedure for the nonfat cells. Statistically significant differences ($P < .05$) are indicated under *P* value.

number of cDNA have lower Cp values, whereas those with lower copy numbers have the reverse.

The data were normalized by the use of cyclophilin mRNA as the recovery standard, and this results in ΔCp values. The distribution of cyclophilin was not significantly different between the nonfat cells and the fat cells or the preadipocytes and adipocytes (Tables 1 and 2). However, in Table 3, the values were not normalized to cyclophilin but rather shown as the ΔCp of the SV minus the matrix in each paired experiment because there was a difference in the distribution of cyclophilin mRNA between the matrix and SV cell fractions. Relative quantification of the data was based on the ratio of mRNA in fat cells to nonfat cells and of preadipocyte to adipocytes. This was calculated using the comparative Cp method, which eliminates the need for standard curves. The arithmetic formula to calculate ratios from ΔCp is based on a $\log_2$ scale ($2^{-\Delta Cp}$). This method is identical to the Comparative C_T procedure described in the *ABI PRISM 7700 Sequence Detection System User Bulletin #2* for quantitative RT-PCR except that Cp is used because Roche uses the crossing point procedure instead of crossing threshold to calculate cycles required for detection of mRNA. The calculation of ratios assumes that the number of target molecules doubles with every PCR cycle. Caution should be used in comparison of the Cp values between different genes because the relative efficiencies of the particular primers and probes used for each gene may be different. A 2-tailed Student *t* test was used to determine whether differences were significant at a *P* value of $< .05$.

2.5. Materials

Bovine serum albumin powder (Bovuminar, containing <0.05 mol of fatty acid per mole of albumin) was obtained from Intergen (Purchase, NY). Bacterial collagenase

Table 2
Comparison of 60 mRNAs in adipocytes differentiated in vitro as compared with preadipocytes derived from human omental fat

	Ratio	ΔΔCp ± SEM	*P* value	Cp
mRNAs significantly enriched in differentiated adipocytes				
Fat-specific protein 27/CIDEC	26 600	−14.7 ± 0.7	.001	31.8
Leptin	5790	−12.5 ± 1.4	.001	34.0
LPL	5040	−12.3 ± 0.9	.001	31.6
AQP7	2702	−11.4 ± 1.2	.001	35.2
Haptoglobin	1550	−10.6 ± 1.8	.001	33.6
Amyloid protein A1	390	−8.6 ± 1.6	.01	29.3
Adipsin/complement D	120	−7.0 ± 1.5	.005	28.3
HSL	119	−6.9 ± 1.2	.01	27.6
Cyclic AMP phosphodiesterase 3B	91	−6.6 ± 1.2	.025	29.2
GPX-3	79	−6.3 ± 0.9	.001	25.6
FAT/CD36	56	−5.8 ± 1.1	.025	25.6
Angiotensinogen	39	−5.3 ± 0.5	.005	28.0
UCP-2	34	−5.1 ± 1.0	.01	26.1
RBP-4	30	−4.9 ± 1.1	.025	25.9
ATGL	18	−4.2 ± 0.7	.001	26.0
AIIR1	15	−3.9 ± 0.5	.001	26.8
Apelin	11	−3.5 ± 0.7	.025	28.8
Endothelin-1	11	−3.4 ± 0.4	.001	32.0
PPARγ	7.5	−2.9 ± 0.8	.05	27.4
Lipin	5.3	−2.4 ± 0.8	.025	28.7
Butyrylcholinesterase	4.9	−2.3 ± 0.4	.025	29.9
OPG	4.6	−2.2 ± 0.6	.05	26.0
Insulin receptor	3.2	−1.7 ± 0.4	.025	30.0
AMPK α2 catalytic subunit	2.0	−1.0 ± 0.3	.01	27.8
mRNAs present in both preadipocytes and differentiated adipocytes				
Thrombospondin 1	3.0	−1.6 ± 0.8		20.5
Complement C3	2.6	−1.4 ± 1.1		22.0
Akt/PKB	1.6	−0.7 ± 0.6		28.4
CD68	1.2	−0.3 ± 0.2		20.7
Cathepsin S	1.2	−0.3 ± 0.5		28.2
TNF-α receptor 2	1.2	−0.3 ± 0.6		29.0
NQO1	1.1	−0.1 ± 0.5		21.0
Renin receptor	1.0	0.0 ± 0.1		19.8
TRB3	0.9	+0.1 ± 0.6		22.8
Vaspin	0.8	+0.3 ± 0.4		36.6
CIDEA	0.8	+0.4 ± 1.4		31.7
RIP 140	0.8	+0.4 ± 0.6		21.8
NFκB p65 subunit	0.8	+0.4 ± 0.2		21.6
11βHSD-1	0.7	+0.6 ± 0.8		21.0
Giα2 guanine nucleotide binding protein	0.6	+0.7 ± 0.5		22.8
eNOS	0.6	+0.7 ± 1.0		34.1
Cyclophilin	0.5	+0.9 ± 0.5		24.0
HIF-1α	0.5	+1.0 ± 0.3	.05	17.9
Map4k4/NIK	0.5	+1.1 ± 0.7		23.4
IL-10	0.5	+1.1 ± 1.2		34.3
TNF-α	0.5	+1.1 ± 1.1		33.8
GAPDH	0.5	+1.1 ± 0.6		16.8
mRNAs enriched in preadipocytes				
NFκB p50 subunit	0.3	+1.6 ± 0.2	.005	24.2
ACE	0.3	+1.9 ± 0.6	.05	25.4
IL-6	0.3	+1.9 ± 0.5	.025	32.8
TGF-β1	0.3	+1.9 ± 0.6	.05	20.9
PAI-1	0.2	+2.0 ± 0.4	.005	15.8
NGF	0.2	+2.6 ± 0.6	.025	23.4
Lipocalin 2	0.1	+2.8 ± 1.9		33.7
MCP-1	0.1	+2.9 ± 1.1	.05	20.1
IL-1β	0.1	+3.2 ± 0.3	.05	25.9
mRNAs enriched in preadipocytes				
IL-24	0.1	+3.1 ± 1.1		34.0
COX-2	<0.1	+4.1 ± 0.6	.001	22.2
RANTES	<0.1	+4.2 ± 1.4		22.4
OPN	<0.1	+5.5 ± 0.5	.001	27.4
IL-8	<0.1	+9.7 ± 0.9	.001	20.6

The ratio is calculated as the $\log_2$ ΔCp of the ΔCp for each mRNA, except cyclophilin, of the preadipocytes derived from human omental adipose tissue subtracted from the ΔCp values for in vitro differentiated adipocytes. The ratio for cyclophilin is based on $\log_2$ of the ΔCp values for cyclophilin. A ratio greater than 1 means that the specific activity of the mRNA is greater in the adipocytes than in the preadipocytes. The values are shown as the means ± SEM of 4 experiments, with each experiment being done with adipocytes and preadipocytes derived from omental adipose tissue of 4 morbidly obese women. The Cp values are the number of cycles based on the second derivative maximum procedure for preadipocytes. Statistically significant differences ($P < .05$) are indicated under *P* value.

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 mRNA in fat cells vs the nonfat cells

The present studies used the quantitative RT-PCR procedure to compare the mRNA specific activity of a

Table 3
Distribution of mRNA between the 2 nonfat cell fractions (the matrix and SV cells) of human omental adipose tissue

Ratio	ΔCp±SEM	*P* value	Cp	mRNA
1.32	−0.4 ± 0.4		26.2	Adipsin/complement D
0.87	0.2 ± 0.5		27.2	Haptoglobin
0.71	0.5 ± 0.3		20.7	IL-8
0.66	0.6 ± 0.3		23.1	CD68
0.49	1.1 ± 0.3	.01	25.9	UCP-2
0.44	1.2 ± 0.5	.05	26.8	NFκB p50 subunit
0.44	1.2 ± 0.4	.05	27.5	Cyclophilin
0.39	1.4 ± 0.3	.005	26.3	HIF-1α
0.33	1.6 ± 0.3	.001	20.1	MCP-1
0.31	1.7 ± 0.5	.025	25.4	RBP-4
0.31	1.7 ± 0.2	.001	23.4	COX-2
0.31	1.7 ± 0.4	.005	27.6	11βHSD-1
0.29	1.8 ± 0.4	.025	26.2	PAI-1
0.29	1.8 ± 0.2	.001	27.4	ACE
0.23	2.1 ± 0.3	.01	32.1	OPN
0.20	2.3 ± 0.3	.001	26.1	eNOS

The ratio is calculated as the $\log_2$ΔCp of the difference between Cp value of the undigested tissue matrix fraction and of the SV fraction obtained by collagenase digestion of human omental adipose tissue. A ratio greater than 1 means that the specific activity of the mRNA is greater in the SV fraction than in the undigested tissue (matrix) fraction. The ΔCp values are the means ± SEM of 8 to 12 paired experiments, and statistically significant differences ($P < .05$) are indicated. The Cp values are the number of cycles based on the second derivative maximum procedure for the matrix fraction.

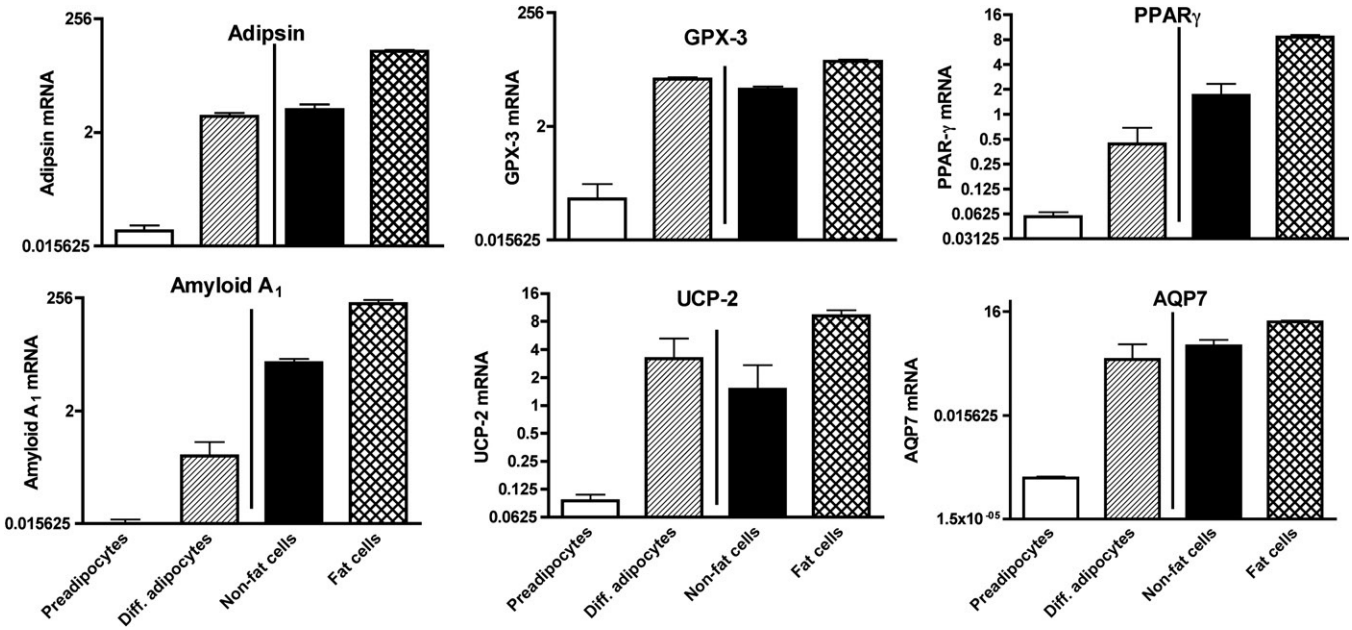

Fig. 1. Messenger RNAs enriched in fat cells and in vitro differentiated adipocytes as compared with freshly isolated nonfat cells and preadipocytes. The values are shown as the means ± SEM for 4 experiments using human omental cultured preadipocytes or in vitro differentiated adipocytes derived from the same 4 individuals, plus 4 to 8 experiments using the nonfat cells (matrix + SV fractions) and adipocytes freshly isolated from the omental adipose tissue of 4 to 8 morbidly obese women. The mRNA values are the ratios ± the SEM of mRNA expression relative to that of cyclophilin that was used as the recovery standard and are plotted on a $\log_2$ scale.

variety of adipokines and other proteins of interest because the appropriate primers and probes for most known transcripts are readily available. This procedure allows one to compare the amount of RNA in nonfat cells to that in isolated fat cells (Table 1) or in preadipocytes as compared with differentiated adipocytes (Table 2). The ratios are based on the ΔΔCp values for the nonfat cells compared with that of the fat cells for fractions derived from each individual. The ΔCp values are relative to that of cyclophilin that was used as a recovery standard in each run. However, we also show the ratio for cyclophilin based on the ΔCp between the 2 fractions to demonstrate that the recovery of cyclophilin was not different between the nonfat cells and fat cells (Table 1) or preadipocytes vs adipocytes (Table 2). In Table 3, the values for the undigested tissue matrix vs isolated nonfat cells of omental adipose tissue are shown as ΔCp values because there was significantly less recovery of cyclophilin in the SV fraction than in the undigested tissue matrix.

The mRNAs for leptin as well as proteins involved in fatty acid uptake and release such as FAT/CD36, lipoprotein lipase (LPL), adipose tissue triglyceride lipase (ATGL), and hormone sensitive lipase (HSL) were preferentially expressed, as expected, in fat cells as compared with the nonfat cells (Table 1). Amyloid protein A1, 11β-hydroxysteroid dehydrogenase 1 (11βHSD-1), retinol binding protein 4 (RBP-4), PPARγ, uncoupling protein 2 (UCP-2), glutathione peroxidase 3 (GPX-3), adipsin, cyclic adenosine monophosphate (AMP) phosphodiesterase 3B, glycerol channel aquaporin 7 (AQP7), fat-specific protein 27 (CIDEC), cell death–inducing DFFA-like effector A (CIDEA), and angiotensinogen were also preferentially expressed by a least 4-fold greater amounts in fat cells than in the nonfat cells (Table 1). The NADPH:quinone oxidoreductase 1 (NQO1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and insulin receptor mRNAs were significantly enriched by 3-fold in freshly isolated fat cells.

The mRNAs for proteins without a defined specific function in fat cells such as Giα2 guanine nucleotide binding protein, osteoprotegerin (OPG), and thrombospondin 1 were expressed at 1.8- to 2.1-fold greater amounts in fat cells, but the differences were not significant (Table 1). Other mRNAs such as AMPK α2 subunit, Akt/protein kinase B (PKB), lipin, cyclophilin, receptor interacting protein 140 (RIP 140), hypoxia inducible factor 1α (HIF-1α), haptoglobin, nuclear factor (NF) κB p50 subunit, CD68, apelin, and renin receptor were expressed to the same extent in fat cells as in nonfat cells (Table 1).

However, the mRNAs for inflammatory cytokines/adipokines such as TNF-α; IL-1β; cyclooxygenase 2 (COX-2); PAI-1; IL-6; IL-8; IL-10; IL-24; regulated on activation, normal T cell expressed and secreted (RANTES); TGF-β1; nerve growth factor (NGF); and MCP-1, as well as Tribbles 3 (TRB3), Map4k4/NIK, lipocalin 2, vaspin, osteopontin (OPN), ACE, cathepsin S, butyrylcholinesterase, and endothelial nitric oxide synthase (eNOS), were significantly enriched by at least 2.5-fold in nonfat cells (Table 1).

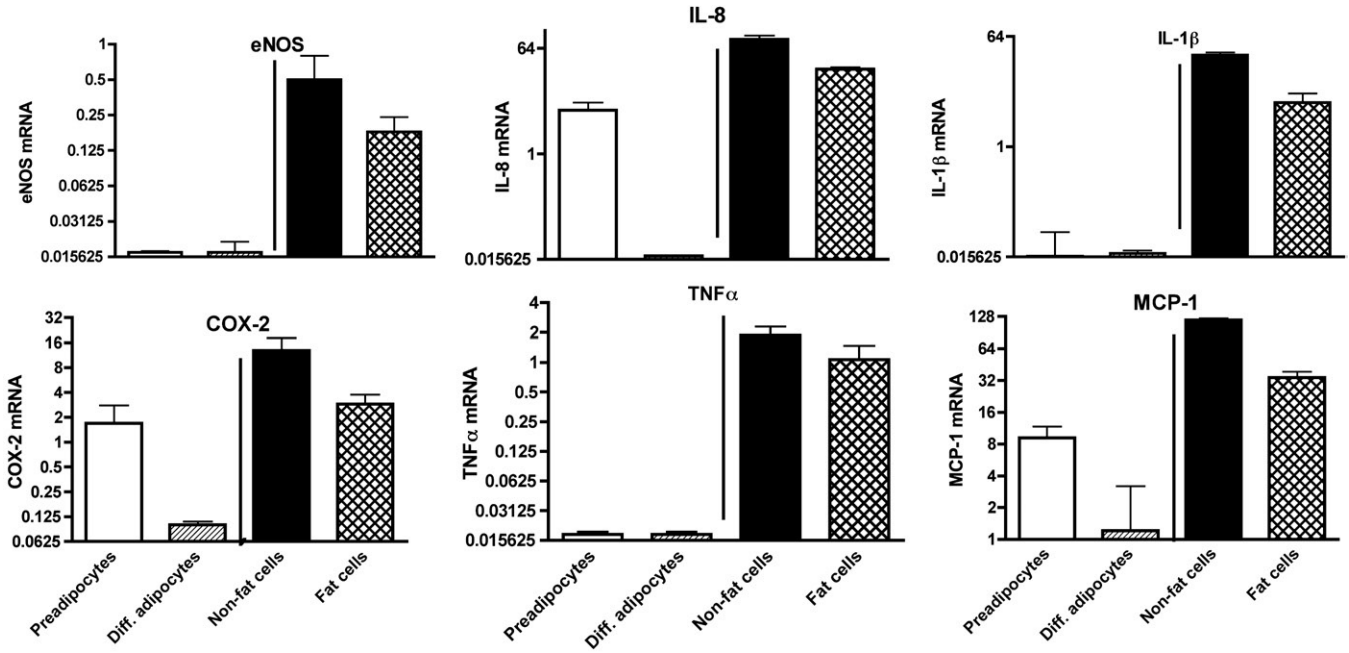

Fig. 2. Messenger RNAs for inflammatory adipokines up-regulated in freshly isolated nonfat cells. The values are derived from the same experiments described in Fig. 1. The mRNA values are the ratios ± the SEM of mRNA expression relative to that of cyclophilin that was used as the recovery standard and are plotted on a $\log_2$ scale.

3.2. Comparison of mRNA in preadipocytes vs differentiated adipocytes

The relative expression of mRNAs in adipocytes that were differentiated from omental adipose tissue preadipocytes was compared with that in preadipocytes (Table 2). The results were generally comparable to those seen when comparing freshly isolated adipocytes to nonfat cells obtained from human omental adipose tissue (Table 1). However, the amount of the mRNAs was far higher (by 2045-, 1550-, 675-, 205-, and 150-fold, respectively, for CIDEC, haptoglobin, AQP7, leptin, and angiotensin II

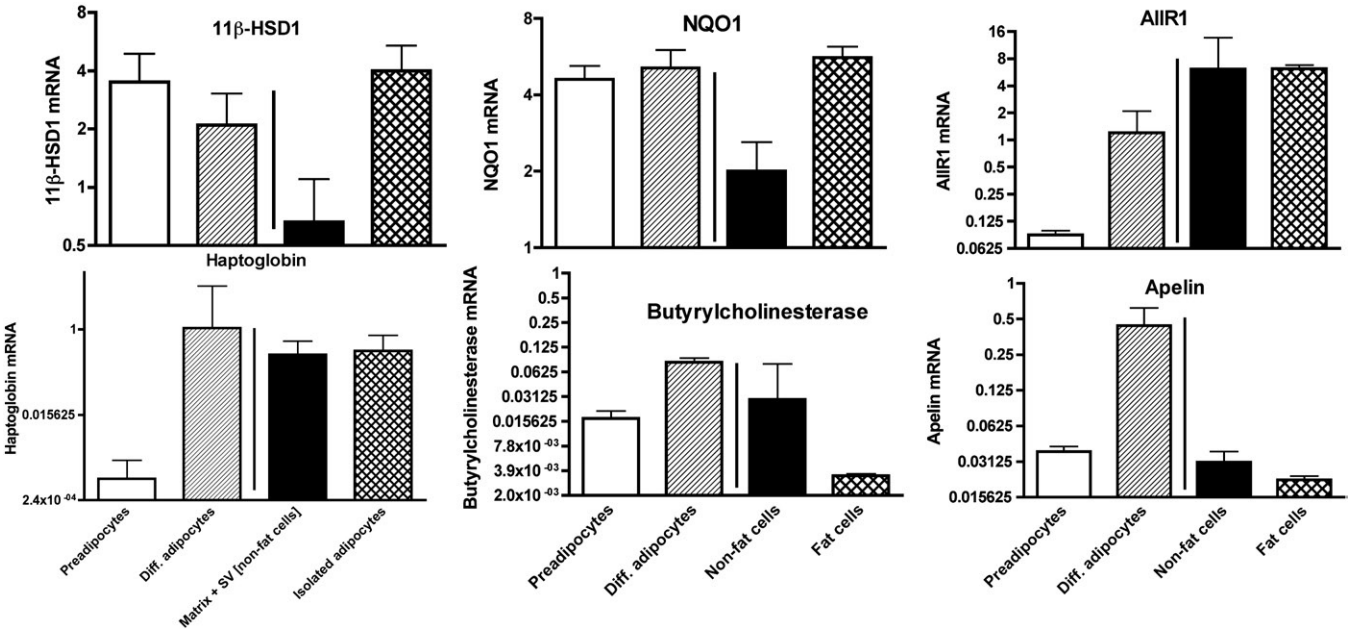

Fig. 3. Messenger RNAs where the ratio in differentiated adipocytes to preadipocytes differs from that in fat cells as compared with freshly isolated nonfat cells. The values are derived from the same experiments described in Fig. 1. The mRNA values are the ratios ± the SEM of mRNA expression relative to that of cyclophilin that was used as the recovery standard and are plotted on a $\log_2$ scale.

receptor 1 [AIIR1]) in differentiated adipocytes as compared with the fat cells. The mRNA ratios were also higher by 60- and 50-fold, respectively, for LPL and butyrylcholinesterase as well as by 8- to 18-fold for amyloid A1, adipsin, cyclic AMP phosphodiesterase 3B, angiotensinogen, and GPX-3 in differentiated adipocytes. However, the ratios of HSL, FAT, UCP-2, RBP-4, PPARγ, and AMPK α2 were comparable in fat cells and differentiated adipocytes. Haptoglobin, AIIR1, apelin, butyrylcholinesterase, OPG, endothelin-1, lipin, and AMPK α2 catalytic subunit were significantly enriched by at least 2-fold in differentiated adipocytes but not in isolated fat cells. In contrast, NQO1, CIDEA, and 11βHSD-1 mRNAs were not significantly enriched in differentiated adipocytes but were in isolated fat cells (Table 2 vs Table 1).

3.3. Comparison of mRNA in the isolated SV cells as compared with the cells present in undigested adipose tissue

The nonfat cells of omental adipose tissue are operationally divided into the cells released by collagenase digestion that are sedimented after centrifugation (SV cells) and the cells in the undigested tissue remaining after digestion [1]. The expression in SV cells of eNOS, OPN, ACE, PAI-1, 11βHSD-1, COX-2, RBP-4, MCP-1, HIF-1α, cyclophilin, NFκB p50 subunit, and UCP-2 was significantly less by 20% to 50% than in the undigested tissue matrix fraction (Table 3). In contrast, the amount of CD68, IL-8, adipsin/complement D, and haptoglobin mRNA was not significantly different between the 2 fractions (Table 3).

3.4. Comparison of relative accumulation of mRNAs in nonfat cells, fat cells, preadipocytes, and differentiated adipocytes

Because the differences in ratios of mRNA between fat cells and nonfat cells as compared with in vitro differentiated adipocytes vs cultured preadipocytes could be due to either decreases or increases in one fraction, the data for 24 mRNAs of interests were compared in the cultured preadipocytes, adipocytes, nonfat cells, and fat cells derived from the omental adipose tissue of morbidly obese women (Figs. 1-4). The data are shown as the ratios ± the SEM of mRNA expression relative to that of cyclophilin that was used as the recovery standard and are plotted on a $\log_2$ scale. It should be emphasized that, although it is possible to compare the data for each mRNA in the 4 fractions, it is not possible to compare the amount of one mRNA to another because the efficiency of each set of primers and probes may be different.

The data in Fig. 1 are for 6 mRNAs preferentially expressed in fat cells and adipocytes. Adipsin, PPARγ, UCP-2, GPX-3, amyloid A1, and AQP7 mRNAs were essentially not found in preadipocytes, but except for PPARγ and amyloid A1 were found in freshly isolated nonfat cells at levels comparable to those of in vitro differentiated adipocytes. However, for all 6 proteins, the amount of mRNA in isolated fat cells was greater than that in nonfat cells.

The data for 6 mRNAs preferentially expressed in nonfat cells are shown in Fig. 2. Endothelial nitric oxide synthase, IL-1β, and TNF-α mRNAs were essentially not present in

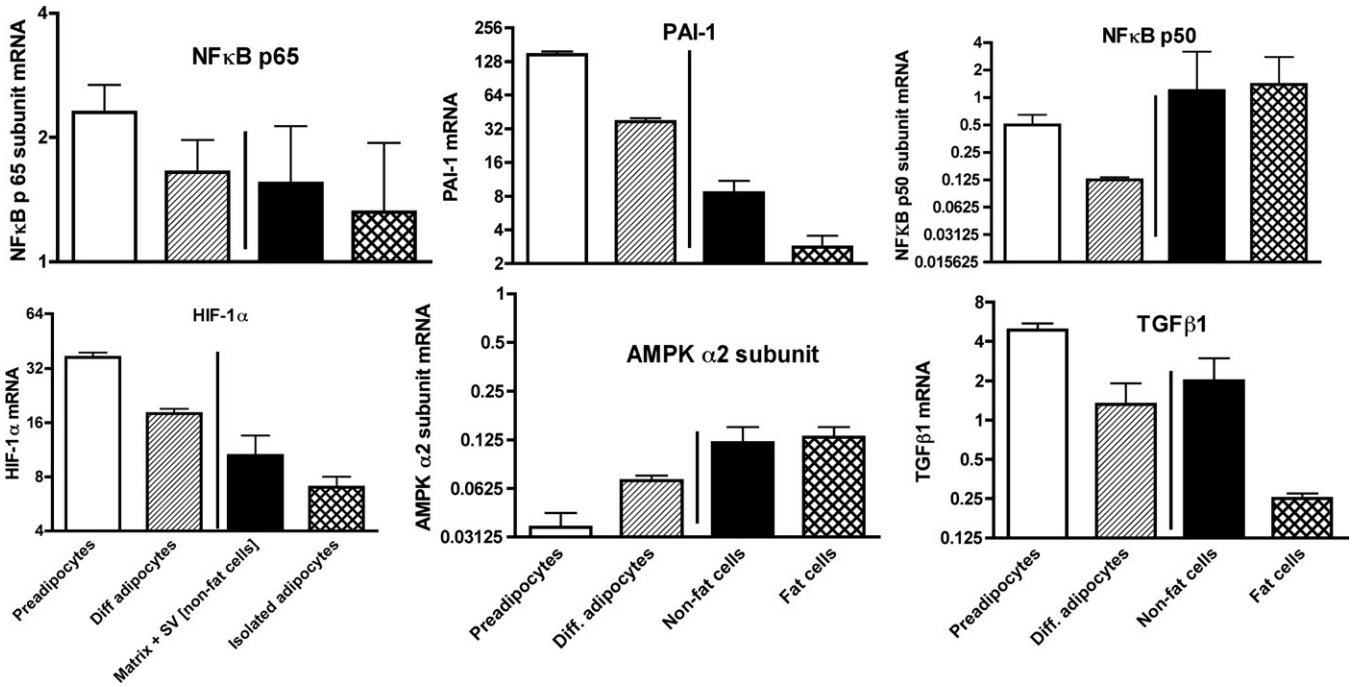

Fig. 4. Messenger RNAs down-regulated in nonfat cells and fat cells and mRNAs for regulatory molecules. The values are derived from the same experiments described in Fig. 1. The mRNA values are the ratios ± the SEM of mRNA expression relative to that of cyclophilin that was used as the recovery standard and are plotted on a $\log_2$ scale.

either preadipocytes or differentiated adipocytes but were up-regulated in both isolated fat cells and nonfat cells. However, the amount of each mRNA was substantially higher in nonfat than in fat cells. Although IL-8, COX-2, and MCP-1 were also up-regulated in both fat cells and nonfat cells, the mRNA levels for these proteins were substantially higher in preadipocytes than was the case for eNOS, IL-1β, and TNF-α mRNAs.

A different pattern was shown for the distribution of the mRNAs for 11βHSD-1 and NQO1 because their mRNA expression was higher in preadipocytes than in isolated nonfat cells and comparable in fat cells to adipocytes (Fig. 3). In contrast, the mRNAs for AIIR1 and haptoglobin were expressed at very low levels in preadipocytes but were up-regulated to about the same extent in both nonfat cells and fat cells (Fig. 3). Butyrylcholinesterase, haptoglobin, AIIR1, and apelin mRNAs were all preferentially expressed in differentiated adipocytes but not in isolated fat cells (Fig. 3). Apelin and butyrylcholinesterase were essentially down-regulated in isolated fat cells, whereas with haptoglobin and AIIR1 their expression was up-regulated in the nonfat cells as compared with preadipocytes.

At least one mRNA, the p65 subunit of NFκB, was expressed to the same extent in all 4 cell types (Fig. 4). In contrast, the p50 subunit of NFκB was expressed at a much higher level in freshly isolated fat cells than in differentiated adipocytes; but the level of expression in nonfat cells was comparable to that in fat cells (Fig. 4). The AMPK α2 catalytic subunit was up-regulated in nonfat cells and fat cells but expressed at a higher level in differentiated adipocytes than in preadipocytes. Yet another pattern was seen with regard to the mRNAs for PAI-1, HIF-1α, and TGF-β1, which were expressed to the greatest extent in preadipocytes and lowest extent in fat cells (Fig. 4).

4. Discussion

The present data indicate that for inflammatory adipokines, such as IL-6, IL-8, IL-1β, MCP-1, and PAI-1, most of their mRNAs are found in the nonfat cells of human omental adipose tissue. This is in agreement with studies on their release by omental adipose tissue over a 48-hour incubation [1-8]. The distribution of the mRNAs in fat cells agrees with in vitro release data with regard to leptin and amyloid A1 release [1]. The one exception was adipsin mRNA, whose level was 9-fold higher in fat cells than in nonfat cells; but its release by fat cells over 48 hours was less than 15% of that by the nonfat cells [8].

Glutathione peroxidase 3 and PPARγ mRNAs were enriched in isolated human fat cells as compared with preadipocytes, but no comparison was made to freshly isolated nonfat cells [11]. Maeda et al [12] originally reported that adipsin, GPX-3, LPL, and serum amyloid A1 mRNAs were expressed in adipose tissue; and our results demonstrate that they are present primarily in fat cells. Amyloid A1 mRNA is preferentially expressed in human omental fat cells [13] and RBP-4 in subcutaneous fat cells [14]. Retinol binding protein 4 has recently been reported to be a molecule secreted by adipose tissue that contributes to insulin resistance in obesity and type 2 diabetes mellitus [15]. Cell death–inducing DFFA-like effector A is localized to adipocytes; and like adiponectin, its gene expression in fat is reduced by obesity in humans [16,17]. CIDEC, also known as *fat-specific protein 27*, colocalizes with perilipin in adipocytes and negatively regulates lipolysis [18].

Our results add 11βHSD-1, UCP-2, cyclic AMP phosphodiesterase 3B, AQP7, and NQO1 to the list of proteins whose mRNAs are preferentially expressed in fat cells. 11β-Hydroxysteroid dehydrogenase 1 was examined because obesity has been reported to increase its gene expression in both subcutaneous [19,20] and omental [21] adipose tissue of obese humans. Glutathione peroxidase 3 is the only one of the 5 known glutathione peroxidases that is secreted by cells and is abundantly expressed in adipose tissue, kidney, and lung [22]. It is interesting that UCP-2 mRNA, like GPX-3, was preferentially localized in fat cells (16- to 18-fold) because UCP-2 reduces mitochondrial reactive oxygen species production [23], whereas GPXs enhance reactive oxygen species degradation [24]. Cyclic AMP phosphodiesterase 3B was examined because it is an important component of insulin and catecholamine action on rat fat cells [25]. Aquaporin-7 was examined because its mRNA expression in subcutaneous adipose tissue has been reported to be down-regulated in obesity [26]. In contrast, the expression of NQO1 mRNA has been reported to be elevated in fat cells from obese human subjects [27]; and both NQO1 [27] and 11βHSD-1 [28] mRNA expressions positively correlate with fat cell size.

Interestingly, we found that AIIR1, apelin, butyrylcholinesterase, and OPG mRNAs were significantly enhanced in differentiated omental adipocytes but not in isolated fat cells. Apelin is an adipokine said to be up-regulated in obesity, and its mRNA is expressed to the same extent in fat cells as in SV cells derived from subcutaneous adipose tissue [29]. Osteoprotegerin was examined because a positive correlation has been reported between serum OPG and visceral obesity in humans [30]. The level of OPG has also been reported to be elevated in subcutaneous adipose tissue from obese humans [31]. Butyrylcholinesterase was examined because elevated levels of butyrylcholinesterase have been postulated to result in low-grade inflammation [32]. The mRNA for this enzyme was found to approximately the same extent in preadipocytes as in nonfat cells but uniquely was elevated in differentiated adipocytes, whereas it was markedly reduced in fat cells. No other mRNA exhibited this kind of response except for apelin. Apelin is up-regulated in human adipocytes by insulin, as is PPARγ [29]. Because insulin and a thiazolidinedione were in the medium used to differentiate preadipocytes to adipocytes, this could explain the enhanced accumulation of both apelin and PPARγ in

differentiated adipocytes. However, this does not explain why apelin mRNA was lower whereas PPARγ mRNA was higher in fat cells than in nonfat cells. These results suggest that further studies should be done to see if butyrylcholinesterase expression is reduced in starvation, a catabolic state, as has been reported for apelin [29].

The p50 and p65 subunits of NFκB play a prominent role in both the immune and inflammatory responses [33]. The present data indicate that the mRNA for the p65 subunit is found in all 4 cell fractions to about the same extent, and similar results were seen for the p50 subunit except that its mRNA expression in differentiated adipocytes was reduced as compared with preadipocytes. The oxygen-dependent transcriptional activator HIF-1α is involved in the up-regulation by hypoxia of visfatin in cultured murine 3T3L1 adipocytes [34]. We found that HIF-α mRNA was expressed in both adipocytes and nonfat cells but that its level in preadipocytes was far higher. However, further experiments will be required to illuminate the physiological importance of this finding.

Vaspin and RANTES mRNAs were measured because it has been reported that the expression of these compounds is enhanced by obesity in visceral adipose tissue; but like most inflammatory adipokines, their mRNAs are predominantly located in the nonfat cells [35,36]. Plasminogen activator inhibitor 1 is another protein synthesized by adipose tissue whose circulating levels are elevated in obesity [37]. Our results indicate that its mRNA is primarily in nonfat cells. Uniquely, expression of PAI-mRNA was far higher in preadipocytes and in vitro differentiated adipocytes than in freshly isolated nonfat cells and fat cells.

One of the aims of our investigations was to compare the distribution of mRNAs between the nonfat cells remaining after the fat cells are released from human omental adipose tissue by collagenase digestion and the cells released during digestion that do not have enough lipid to float. After collagenase digestion of rodent adipose tissue, almost all the tissue passes through a 200-μm filter; and brief centrifugation separates the digest into those cells that float (adipocytes) and the cells without enough lipid to float [38]. These are the so-called nonfat cells commonly described as SV cells and composed of preadipocytes, stem cells, mononuclear blood cells, macrophages, endothelial cells, and some small pieces of blood vessels. In human adipose tissue, this is not the case because after a 2-hour digestion of adipose tissue, approximately 60% of total RNA is recovered in the undigested tissue that does not pass through a 200-μm mesh, whereas the rest is almost equally divided between the SV and adipocytes/fat cells [7]. The present results indicate that the mRNAs for adipsin/complement D and haptoglobin were equally distributed between the matrix and SV cells. For mRNAs such as OPN, eNOS, ACE, PAI-1, 11βHSD-1, COX-2, RBP-4, MCP-1, HIF-1α, cyclophilin, NFκB p50 subunit, UCP-2, IL-8, and GAPDH, the specific activity of their mRNAs was 20% to 50% in the SV cells to that in the cells present in the undigested tissue. These differences were all statistically significant. This demonstrates that SV cells liberated during collagenase digestion are not enriched in the mRNAs for proteins involved in the inflammatory response. The mRNA for CD68, which has been considered as a macrophage marker, did not have a statistically significant difference between the undigested tissue matrix fraction and the free SV cells. However, CD68 is also found in human fibroblasts [39]. Khazen et al [40] have reported that there was no difference in its expression in adipocytes as compared with SV cells derived from human subcutaneous adipose tissue. In mouse adipose tissue, CD68 mRNA was much more highly expressed in the SV cell fraction than in adipocytes [40].

Osteopontin was examined because its circulating levels as well as mRNA expression have been reported to be elevated in human obesity [41]. Furthermore, OPN is said to be involved in obesity-induce macrophage infiltration in mice [42]. Our data extend to humans the report that OPN is preferentially localized in nonfat cells isolated from mouse adipose tissue [42]. Osteopontin mRNA was found only in the macrophages but not in endothelial cells or other cells isolated from the SV fraction of collagenase-digested mouse adipose tissue [42]. If OPN is a specific marker for macrophages in human fat, then our finding that the level of OPN mRNA was 4-fold higher in the undigested tissue matrix than in the isolated human SV cells suggests that most of the macrophages in adipose tissue from morbidly obese humans are not released by collagenase digestion.

It is hardly surprising that the mRNAs for proteins such as Giα2, NHE1, cyclophilin, AMPKα, HIF-1α, and NFκB p50 subunit were present to the same extent in fat cells as in nonfat cells because they have no specific function related to adipocyte metabolism. Furthermore, the presence of elevated amounts of the mRNAs in the nonfat cells for inflammatory cytokines is as expected if most of the inflammatory response in adipose tissue is due to the cells other than fat cells.

However, interesting differences were found between the freshly isolated nonfat cells of human omental adipose tissue and preadipocytes derived from them by prolonged culture. The lower level of expression of the mRNAs for proteins such as adipsin/complement D, GPX-3, haptoglobin, and TNF-α suggests that during in vitro culture these mRNAs are down-regulated in nonfat cells. In contrast, the mRNAs for PAI-1, HIF-1α, and 11βHSD-1 were elevated in preadipocytes. The role of the 11βHSD-1 enzymes in visceral obesity is as yet unclear, but the expression of 11βHSD-1 mRNA in omental adipose tissue positively correlates with BMI [43,44]. This enzyme converts cortisone to cortisol within adipose tissue, and its activity is enhanced by incubation of omental adipose tissue in primary culture by cytokines [43]. The present results indicate that this enzyme is up-regulated in fat cells of omental adipose tissue. The higher levels of

11βHSD-1 mRNA in fat cells may be secondary to an enhanced inflammatory response in adipose tissue from morbidly obese women. If 11βHSD-1 is a protein involved in the inflammatory response, it probably acts as feedback regulator to reduce inflammation because it enhances the formation of cortisol, a known anti-inflammatory agent. A unique feature of 11βHSD-1 mRNA is that it is preferentially up-regulated in adipocytes unlike the mRNAs for cytokines such as TNF-α, IL-1β, IL-8, and IL-6 that were preferentially up-regulated in nonfat cells.

Lipin was examined because its expression in transgenic mice causes obesity [45] but its expression in adipose tissue is reduced in obesity [46]. Endothelin-1 is a potent vasoconstrictor peptide [47], and human adipose tissue was found to have a marked release of endothelin-1 in vivo that was increased in obesity [48]. Lipin mRNA expression was slightly, but not significantly, enhanced in human fat cells as compared with the nonfat cells but was enhanced in differentiated adipocytes as compared with preadipocytes. Endothelin-1 mRNA was also preferentially expressed, by 11-fold, in differentiated adipocytes as compared with preadipocytes but in isolated fat cells was expressed at levels less than 6% of that in nonfat cells. Clearly, neither lipin nor endothelin-1 mRNA is preferentially expressed in freshly isolated fat cells from morbidly obese individuals.

There are several caveats with regard to these studies. The first is that what was measured was mRNA expression, which does not necessarily correspond with the level of protein. However, in studies using omental adipose tissue from humans, it is difficult to obtain sufficient tissue and specific antibodies to measure the amount of protein. The second caution is that to obtain fat cells from human adipose tissue it is necessary to incubate the tissue with collagenase, and we have shown that the time required for this results in up-regulation of the mRNAs for proteins involved in the inflammatory process [9]. However, this does not affect the comparison of mRNA distribution in nonfat cells vs fat cells because both are up-regulated during this process [9]. Another caveat is that differentiated adipocytes were obtained by incubation of preadipocytes in medium containing insulin, dexamethasone, a methyl xanthine, and a thiazolidinedione, which is in effect exposing them to an anabolic condition.

We conclude that the mRNAs for inflammatory proteins are preferentially expressed in the nonfat cells, especially those not released during collagenase digestion of human omental adipose tissue. Novel adipokines that may be secreted by omental adipose tissue include endothelin-1, OPN, butyrylcholinesterase, lipocalin 2, vaspin, RANTES, apelin, and NGF; but they are all preferentially expressed in nonfat cells. Fat cells preferentially express those mRNAs for fatty acid storage and release proteins as well as RBP-4, amyloid A1, UCP-2, adipsin, PPARγ, AQP7, NQO1, GPX-3, angiotensinogen, CIDEC, CIDEA, and 11βHSD-1 whose roles in adipocyte metabolism and the inflammatory response are incompletely understood.

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References

[1] Fain JN. Release of interleukins and other inflammatory cytokine by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm 2006;74:443-77.
[2] Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue matrix and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004;145:2273-82.
[3] Fain JN, Cheema PS, Bahouth SW, Hiler ML. Resistin release by human adipose tissue explants in primary culture. Biochem Biophys Res Commun 2003;300:674-8.
[4] Fain JN, Bahouth SW, Madan AK. TNFα release by the nonfat cells of human adipose tissue. Intl J Obes 2004;28:616-22.
[5] Fain JN, Madan AK. Regulation of monocyte chemoattractant protein 1 (MCP-1) release by explants of human visceral adipose tissue. Int J Obes 2005;29:1299-307.
[6] Fain JN, Tichansky DS, Madan AK. TGF-β1 release by human adipose tissue is enhanced in obesity. Metabolism 2005;54:1546-51.
[7] Fain JN, Tichansky DS, Madan AK. 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. Metabolism 2006; 55:1113-21.
[8] Fain JN, Nesbit AS, Sudlow FF, Cheema P, Peeples JM, Madan AK, et al. Release in vitro of adipsin, VCAM-1, angiotensin 1 converting enzyme [ACE] and soluble tumor necrosis factor receptor 2 [sTNFR2] by human omental adipose tissue as well as by the non-fat cells and adipocytes. Metabolism 2007;56:1583-90.
[9] Fain JN, Bahouth SW, Madan AK. Involvement of multiple signaling pathways in the post bariatric induction of IL-6 and IL-8 mRNA and release in human visceral adipose tissue. Biochem Pharmacol 2005;69: 1315-24.
[10] Halvorsen YD, Bond A, Sen A, Franklin DM, Lea-Currie YR, Sujkowski D, et al. Thiazolidinediones and glucocorticoids synergistically induce differentiation of human adipose tissue stromal cells: biochemical, cellular and molecular analysis. Metabolism 2001;50:407-13.
[11] Urs S, Smith C, Campbell B, Saxton AM, Taylor J, Zhang B, et al. Gene expression profiling in human preadipocytes and adipocytes by microarray analysis. J Nutr 2004;134:762-70.
[12] Maeda K, Okubo K, Shimomura I, Mizuno K, Matsuzawa Y, Matsubara K. Analysis of an expression profile of genes in the human adipose tissue. Gene 1997;190:227-35.
[13] Sjoholm K, Palming J, Olofsson LE, Gummesson A, Svensson PA, Lystig TC, et al. A microarray search for genes predominantly expressed in human omental adipocytes: adipose tissue as a major production site of serum amyloid A. J Clin Endocrinol Metab 2005;90: 2233-9.
[14] Yao-Borengasser A, Varma V, Bodles AM, Rasouli N, Phanavanh B, Lee MJ, et al. Retinol binding protein 4 expression in humans: relationship to insulin resistance, inflammation, and response to pioglitazone. J Clin Endocrinol Metab 2007;92:2590-7.
[15] Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436:356-62.

[16] Nordstrom EA, Ryden M, Backlund EC, Dahlman I, Kaaman M, Blomqvist L, et al. A human-specific role of cell death-inducing DFFA (DNA fragmentation factor-*a*)-like effector A (CIDEA) in adipocyte lipolysis and obesity. Diabetes 2005;54:1726-34.

[17] Gummesson A, Jernas M, Svensson PA, Larsson I, Glad CA, Schele E, et al. Relations of adipose tissue cell death-inducing DFFA-like effector A gene expression to basal metabolic rate, energy restriction and obesity: population-based and dietary intervention studies. J Clin Endocrinol Metab 2007;92:4759-65.

[18] Puri V, Konda S, Ranjit S, Aouadi M, Chawla A, Chouinard M, et al. Fat specific protein 27: a novel lipid droplet protein that enhances triglyceride storage. J Biol Chem 2007;282:34213-8.

[19] Rask E, Walker BR, Soderberg S, Livingstone DEW, Eliasson M, Johnson O, et al. Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11*b*-hydroxysteroid dehydrogenase type 1 activity. J Clin Endocrinol Metab 2002;87:3330-6.

[20] Engeli S, Bohnke J, Feldpausch M, Gorzelniak K, Heintze U, Janke J, et al. Regulation of 11β-HSD genes in human adipose tissue: influence of central obesity and weight loss. Obes Res 2004;12:9-17.

[21] Mariniello B, Ronconi V, Rilli S, Bernante P, Boscaro M, Mantero F, et al. Adipose tissue 11β-hydroxysteroid dehydrogenase type 1 expression in obesity and Cushing's syndrome. Eur J Endocrinol 2006;155:435-41.

[22] Bierl C, Voetsch V, Jin RC, Handy DE, Loscalzo J. Determinants of human plasma glutathione peroxidase (GPx-3) expression. J Biol Chem 2004;279:26839-45.

[23] Brand MD. The efficiency and plasticity of mitochondrial energy transduction. Biochem Soc Trans 2005;33:897-904.

[24] Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. Biochemistry (Mosc) 2005;70:200-14.

[25] Cong L, Chen K, Li J, Gao P, Li Q, Mi S, et al. Regulation of adiponectin and leptin secretion and expression by insulin through a PI3K-PDE3B dependent mechanism in rat primary adipocytes. Biochem J 2007;403:419-25.

[26] Ceperuelo-Mallafre V, Miranda M, Chacon MR, Vilarrasa N, Megia A, Guiterrez C, et al. Adipose tissue expression of the glycerol channel aquaporin-7 gene is altered in severe obesity but not in type 2 diabetes. J Clin Endocrinol Metab 2007;92:3640-5.

[27] Palming J, Sjoholm K, Jernas M, Lystig TC, Gummesson A, Romeo S, et al. The expression of NAD(P)H:quinone oxidoreductase 1 is high in human adipose tissue, reduced by weight loss, and correlates with adiposity, insulin sensitivity, and markers of liver dysfunction. J Clin Endocrinol Metab 2007;92:2346-52.

[28] Michailidou Z, Jensen MD, Dumesic DA, Chapman KE, Seckl JR, Walker BR, et al. Omental 11β-hydroxysteroid dehydrogenase 1 correlates with fat cell size independently of obesity. Obesity (Silver Spring) 2007;15:1155-63.

[29] Boucher J, Masri B, Daviaud D, Gesta S, Guigne C, Mazzucotelli A, et al. Apelin, a newly identified adipokine is up-regulated by insulin and obesity. Endocrinol 2005;146:1764-71.

[30] Golledge J, Jayalath R, Oliver L, Parr A, Schurgers L, Clancy P. Relationship between CT anthropometric measurements, adipokines and abdominal aortic calcification. Atherosclerosis 2008;197:428-34.

[31] Skopkova M, Penesova A, Sell H, Radikova Z, Vicek M, Imrich R, et al. Protein array reveals differentially expressed proteins in subcutaneous adipose tissue in obesity. Obesity (Silver Spring) 2007;15:2396-2406.

[32] Rao AA, Sridhar GR, Das UN. Elevated butyrylcholinesterase and acetylcholinesterase may predict the development of type 2 diabetes mellitus and Alzheimer's disease. Med Hypotheses 2007;69:1272-6.

[33] Li X, Stark GR. NF*k*B-dependent signaling pathways. Exp Hematol 2002;30:285-96.

[34] Segawa K, Fukuhara A, Hosogai N, Morita K, Okuno Y, Tanaka M, et al. Visfatin in adipocytes is upregulated by hypoxia through HIF1*a*-dependent mechanism. Biochem Biophys Res Commun 2006;349: 875-82.

[35] Kloting N, Berndt J, Kralisch S, Kovacs P, Fasshauer M, Schon MR, et al. Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. Biochem Biophys Res Commun 2006;339: 430-6.

[36] Wu H, Ghosh S, Perrard XD, Feng L, Garcia GE, Perrard JL, et al. T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. Circulation 2007;115: 1029-38.

[37] Alessi MC, Poggi M, Juhan-Vague L. Plasminogen activator inhibitor–1, adipose tissue and insulin resistance. Curr Opin Lipidol 2007;18: 240-5.

[38] Rodbell M. Localization of lipoprotein lipase in fat cells of rat adipose tissue. J Biol Chem 1964;239:753-5.

[39] Kunz-Schughart LA, Weber A, Rehli M, Gottfried E, Brockhoff G, Krause SW, et al. The "classical" macrophage marker CD68 is strongly expressed in primary human fibroblasts. Verh Dtsch Ges Pathol 2003; 87:215-23.

[40] Khazen W, M'Bika J, Tomkiewicz C, Benelli C, Chany C, Achour A, et al. Expression of macrophage-selective markers in human and rodent adipocytes. FEBS Lett 2005;579:5631-4.

[41] Gomez-Ambrosi J, Catalan V, Ramirez B, Rodriguez A, Colina I, Silva C, et al. Plasma osteopontin levels and expression in adipose tissue are increased in obesity. J Clin Endocrin Metab 2007;92: 3719-27.

[42] Nomiyama T, Perez-Tilve D, Ogawa D, Gizard F, Zhao Y, Heywood EB, et al. Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. J Clin Inves 2007;117:2877-88.

[43] Tomlinson JW, Moore J, Cooper MS, Bujalska I, Shahmanesh M, Burt C, et al. Regulation of expression of 11β-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. Endocrinology 2001;142:1982-9.

[44] Desbriere R, Vuaroqueaux V, Archard V, Boullu-Ciocca S, Labuhn M, Dutour A, et al. 11β-hydroxysteroid dehydrogenase type 1 mRNA is increased in both visceral and subcutaneous adipose tissue of obese patients. Obesity (Silver Spring) 2006;14:794-8.

[45] Reue K, Zhang P. The lipin protein family: dual roles in lipid biosynthesis and gene expression. FEBS Lett 2008;582:90-6.

[46] van Harmelen V, Ryden M, Sjolin E, Hoffstedt J. A role of lipin in human obesity and insulin resistance: relation to adipocyte glucose transport and GLUT 4 expression. J Lipid Res 2007;48:201-6.

[47] Masaki T. Historical review: endothelin. TRENDS Pharmacol Sci 2004;25:219-24.

[48] Van Harmelen V, Eriksson A, Astrom G, Wahlen K, Naslund E, Karpe F, et al. The vascular peptide endothelin-1 links fat accumulation with alterations of visceral adipocyte lipolysis. Diabetes 2008;57:378-86.