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Glucocorticoid Receptor Messenger Ribonucleic Acid in Different Regions of Human Adipose Tissue*

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ABSTRACT. The expression of glucocorticoid receptor (GR) messenger RNA (mRNA) was investigated in sc adipose tissue and isolated adipocytes from the abdominal and gluteal regions in men and women using a human GR complementary RNA probe. GR mRNA levels were 2-fold higher in female than in male abdominal tissue or adipocytes, whereas in gluteal tissue or adipocytes no sex differences were observed. GR mRNA levels in female abdominal adipocytes were 50% higher than in corresponding female gluteal adipocytes; the opposite was observed in males. Northern blot analysis of total cellular RNA isolated from abdominal and gluteal adipocytes showed hybridization of the human GR probe to an RNA species of approximately 7.1 kilobases in both regions. No sex or regional differences in GR mRNA stability were observed. The human metallothionein II (hMTII) mRNA, which is regulated by glucocorticoids at the transcriptional level, showed an opposite sex and regional pattern as GR mRNA. However, in gluteal adipose tissue no sex differences were observed in hMTII mRNA levels. The expression of β-actin mRNA, which is not regulated by glucocorticoids, showed no sex or regional variation. By immunocytochemistry, using an anti-GR-monoclonal antibody, cytoplasmic as well as nuclear staining for GR was demonstrated in both sexes and both regions. In conclusion, variations in GR mRNA levels between sexes and body regions may explain the well known sex and tissue differences in effects of glucocorticoids on human adipose tissue. (*Endocrinology* **127: 1689–1696, 1990**)

THE DISTRIBUTION of body fat differs between men and women. Males tend to accumulate fat in the abdominal area, whereas females are prone to deposit fat in the gluteal-femoral area. Epidemiological studies have strongly indicated the importance of abdominal fat deposition as a risk factor for cardiovascular disease and type II diabetes mellitus, whereas fat localized to the gluteal-femoral region does not correlate to these diseases (1, 2). The mechanisms behind regional adipose tissue distribution are unknown. Whether fat cells in different areas originate from the same or separate cell populations is not known. However, it is well established that there exist marked differences in metabolic activity between different regions of adipose tissue, suggesting that human adipose tissue may not be a uniform tissue (2–4). In particular, the rates of synthesis and breakdown (lipolysis) of adipocyte triglycerides differ between adipose regions. This may be important for regional variations in fat deposition, because triacylglycerol constitutes more than 95% of the total fat cell volume. β-Adrenergic receptors and lipoprotein lipase may play key roles in this respect, because these proteins are major regulators of triglyceride turnover in human fat cells (2–4). Catecholamines, which are the major lipid mobilizing hormones, stimulate lipolysis through β-adrenoceptors, whereas lipoprotein lipase regulates the uptake of circulating triglycerides by fat cells. Indeed, variations in the number of β-adrenoceptors and in the activity of lipoprotein lipase appear to be major factors behind regional variations in lipid mobilization and storage by subcutaneous fat cells (5, 6). In addition, regional variations in the properties of the insulin receptor may explain why the action of insulin on adipocyte metabolism also varies in different regions (7).

There is strong evidence that glucocorticoids play an important role in the regulation of adipose tissue metabolism and distribution (8, 9). Dexamethasone has been reported to alter lipid and glucose metabolism in adipocytes (10). Glucocorticoids also play important roles in the differentiation of adipocytes triggering the differentiation program of uncommitted adipoblasts into committed preadipocytes by regulating specific gene expression (11). Furthermore, patients with Cushing's syn-

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drome characteristically redistribute their fat deposits and show atrophy of peripheral fat and accumulation of central fat. Finally, glucocorticoids modulate the expression of several of the key proteins involved in the regulation of adipocyte triglyceride metabolism, such as insulin receptors, β-adrenergic receptors and lipoprotein lipase (12, 13).

The mechanisms by which glucocorticoids might influence adipose tissue metabolism in different regions are not known. Regional variations in glucocorticoid receptor (GR) concentration and/or in the expression of glucocorticoid regulated genes may be of importance for the metabolic differences observed. A previous report has indeed indicated that there exist regional differences in binding capacity of glucocorticoids between adipocytes from various human fat deposits in both sexes. Much higher binding to omental adipocytes than to sc abdominal fat cells was reported (14).

In addition to their effect on adipocyte metabolism it has been suggested that glucocorticoids participate in the pathogenesis of human hyperplastic obesity by converting a pool of precursor cells in human adipose tissue to adipocytes (15). This event is probably governed by the regulation of specific gene expression mediated via binding of GR complexes to specific gene sequences (11, 16). Interestingly, only steroid hormones exhibiting glucocorticoid activity have been shown to stimulate the adipose conversion process (15). We have presently investigated whether regional and sex differences exist in the expression of GR in sc adipose tissue. GR mRNA levels and the mRNA levels of the glucocorticoid dependent metallothionein II gene (hMTII) have been compared in abdominal and gluteal adipose tissue segments as well as in isolated adipocytes obtained from nonobese men and women. In addition, anti-GR-antibodies were used to demonstrate the presence of the GR protein in these two regions.

Materials and Methods

Subjects

Eleven female and 11 male nonobese and healthy volunteers were investigated. They were all drug free. None of the women took oral contraceptives over the last 6 months. The age of the females was 24–55 yr (mean ± SE: 34 ± 3 yr). The age of the males was 26–54 yr (37 ± 2 yr). One woman was menopausal; all other women had regular menstruations and were investigated in the middle of their menstruation cycle. Body mass index (kg/m^2) was 23.1 ± 0.6 and 23.4 ± 0.4 in the female and male groups, respectively. The proportion of body fat assessed as described previously (6) was 34 ± 2% in the female group and 23 ± 1% in the male group ($P < 0.01$). The waist to hip ratio in females and males was 0.91 ± 0.01 and 0.94 ± 0.01, respectively ($P < 0.05$). On the day preceding the investigation all subjects collected urine for 24 h. After the subjects had an overnight fast a venous blood sample was obtained, and sc fat specimens were excised from the abdominal and gluteal areas as described in detail previously (6). The study was approved by the hospital's Ethics Committee, and informed consent was obtained.

Cortisol determinations

Cortisol in urine and plasma was measured by RIA at the hospital's routine clinical chemistry laboratory.

Adipose tissue preparations

One or two pieces (about 50 mg) of adipose tissue were stored at room temperature in formaldehyde to be used for immunohistochemistry. The remaining tissue was used for molecular biology studies and was stored at −70 C either as intact pieces (25–50 mg) or as isolated fat cells, which were prepared as described (6).

Immunocytochemical studies of GR

Biopsy samples were taken from the abdominal and gluteal regions of healthy humans by means of a needle biopsy. The samples were fixed in formaldehyde (2%), embedded in paraffin, cut by a microtome attached onto glass slides, and deparaffinized in graded ethanol solutions, followed by rehydration in PBS. We used a monoclonal mouse-antirat GR immunoglobulin G2a (IgG2a) antibody designated mab 7 (17) in ascites, diluted 1/100. This antibody has been tested in our laboratory for monospecificity and cross-reacts with the human GR but not with other steroid hormone receptors such as the progesterone and the estrogen receptor (18). Control stainings where PBS substituted for mab 7 were always negative. The second and third antibodies were from Vector Laboratories (Burlingame, CA) Vectastain ABC-kit, containing a biotinylated goat-antimouse Ig as second antibody and a macromolecular complex between avidin and biotinylated horseradish peroxidase as the third component, both in dilutions recommended by the manufacturer.

All incubations and stainings were performed at room temperature, and the whole staining procedure was carried out in sequence during the same day. After incubation for 15 min with 3% (vol/vol) normal rabbit serum (NRS), the samples were incubated with mab 7 at an optimal dilution of 1/100 in PBS/1% (vol/vol) NRS for 60 min followed by two 10-min washes in PBS. The second biotinylated goat-antimouse Ig was added and incubated for another 60 min followed by two 10-min washes in PBS. Then the macromolecular complex between avidin and biotinylated horseradish peroxidase was added for 45 min. The peroxidase substrate solution was incubated for 15 min followed by two 10-min washes in distilled water. The samples were mounted in 50% (wt/vol) glycerol in PBS. The immunostained lipocyte samples were studied by a Nikon Labophot light microscope (Nikon Inc., Garden City, NY) using conventional transmitted light technique.

The staining was visualized by using a peroxidase substrate solution made up of 0.02% (wt/vol) 2-amino-9-ethyl-carbazole, 5% (wt/vol) *N'-N*-diethylformamide, 0.015% (wt/vol) hydrogen peroxide in 50 mM aetate buffer, pH 5.0. The buffer used

expression when compared to gluteal fat cells.

The hMTII gene is known to be positively regulated by both glucocorticoids and progesterone (20, 33). However, in spite of significantly higher GR mRNA expression in female as compared to male abdominal adipocytes, male as compared to female abdominal adipocytes showed a 2-fold higher expression of hMTII mRNA. One explanation for this might be that the hMTII gene in abdominal adipocytes is differently regulated in men and women.

In cell lines that contain functional glucocorticoid hormone receptors, MCF-7 and HeLa, it has been shown that treatment with dexamethasone results in increased levels of MTII mRNA (33). Only in cell lines containing progesterone receptors, such as MCF-7 and T47D, does treatment with progesterone result in increased levels of hMTIIA mRNA. Interestingly, in the HeLa cell line that contains only GR, there is a decrease in the level of hMTIIA mRNA when dexamethasone and progesterone are added together (33). Thus, the lower expression of hMTIIA mRNA in female as compared to male abdominal adipocytes could be explained by a competition of progesterone, whose receptor is not present in the adipocyte, with dexamethasone for the GR. Of course, other more complicated explanations are also plausible.

The influence of region and sex on adipose expression of the GR gene as well as of a glucocorticoid regulated gene (hMTII) may have implications for clinical medicine. For example, the enlargement of abdominal but not gluteal fat deposits in cases of excess levels of glucocorticoids in blood as in Cushing's disease (36) may be explained by regional variations in GR expression and glucocorticoid controlled gene expression. Furthermore, it has been suggested that metallothioneins, which are low molecular weight, highly inducible, heavy metal metabolism binding proteins, serve in the regulation of intracellular Zn^{++} metabolism. Among the Zn^{++} requiring systems are several enzymes involved in DNA replication and repair. Therefore, during periods of active DNA synthesis there is likely to be an increased demand for Zn^{++}, which could be met by elevated metallothionein synthesis (37). During pregnancy, when DNA synthesis is active and progesterone levels are elevated, the embryonic levels of metallothionein are induced. However, neither the functional significance of the progesterone induction of hMTII gene expression nor the exact physiological role of metallothionein are clear (38). In addition, the mechanisms by which sex steroid hormones might influence adipose tissue metabolism and interact with the glucocorticoid receptor system in different regions need to be further evaluated.

In summary, the present study shows marked regional and sex variations in adipocyte GR and metallothionein gene expression, possibly indicating that the biological effects of different steroid hormones such as glucocorticoids and progesterone might differ between male and female abdominal adipocytes. The variations in GR mRNA between fat cells from different fat deposits may be involved in regional variations of adipose depositions between sexes and during glucocorticoid excess.

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marcations in all directions. The nuclear staining was usually much weaker than the cytoplasmic, but in some cells (figure not shown), there was intense nuclear staining surrounded by a cytoplasmic staining of the same intensity or less. In these cells, endogenous cortisol might have mediated an increased nuclear translocation of GR. The major central part of the cell where the triglycerides are stored remained unstained.

The staining intensity varied a lot between different subjects and also between the abdominal and the gluteal areas in the same subject, however, without consistent differences in any direction. In some cells the GR staining was intensely strong, whereas in other cells nearby the intensity could be much weaker. The average staining intensity also exhibited similar large variations between different subjects. These differences were seen even though all samples were stained during the same day, using the same reagents and exposure times to antibodies and peroxidase substrate solutions. Although the observed variations might reflect *in vivo* differences in GR content between different subjects and various areas of the body, no attempts were made to directly quantitate the immunohistochemical results, because the method is not quantitative.

Cortisol determinations

There were no significant sex differences in the plasma or urinary levels of cortisol. The concentration of cortisol in plasma of men and women was 378 ± 23 and 450 ± 47 nmol/24 h, respectively. The corresponding values in urine were 112 ± 42 and 51 ± 7 nmol/24 h. GR mRNA levels measured did not correlate with cortisol in plasma or urine.

Discussion

The most prominent finding in our study is the marked regional variation in GR gene expression between adipocytes of various tissue localization. In women 50% higher GR mRNA levels were found in abdominal adipose tissue as compared to gluteal adipose tissue. This ratio was reversed in men, who had 50% lower GR mRNA values in abdominal as compared to gluteal adipose tissue. The difference in GR expression between the two regions is further emphasized by the fact that the GR mRNA levels were almost identical in the gluteal region of men or women. The site variations in GR mRNA expression are most likely real, because the apparent half-life for GR mRNA was similar in both regions. Unfortunately, it is not possible at present to directly measure gene transcription rate in freshly isolated human adipocytes.

It is unknown at present if our findings are directly related to regional variations in GR protein content. However, previous studies have shown a strong correlation between mRNA and protein levels for GR in other cell systems (25–27). In order to further address this issue we visualized GR in abdominal and gluteal fat cells of both sexes using immunohistochemistry. Unfortunately, the latter technique does not allow a quantification of the receptor protein content. Some controversy exists as to what extent steroid hormone receptors such as the GR are located in the cytoplasm or in the nucleas of cells (30). The present immunohistochemical method showed significant staining of GR in both cellular compartments of the adipocyte. Although no apparent sex or regional variation with regard to GR localization was observed in these immunohistochemical studies, they confirmed the presence of GR protein in adipocytes.

Some of the sex differences in GR mRNA expression observed in human adipocytes might be explained by the action of sex hormones upon the glucocorticoid receptor system (8). Pfeiffer and Barden (31) have shown that estrogens indeed act directly at the level of gene transcription to modify GR mRNA content of anterior pituitary gland in rat. Sex-related differences have also been found for GR affinity, binding capacity, and nuclear translocation in rat brain, and recently Turner and Weaver (32) have shown that ovariectomy increases the number of corticosterone binding sites in hypothalamus. Furthermore, it is known that the glucocorticoid and progesterone receptors bind to the same specific DNA sequences within GR-regulated genes (33, 34) and that progesterone may bind to the glucocorticoid receptor (35). Interestingly, both the progesterone and the estrogen receptor seem to be absent or expressed in extremely low quantities in human adipocytes (Rebuffé-Scrive M, M. Brönnegård, A. Nilsson, J. Eldh, J.-Å. Gustafsson, and P. Björntorp, submitted for publication). It is therefore possible that sex hormones, either directly or indirectly via control of glucocorticoid receptor synthesis and bioavailability, might modulate GR concentrations.

The sex and regional differences in gene expression are apparently not found for all genes. Thus, mRNA for β-actin, which is a gene that is not influenced by glucocorticoids, showed the same level in both regions and both sexes. The present data also suggest that GR and hMTII gene expression is influenced by sex only in the abdominal area, whereas both the GR and hMTII mRNA levels differed by a factor of two between males and females. In the gluteal area of men and women, however, mRNA levels for both genes were in the same order of magnitude. These results are in consonance with recent data showing similar lipolytic activity in gluteal adipocytes of men and women but higher lipolytic activity in female as compared to male abdominal adipocytes (3, 6, 9). This indicates that abdominal adipocytes have different metabolic activity as well as different levels of gene

for dilution, washes, and as a substitute for primary antibodies was PBS composed of 137 mM NaCl, 8 mM Na_2HPO_4, 3 mM KCl, 1.5 mM KH_2Po_4, pH 7.35.

Determination of glucocorticoid receptor and metallothionein mRNA

For construction of the human GR probe, a 50-basepair (bp) double-stranded oligonucleotide corresponding to nucleotides 150–209 (or amino acid 10–26) in the human GR sequence (19) was synthesized and cloned into the PstI/HindIII sites in pGEM TM I. The human MT II probe was constructed in an identical way, the sequence of the oligonucleotide used corresponding to nucleotides 406–456, second exon (20). The sequences of these inserts were confirmed by DNA sequencing. Vectors and reagents required for *in vitro* synthesis of cRNA and mRNA using the Sp6/T7 riboprobe system were obtained from Promega Biotech (Madison, WI).

For preparation of total nucleic acids (TNA), adipose tissue (250–500 mg) was homogenized in 1 × SET [1% sodium dodecyl sulfate (SDS), 20 mM Tris HCl, pH 7.5, and 10 mM EDTA] and digested with 10 mg/ml Proteinase K for 45 min at 45 C, after which the adipose tissue was phenol-chloroform extracted by the addition of isoamylic ethanol (vol 24:1), reextracted with chloroform, ethanol precipitated, dried, and dissolved in 0.2 × SET as described (21, 22). TNA concentrations were determined spectrophotometrically (1A at 260 nm equivalent to 50 μg TNA). For solution hybridization TNA samples were hybridized to approximately 20,000 cpm ^{35}S-cRNA probe in 40 μl 0.6 M NaCl, 20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 0.1% SDS, 1 mM dichlorotriphenyltrichloroethane, and 25% formamide at 68 C for 12–16 h, after which the hybridization samples were diluted with 1 ml 0.3 M NaCl, 10 mM Tris-HCl, pH 7.5, 2 mM EDTA, 100 μg/ml denatured salmon sperm DNA containing 40 μg RNase A, and 5.9 × 10^5 U RNase T_1. Digestion was performed for 45 min at 45 C, after which RNAse resistant RNA was precipitated with 100 μl 6 M CCl_3 COOH. Precipitates were collected for scintillation counting by filtration on Whatman GF/C filters (Whatman Inc., Clifton, NJ).

The amount of GR and MT II mRNA of a sample was calculated from a linear standard curve constructed from incubations with known amounts of *in vitro* synthesized mRNA, complementary to the ^{35}S-labeled probe. A standard curve (3–3000 × 10^{-16} mol mRNA/incubation) was included in each assay, and each TNA sample was analyzed in triplicate. For each specific probe, the corresponding amount of sample mRNA was calculated from the linear part of the respective standard curve. Results are expressed as attomoles (amol) mRNA/μg TNA calculated from the standard curve and spectrophotometric measurements. RNase resistant counts per min were less than 3% of input counts per min.

Northern blot analysis

Northern blot analysis was performed using total RNA from adipose tissue, which was prepared by the acid guanidinium thiocyanate method combined with the $CsCl_2$ centrifugation procedure (23). The integrity of RNA samples was verified after gel electrophoresis by ethidium bromide staining. Northern blot hybridizations were performed essentially as described by Maniatis *et al.* (24). Nylon membranes (Hybond, Amersham Corp, Buckinghamshire, England) to which RNA had been transferred were prehybridized for 2 h at 55 C in 50% formamide, 5 × SSC (1.0 M NaCl, 0.1 M sodium citrate), 5 × Denhardt's solution (0.02% Ficoll, 0.02% polyvinyl pyrrolidone, and 0.02% BSA), 5 mM phosphate buffer (pH 6.5), 5 mM EDTA, 0.1% SDS, and 200 μg/ml salmon testis DNA. Hybridization was carried out for 48 h at 55 C in an identical solution containing 4–6 × 10^6 cpm/ml ^{32}P-labeled cRNA probe. After hybridization, the filter was washed serially with 0.1 × SSC and 0.1% SDS at 68 C to eliminate nonspecific binding of the probes to the filter and ribosomal RNA. Autoradiographs were obtained by exposure to Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY) with an intensifying screen for 72 h at −70 C.

Measurement of RNA stability

The stability of GR mRNA was measured by actinomycin D chase. Pieces (10–15 mg) of abdominal and gluteal adipose tissue were incubated at 37 C in Krebs Ringer phosphate buffer (pH 7.4) containing dialyzed bovine albumin (2%) with or without actinomycin D (5 μg/ml). TNA was isolated from the tissue samples at 0, 2, 4, and 6 h of incubation, and TNA solution hybridization was performed to determine mRNA levels, as described above.

Statistical methods

Values are mean ± SE Student's paired or unpaired *t*-test, analysis of variance, and linear regression analysis were used to evaluate the results statistically.

Results

mRNA measurements

In the present study we have used RNA probes derived from synthetic oligonucleotides (50 bases), for the detection and quantitation of specific mRNA species. To ensure that these probes detect the correct transcripts, initial experiments using Northern blot analysis were carried out. As shown in Fig. 1, the hGR probe used hybridized to a single RNA species of 7.0–7.1 kilobases in both abdominal and gluteal adipose tissue. A GR mRNA species of similar size has previously been demonstrated in other human tissues (25).

To facilitate further studies of hGR mRNA in human adipose tissue, a solution hybridization assay was used. We find this procedure advantageous when analyzing gene expression in human tissues, because the assay is sensitive and requires small amounts of total cellular RNA. In addition, significant correlations have been established between the levels of steroid hormone receptor, as measured by radioligand binding, and the concentrations of steroid hormone receptor mRNA (26, 27).

Steady state levels of GR mRNA could be detected in

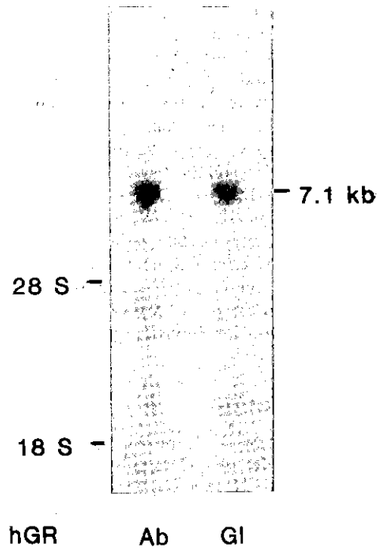

FIG. 1. Northern blot analysis of RNA from human adipose tissue. Thirty micrograms of total RNA was prepared from abdominal (Ab) and gluteal (Gl) adipose tissue, subjected to RNA blot analysis and probed with a ^{32}P-labeled GR cRNA probe as described in *Materials and Methods*. Positions of RNA markers are indicated.

both female and male adipose tissue regardless of the origin of the sample (Table 1). GR mRNA levels in gluteal adipose tissue were similar in both sexes, whereas GR mRNA levels in abdominal adipose tissue were 2-fold higher in women compared to men ($P < 0.025$). In women, abdominal adipose tissue expressed 50% more GR mRNA than gluteal adipose tissue ($P < 0.01$), whereas the opposite was observed in men ($P < 0.01$). Table 2 shows the recovery of total nucleic acids and GR mRNA from different adipocyte preparations.

In order to exclude the influence of other cell types in analysis of GR mRNA levels in adipose tissue we also performed analysis of GR mRNA expression in isolated adipocytes. As shown in Table 1, the regional differences obtained in adipose tissue correlated well with corresponding regional differences in GR mRNA levels in isolated adipocytes. There was a tendency of slightly higher GR mRNA values obtained in isolated adipocytes as compared to segments of adipose tissue. These differences, however, were not statistically significant. The regional and sex variations of GR mRNA were the same whether mRNA was expressed per amount of TNA or as molecules per cell (Table 1).

Incubation of adipose tissue pieces from the abdominal and gluteal regions and from both sexes with or without the RNA polymerase inhibitor actinomycin D were undertaken in order to evaluate GR mRNA stability. Figure 2 shows the time-dependent decay of GR mRNA levels in abdominal and gluteal male adipose tissue, indicating an apparent half-life of 6 h in both regions. Similar results were obtained in corresponding female adipose tissue and with isolated adipocytes that were incubated with actinomycin D for the same length of time (data not shown). This method may not be sufficient for evaluating true mRNA half-life, because actinomycin D may itself influence the results. However, there are no better methods yet developed designed for determination of RNA half-life in human fat cells. Other exact methods for determination of mRNA half-lives, such as pulse-chase analysis (28), require incubation of adipocytes for longer periods than 6 h and can only be applied to adipocyte cultures. However, previous investigations have indicated a GR mRNA half-life of approximately 6–11 h in other cell types (29). These values are similar to the present findings with actinomycin D. Thus, our data indicate that there are no regional or sex differences in stability of GR mRNA in sc adipose tissue.

Table 3 shows the basal expression of hMTII mRNA in adipose tissue. The hMTII gene is known to be positively regulated by glucocorticoids, progesterone, and heavy metals in most cells (20). Therefore, it was of interest to evaluate if this glucocorticoid regulated gene was differently expressed in the two adipose regions or if there existed any sex differences in its mRNA expression. hMTII mRNA levels in gluteal adipocytes were similar in both sexes, whereas hMTII mRNA levels in abdominal adipocytes were significantly higher in men compared to women ($P < 0.001$). In men, abdominal adipose tissue expressed significantly more hMTII mRNA than gluteal adipose tissue ($P < 0.05$), whereas the opposite was observed in women. In addition, the levels of hMTII mRNA in abdominal adipose tissue were two times higher in men than in women ($P < 0.01$). Similar significant results were obtained whether Student's t test or analysis of variance were used as statistical method.

In separate experiments (data not shown) the mRNA levels for the β-actin gene were determined in adipose tissue segments. This gene is not regulated by GR. No differences in β-actin mRNA between the regions or between the sexes were observed.

TABLE 1. Glucocorticoid mRNA expression in human adipose tissue

		TNA (amol/μg)		Molecules/cell	
		Adipose segments (n = 11)	Adipocytes (n = 8)	Adipose segments (n = 11)	Adipocytes (n = 8)
Men	Abdominal	30 ± 4[a]	48 ± 12[a]	7 ± 1[a]	10 ± 1[b]
	Gluteal	46 ± 5[c]	69 ± 12[c]	12 ± 2[c]	15 ± 1[c]
Women	Abdominal	67 ± 12	90 ± 12	14 ± 2	19 ± 2
	Gluteal	47 ± 9[d]	67 ± 10[c]	9 ± 2[d]	13 ± 3[d]

mRNA for GR was determined in adipose tissue segments of 11 men and 11 women. In 8 women and 8 men GR mRNA was determined in isolated adipocytes as well. Student's paired t test was used for statistical comparison of abdominal vs gluteal within sexes; Student's unpaired t test was used for statistical comparison of gluteal or abdominal values between sexes. mRNA was expressed as amol GR mRNA per amount of TNA or as molecules per cell. Values are mean ± SE.

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.01$.
[d] $P < 0.05$.

TABLE 2. Recovery of glucocorticoid receptor mRNA and total nucleic acids in human adipose segments

		GR mRNA (amol)	TNA (μg)
Men	Abdominal	908 ± 95	75 ± 22
	Gluteal	1226 ± 158	76 ± 15
Women	Abdominal	1667 ± 106	78 ± 19
	Gluteal	1198 ± 126	74 ± 16

GR mRNA levels were determined by solution hybridization in segments of adipose tissue of eight men and eight women. Recovery of TNA was determined spectrophotometrically. Values are mean ± SE.

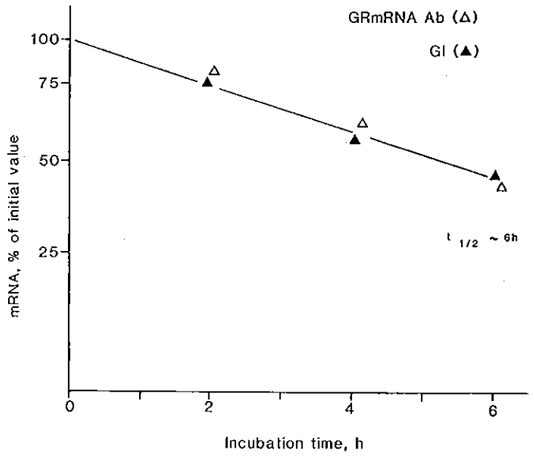

FIG. 2. Half-life of GR mRNA in human adipose tissue. Tissue was incubated in the presence or absence of 5 μg/ml actinomycin D. TNA was extracted after 0, 2, 4, and 6 h of incubation, and mRNA levels determined by solution hybridization. The figure shows the mean values (in percent of the GR mRNA levels at the start time of actinomycin D treatment) from the results of two separate experiments.

TABLE 3. Metallothionein II mRNA expression in human adipose tissue

	TNA (amol/mg)	
	Abdominal	Gluteal
Men	1688 ± 229	1112 ± 235[a]
Women	805 ± 126	1322 ± 235[a]

mRNA was determined in segments of adipose tissue of five men and five women and related to total nucleic acids TNA. Abdominal and gluteal values were compared within sexes using Student's unpaired t test. Values are mean ± SE.

[a] $P < 0.01$.

Immunohistochemical studies

The typical GR-staining of normal human adipose tissue is shown in Fig. 3. GR was found predominantly in the perinuclear area with fairly sharp peripheral de-

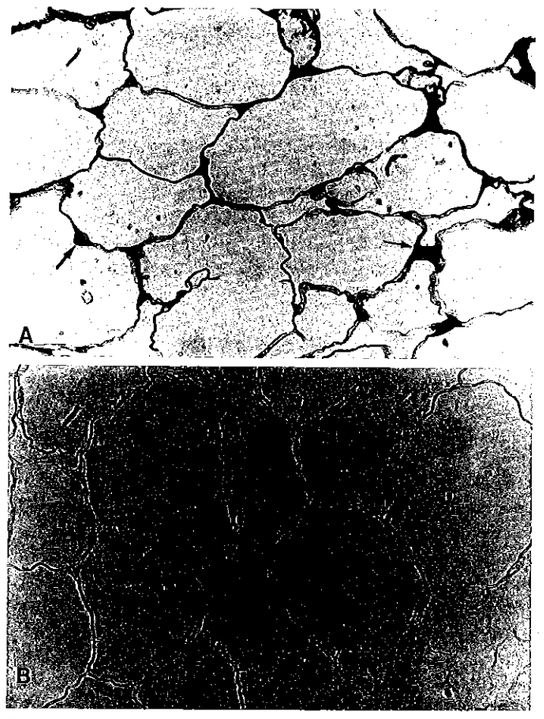

FIG. 3. GR staining in human adipose tissue. A, GR is mainly localized in the cytoplasm in close association with the nuclear membrane (*arrow*). In some cells the nuclei show strong staining intensity. The rest of the cytoplasm is essentially unstained. B, Negative control where PBS replaced the first antibody (monoclonal anti-GR antibody).