Stimulation of Leptin Release by Arachidonic Acid and Prostaglandin E_2 in Adipose Tissue From Obese Humans

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The purpose of this study was to examine the effect of arachidonic acid and its metabolites on leptin formation by explants of human adipose tissue over a 48-hour incubation in primary culture. We found that arachidonic acid or prostaglandin E_2 (PGE_2) stimulated leptin release by explants of subcutaneous adipose tissue from obese humans. The stimulatory effect of arachidonic acid on leptin formation was blocked by NS-398, a cyclooxygenase-2 (COX-2) inhibitor. There was appreciable release of PGE_2 to the medium over 48 hours, and this was inhibited by 99% in the presence of 200 nmol/L dexamethasone or 5 μmol/L NS-398. The increase in PGE_2 release correlated with induction of COX-2 activity during the 48-hour incubation. The increase in COX-2 activity was blocked by 200nmol/L dexamethasone. The level of leptin mRNA at 48 hours was reduced by 28% if PGE_2 was added in the absence of dexamethasone, while in the presence of dexamethasone, the amount of leptin mRNA was enhanced by 156%. These data suggest that when upregulation of COX-2 is blocked by dexamethasone, exogenous PGE_2 enhances both leptin release and leptin mRNA accumulation by explants of human adipose tissue in primary culture.

THE FACTORS THAT regulate leptin are not well understood. There is general agreement that glucocorticoids stimulate leptin synthesis and that inhibitory effects of catecholamines on leptin synthesis by explants of human adipose tissue can be seen in primary culture.[1] Because the fasting state results in decreased leptin release and the fed state in an enhanced leptin release, it has been presumed that insulin is a potent stimulus for leptin formation. However, both stimulatory and inhibitory effects of insulin have been reported on leptin synthesis by human adipose tissue in primary culture.[2-5] The present studies were designed to investigate other possibilities for the regulation of leptin formation by cut pieces of human adipose tissue incubated in primary culture for 48 hours.

The initial impetus for the present studies was our finding that arachidonic acid is a potent stimulator of leptin release by explants of human adipose tissue in primary culture. One mechanism by which arachidonic acid could stimulate leptin release is by conversion to prostanoids such as prostaglandin E_2 (PGE_2).[6] It is possible that the inhibition by PGE_2 of lipolysis affects leptin release since PGE_2 inhibits lipolysis by human adipose tissue.[6] The present report indicates that PGE_2 can stimulate leptin release by human fat in primary culture, and that the effects of arachidonic acid on leptin release are secondary to enhanced formation of prostanoids via cyclooxygenase-2 (COX-2).

SUBJECTS AND METHODS

Subjects

Subcutaneous abdominal adipose tissue was obtained from 29 obese females and 4 obese males undergoing elective open abdominal surgery (gastric bypass) under general anesthesia after an overnight fast. The mean body mass index (BMI) was 49 ± 1.3 (mean ± SEM). The average age of the patients was 39 ± 1.2 years (mean ± SEM). Fasting blood glucose averaged 5.4 ± 0.2 mmol/L. The fasting plasma insulin averaged .15 ± .02 nmol/L (mean ± SEM).

Each experimental replication involved tissue from a different individual. The study had the approval of the local ethics committee, and all patients involved gave their informed consent. The patients were fasted overnight before surgery, but had not been on any type of dietary restriction just prior to surgery.

A total of 10 to 20 g of abdominal subcutaneous adipose tissue was placed in saline at 37°C and immediately (5 to 10 minutes) transported to the laboratory. The tissue was cut with scissors into small pieces (20 to 30 mg). The explants of adipose tissue (400 mg) were incubated in 50 mL polypropylene tubes containing 5 mL of incubation buffer in an upright position in a gyratory water bath shaker for 48 hours (100 rpm). The buffer for incubation of adipose tissue was Dulbecco's modified Eagle's medium/Ham's F12 (1:1, Sigma No. 2906; Sigma-Aldrich, St Louis, MO) as described by Fain et al.[7] The pH of the buffer was adjusted to 7.4, and the buffer was then filtered through a 0.2 μm filter. The adipose tissue was collected, cut, and incubated under sterile conditions with air as the gas phase.

Human adipocytes were isolated by digestion of cut pieces of human adipose tissue (1 g/mL) with bacterial collagenase (0.66 mg/mL) in buffer containing albumin for 1hour in a gyratory water bath shaker (40 rpm). The adipocytes were separated from undigested tissue by filtration through 250 μm Nylon mesh fabric. There was some lysis of adipocytes during digestion, and the lipid on top of the intact adipocytes was removed, as well, at each washing step. The cells were subjected to 4 cycles of washing and centrifugation. The same weight of adipocytes was added to the tubes as was used for incubations with tissue intact. The yield of lysed and intact adipocytes based on lipid content was 67% from obese subjects (average of 16 different isolations), and the approximate yield of unbroken adipocytes after 4 cycles of washing and centrifugation was 50% of the tissue weight. The isolated adipocytes were incubated under the same conditions as the tissue except that due to their fragility, they were shaken at 40 rpm in the water bath.

The leptin content of 20 to 50-μL aliquots of the incubation medium was determined using radioimmunoassay kits from Linco Research, St Charles, MO. Lipolysis was based on analysis of glycerol release into the medium and determined on 20 to 50-μL aliquots as described by

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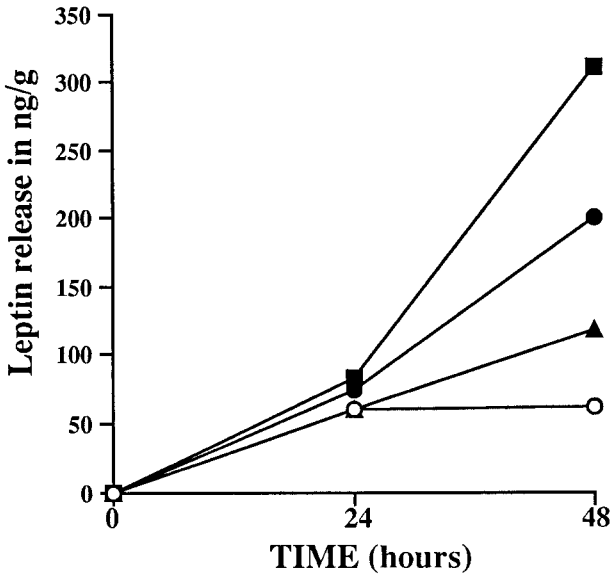

Fig 1. Time course for stimulation of leptin release by human adipose tissue. Fragments of human subcutaneous adipose tissue (approximately 400 mg) were incubated for 48 hours in 5 mL of medium either without (○), with 200 nmol/L dexamethasone (●), dexamethasone + 0.1 μmol/L PGE_2 (■) or dexamethasone plus 1 μmol/L NS-398 (▲). The values are shown as the means of 2 paired experimental replications

Fain et al.[7] PGE_2 was measured using 2 to 50 μL-aliquots of the medium or of tissue homogenates by the procedure of Parfenova et al.[8] Prostacyclin formation, because of its short half-life, was measured by the accumulation of its primary metabolite 6-keto $PGF_{1\alpha}$.[8]

COX-2 activity was measured by the formation of PGE_2 in homogenates during a 1-hour incubation in the presence of 20 μmol/L arachidonic acid. The original medium was removed, the tissue (300 to 400 mg) was then resuspended in 5 mL of the fresh incubation buffer and homogenized using a Polytron. The homogenate was incubated for 1 hour at 37°C. Aliquots of the medium were removed for PGE_2 analysis at the start of the incubation and at 60 minutes. The aliquots were added to tubes containing 10 μmol/L indomethacin to stop further COX-2 activity. PGE_2 was measured on 5 to 50 μL aliquots of homogenate.

In the studies in which the amount of COX-1 and COX-2 were determined by Western blotting, the adipose tissue was homogenized with a Polytron in 0.5 mL of lysis buffer containing: 40 mmol/L Tris-HCl, pH 8.0, 274 mmol/L NaCl, 2 mmol/L EGTA, 2% Triton X-100, 20% glycerol, 3 mmol/L $MgCl_2$, 10 μg/mL of aprotinin, 20 μg/mL of leupeptin, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 μmol/L sodium vanadate, and 50 mmol/L sodium fluoride. Protein was determined by the method of Bradford,[9] and 15 μg of protein/lane of each extract were subjected to polyacrylamide electrophoresis followed by Western blotting with antibodies against COX-2 (goat polyclonal from Santa Cruz, Santa Cruz, CA or COX-1 rabbit polyclonal from Cayman, Ann Arbor, Ml) as described by Bahouth.[10]

Total RNA was extracted from adipose tissue at the start and at the end of the incubation.[11,12] Leptin mRNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were analyzed simultaneously by Northern blot analysis using a ^{32}P-labeled mouse leptin cDNA probe and a human GAPDH cDNA probe. It was necessary to increase the sensitivity of the Northern blot analysis by the inclusion of ULTRAhyb (Ambion, Austin, TX) in the hybridization buffer. The radioactivity in leptin mRNA in each experiment was corrected for recovery of 18S RNA and also expressed as the ratio of cpm recovered in leptin mRNA divided by that in GAPDH mRNA.

Bovine serum albumin powder (Bovuminar, lot L59410) was obtained from Intergen (Purchase, NY). PGE_2 and NS-398 were obtained from Cayman. Stock solutions of PGE_2 were prepared in 100% ethanol. PGE_2 was added in a volume of 5 μL to 5 mL of medium just before the addition of adipose tissue, and an equal volume of ethanol was added to controls. Other chemicals were from Sigma-Aldrich. All agents were added at the start of the 48-hour incubation period.

The effects of added agents are generally shown as the percentage change from the incubation control in each experiment. This transformation resulted in a more normal distribution of the data since the basal value for leptin release at 48 hours was quite variable. Statistical comparisons were made using Student's *t* test on the paired differences.

RESULTS

PGE_2 enhanced leptin release by explants of human adipose tissue over the second 24 hours of a 48-hour incubation in the presence of 200 nmol/L dexamethasone (Fig 1). NS-398, which is a specific inhibitor of COX-2,[13] inhibited leptin release between 24 and 48 hours in the presence of 200 nmol/L dexamethasone (Fig 1). Little effect of either PGE_2 or NS-398 was seen during the first 24 hours of the 48-hour incubation (Fig 1). The lag period of around 24hours before the release of leptin to the medium was affected by dexamethasone was comparable to what we have previously reported.[7] Maximal stimulation of leptin release was seen with 2 μmol/L PGE_2 in the presence of dexamethasone (Fig 2). A significant stimulation of leptin release (43%) was seen with 0.1 μmol/L PGE_2 (Fig 2).

Because PGE_2 stimulated leptin release, the question arose

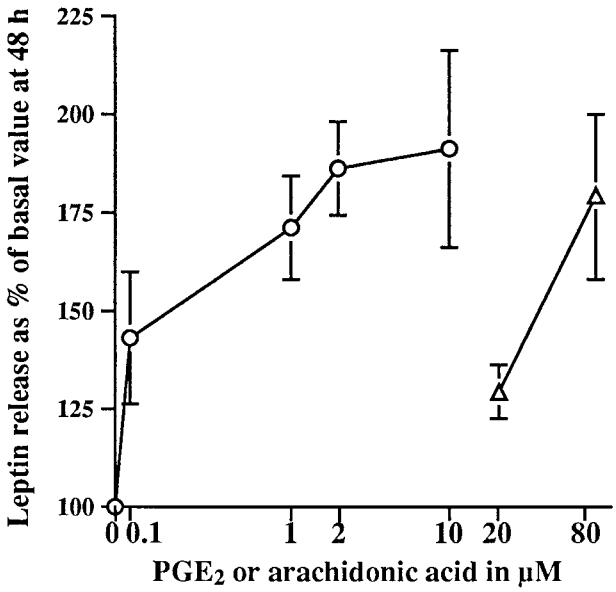

Fig 2. Stimulation of leptin release by arachidonic acid and PGE_2. Fragments of adipose tissue (450 mg) were incubated for 48 hours in the presence of 200 nmol/L dexamethasone. The values are the means ± SEM of 14 paired experimental replications for PGE_2 (○) and 7 for arachidonic acid (△). The basal release of leptin at 48 hours was 155 ng/g of adipose tissue. All effects of PGE_2 or arachidonic acid were statistically significant: $P < .05$.

whether exogenous arachidonic acid, which is a precursor of PGE_2, could increase leptin release by explants of human adipose tissue incubated for 48 hours. The addition of 20 μmol/L arachidonic acid resulted in a 30% increase in leptin release in the presence of 200 nmol/L dexamethasone, while a 80% increase in leptin release was seen with 80 μmol/L arachidonic acid (Fig 2). A 45% increase in leptin release was seen with 0.1 μmol/L PGE_2, while 2 μmol/L PGE_2 resulted in a 90% increase in leptin release (Fig 2).

How much arachidonic acid is released during a 48-hour incubation of adipose tissue? Basal lipolysis in the presence of 200 nmol/L dexamethasone was approximately 6.2 ± 0.6 μmol of glycerol/g of fat (mean ± SEM for adipose tissue from 14 subjects over a 48-hour incubation) in the experiments shown in Fig 3. The arachidonic acid content of human fat has been reported to be between 0.4% to 0.6% of total fatty acids.[14,15] This would result in a release of sufficient arachidonic acid to the medium to give a final concentration of 7.4 μmol/L in the medium if none was re-esterified. Another question is whether PGE_2 formation is enhanced in the presence of added arachidonic acid. At least 0.51% ± 0.11% (mean ± SEM for 7 paired experiments) of added arachidonic acid (20 μmol/L) was converted to PGE_2 over a 48-hour incubation of human adipose tissue in the absence of dexamethasone since the final concentration of PGE_2 was 180 nmol/L in the absence of arachidonic acid and 315 nmol/L in the presence of 20 μmol/L arachidonic acid. Our results indicate that the addition of 20 μmol/L arachidonic acid to explants of human adipose tissue produced a 74% ± 16% increase over basal in the PGE_2 content of the medium at 48 hours in these 7 experiments.

We found very little PGE_2 in human adipose tissue at the start of a 48-hour incubation (less than 10 pmol/g of fat). In 4 experimental replications, we measured PGE_2 formation at both 24 hours and 48 hours and found that only 26% ± 4% of the total accumulation of PGE_2 was seen at 24 hours. We also found that the amount of PGE_2 present in the tissue after 48 hours was 28% ± 2% (n = 11, mean ± SEM) of that released to the medium. PGE_2 formation at 48 hours averaged 2,230 ± 250 pmol/g of fat (n = 16 different experimental replications, mean ± SEM).

In a different experimental design, we examined the question of whether the marked reduction in PGE_2 release due to dexamethasone that was seen over a 48-hour incubation could also be seen with respect to the COX activity of homogenates at 48 hours. The COX activity of homogenates made from cut pieces of human adipose tissue both at the beginning and after a 48-hour incubation was measured in the presence of 20 μmol/L arachidonic acid. In tissue homogenates taken at the start of the

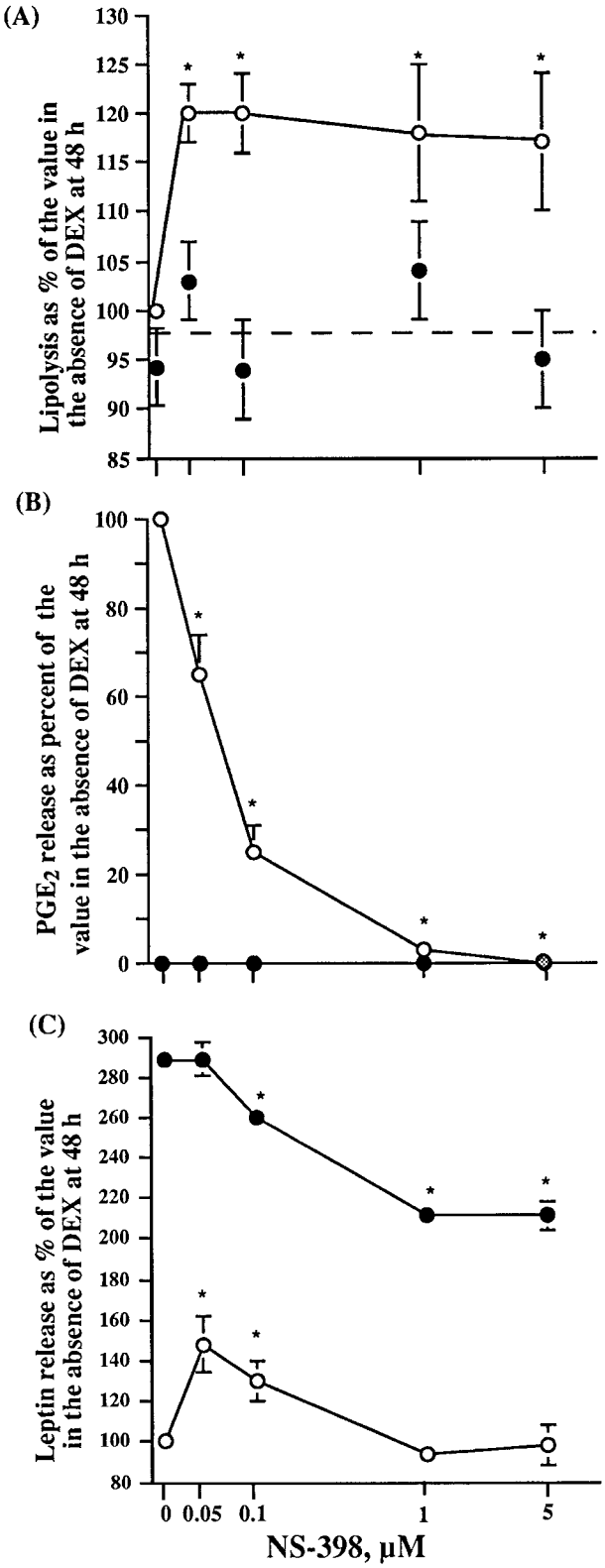

Fig 3. Effect of the COX-2 inhibitor NS-398 on lipolysis, leptin release and PGE_2 release by human adipose tissue. Fragments of human subcutaneous adipose tissue (average of 390 mg) were incubated for 48 hours in 5 mL of medium either without (○), with 200 nmol/L dexamethasone (●), and the indicated concentrations of NS-398. The values are shown as the means ± SEM of 14 paired experimental replications and are shown as percentage of the 48-hour value for tissue incubated in the absence of dexamethasone. The release of leptin in the absence of dexamethasone at 48 hours was 121 ± 20 ng/g of fat. Basal lipolysis in the absence of dexamethasone was 6.6 ± 0.4 μmol/g of fat. The basal value for PGE_2 release was 2.2 ± 0.3nmol/g in the absence of dexamethasone. Statistically significant effects of NS-398 are indicated by an asterisk ($P < .05$ based on paired comparisons).

incubation, PGE_2 formation was 3 ± 3 pmol/g of tissue homogenate (mean ± SEM of 4 experiments). In contrast, after a 48-hour incubation of fat, PGE_2 formation by the tissue homogenate was 85 ± 24 pmol/g over 1 hour in the absence of dexamethasone, but only 18 ± 11 pmol/g after a 48-hour incubation in the presence of 200 nmol/L dexamethasone. These data suggest that the incubation of human fat for 48 hours results in an upregulation of COX activity, and this upregulation was markedly reduced (79%) by 200 nmol/L dexamethasone.

The question of whether COX-1 or COX-2 was responsible for the upregulation of COX activity seen during a 48-hour incubation was examined in the studies shown in Fig 4. We found both COX-1 and COX-2 protein at the end of a 48-hour incubation of explants of human adipose tissue, but no detectable amounts of COX-2 by Western blotting at the start of the incubations (Fig 4). The amount of COX-1 decreased over the 48-hour incubation, while there was a remarkable upregulation of the amount of COX-2 protein (Fig 4). The increase in the amount of COX-2 protein was abolished in the presence of dexamethasone (Fig 4), which is a well-established inhibitor of COX-2 upregulation.[16] Furthermore, the amount of COX-1 was little affected by dexamethasone (Fig 4). In the adipose tissue from this individual, we also measured the release of leptin and PGE_2 to the medium over the 48-hour incubation. The release of leptin was elevated almost 3-fold, and the release of PGE_2 was abolished by dexamethasone (Fig 4). These data indicate that the PGE_2 formation by explants of human adipose tissue is accompanied by an increase in the amount of COX-2 protein.

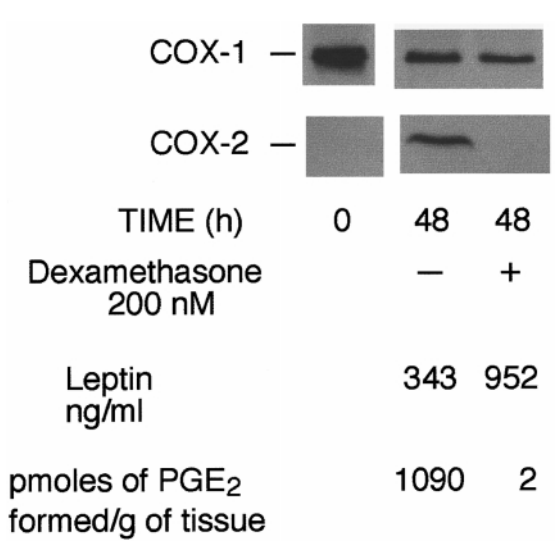

Fig 4. Western blotting of COX-1 and COX-2 activity in adipose tissue homogenates. Homogenates of adipose tissue explants (0.4 g) were made at the start and end of a 48-hour incubation both in the absence or presence of 200 nmol/L dexamethasone. Equal amounts of homogenate protein (15 μg) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting with polyclonal antibodies to COX-1 and COX-2. Detection of immunoreactive COX was determined by the ECL-chemiluminescence. The release of leptin and PGE_2 by the adipose tissue from this patient after 48 hours in culture in the absence and presence of dexamethasone is also shown in the figure.

Table 1. Comparison of Effects of Arachidonic Acid on Leptin Release in the Absence and Presence of NS-398

	Without	+ 20 μmol/L Arachidonic Acid	Percentage Change due to Arachidonic Acid
	Leptin Release in ng/g of Fat		
Basal	80 ± 7	114 ± 6	+42 ± 10
+ NS-398			
5 μmol/L	80 ± 11	76 ± 15	−5 ± 7

NOTE. The data are from pieces of human adipose tissue (380 mg) incubated in primary culture for 48 hours in the absence of dexamethasone. The effect of arachidonic acid (20 μmol/L) is shown as the paired percentage changes from basal in 7 paired experimental replications and was statistically significant in the absence of NS-398 ($P < .01$).

The upregulation of COX-2 activity was not required for the stimulation of leptin release in adipose tissue due to exogenous arachidonic acid, because those studies were performed in the presence of 200 nmol/L dexamethasone (Fig 2). There was a 42% increase in leptin release due to 20 μmol/L arachidonic acid in the absence of dexamethasone (Table 1). The increase in leptin release was apparently due to prostanoid formation, because it was blocked by 5 μmol/L NS-398, a specific inhibitor of the COX-2 enzyme[13] that converts arachidonic acid to PGE_2 and other prostanoids (Table 1).

We also examined PGE_2 formation in the presence of arachidonic acid and NS-398. PGE_2 formation by human adipose tissue incubated for 48 hours in the presence of 5 μmol/L NS-398 was 30 ± 6 pmol/g in the absence of arachidonic acid and unchanged by the presence of 20 μmol/L arachidonic acid (30 ± 6 pmol/g as the mean ± SEM of 7 paired experiments). These data demonstrate that the formation of PGE_2 from arachidonic acid by human adipose tissue requires COX-2 activity.

If endogenous prostanoids formed via COX-2 stimulate endogenous leptin release and inhibit lipolysis, then the inhibition of their formation by NS-398 should reduce leptin release and stimulate lipolysis. In fact, this is what we found in the presence of dexamethasone, in which 1 or 5 μmol/L NS-398 reduced leptin release by 27% (Fig 3). Lipolysis was stimulated by as little as 0.05 μmol/L NS-398, but only in the absence of dexamethasone, where it inhibited PGE_2 formation by 35% (Fig 3). It is unclear why leptin release was unaffected by 5 μmol/L NS-398 in the absence of dexamethasone. What is clear is that while 5 μmol/L NS-398 mimicked the inhibition by 200 nmol/L dexamethasone of PGE_2 release, it failed to affect leptin release.

In the next series of experiments, we examined the effect of PGE_2 on leptin mRNA accumulation after a 48-hour incubation of adipose tissue explants. The increase in leptin release by PGE_2 was greater in the presence of dexamethasone than in its absence (Fig 5). There was a 156% ± 19% increase ($P < .025$ based on paired comparisons) in leptin mRNA due to PGE_2 in the presence of dexamethasone as contrasted to a 28% ± 8% ($P < .005$) inhibition of leptin mRNA accumulation by PGE_2 in the absence of dexamethasone (Fig 5). The level of leptin mRNA after a 48-hour incubation of human adipose tissue averaged 51% of that present at the start of the incubation in the absence and 112% in the presence of dexamethasone (Fig 5).

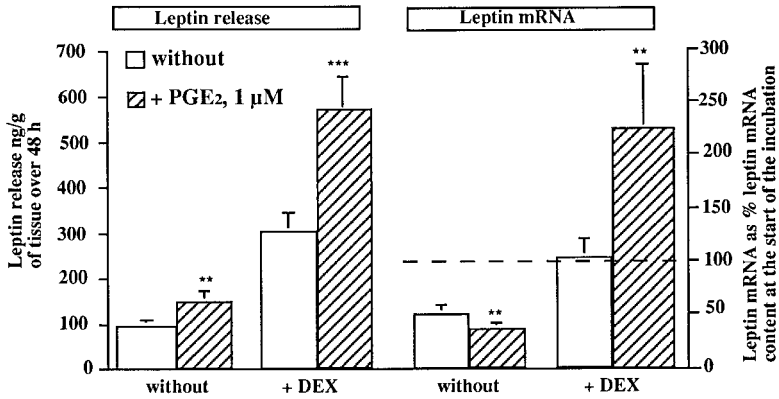

Fig 5. PGE_2 enhances leptin mRNA accumulation in the presence of dexamethasone. Fragments of human subcutaneous adipose tissue (≃ 400 ng) were incubated for 48 hours in 5 mL of medium either without or with 200 nmol/L dexamethasone in both the absence (□) and presence of 1 μmol/L PGE_2 (▨). Values are means ± SEM of 14 paired experimental replications. Statistical significance of the effects of PGE_2 was determined on the paired percentage differences: **$P < .01$, ***$P < .001$.

We also examined the effects of 1 μmol/L PGE_2 on the ratio of leptin mRNA/GAPDH mRNA, which was 0.3 in the absence and 0.7 in the presence of dexamethasone in the 14 experiments shown in Fig 5. There was no effect of PGE_2 on the ratio of leptin mRNA to that of GAPDH mRNA (−1% ± 25%) in the absence, but an increase was seen in the ratio due to PGE_2 (+56% ± 19%, $P < .025$) in the presence of dexamethasone. The data showing leptin and GAPDH mRNA from 1 of the 14 experiments are shown in Fig 6. These data suggest that PGE_2 increased the amount of GAPDH mRNA to about the same extent as it decreased leptin mRNA in the absence of dexamethasone.

There was a marked stimulation of leptin mRNA accumulation at 48 hours by 200 nmol/L dexamethasone (which averaged 50% in the data shown in Fig 5). The amount of leptin release by adipose tissue from 1 individual to the next was quite variable either in the absence or presence of dexamethasone (Fig 7). However, if the data for fat from each human donor were plotted either without or with 200 nmol/L dexamethasone, there was a close correlation in each experiment (0.91) between leptin release and leptin mRNA (Fig 7). This correlation was most evident at high values for leptin release. These data indicate that dexamethasone effects on leptin release are probably secondary to enhancing the level of leptin mRNA, but whether this involves enhanced transcription of leptin mRNA, decreased breakdown, or both remains to be established.

The question arises as to whether the effects of arachidonic acid and NS-398 on leptin release over a 48-hour incubation of explants of adipose tissue are due to their actions on other cells, such as preadipocytes, present in adipose tissue. We examined this question via 2 approaches. It has been shown that 1 μmol/L carbaprostacylin, which is a stable analog of prostacyclin, acts as an intracrine stimulator for differentiation of Ob1771 and 3T3-F442A cells (clonal murine preadipocyte cell lines) over a 24-hour incubation based on accumulation of adipocyte fatty acid binding protein mRNA.[17] Carbaprostacyclin,[18] arachidonic acid,[19] and glucocorticoids[19] all stimulate the formation of late markers of adipose conversion such as GAPDH over a 12-day incubation of Ob1771 clonal cell lines.

While arachidonic acid (Fig 2) and dexamethasone, a synthetic glucocorticoid (Fig 3) stimulated leptin release, we found that the addition of 1 μmol/L carbaprostacyclin to explants of human adipose tissue actually inhibited leptin release by −31%

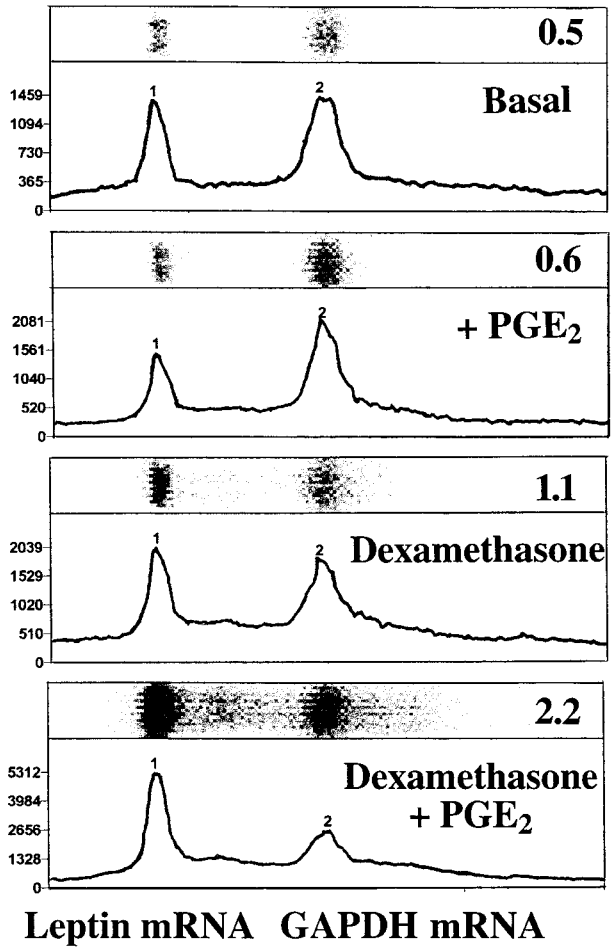

Fig 6. Effect of PGE_2 on the ratio of radioactivity in leptin mRNA to that in GAPDH mRNA. The data are from 1 of the 14 experiments summarized in Fig 5 and represent the electronic autoradiographic analysis of Northern blots. The samples were first electrophoresed on the same gel and then transferred to Nytran membranes and simultaneously analyzed with probes for leptin and GAPDH mRNA. The results are shown both as digital images and graphed as cpm.

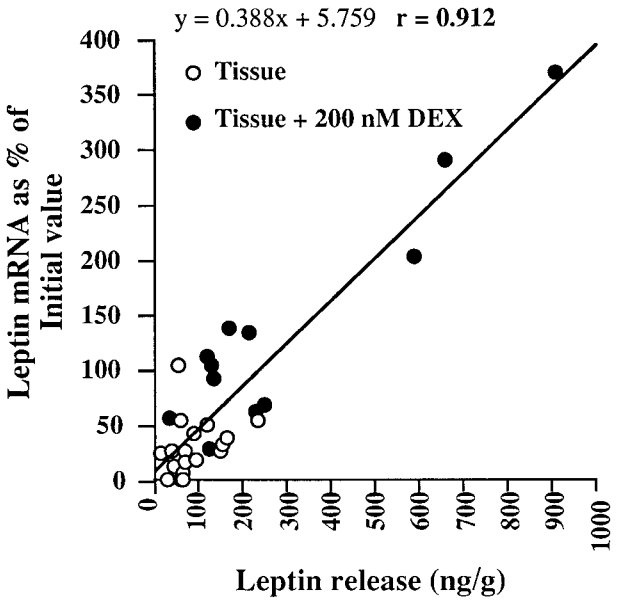

Fig 7. Correlation between leptin mRNA accumulation and leptin release by human adipocytes or adipose tissue. Each point is from a different human fat donor either without or with 200 nmol/L dexamethasone. The correlation coefficient was 0.912 for the pooled data.

± 4% over a 48-hour incubation as the means ± SEM of 3 paired experiments ($P < .025$). We also incubated human adipose tissue explants with 5 μmol/L beraprost, another stable analog of prostacyclin, which is in clinical use.[20,21] In the presence of 5 μmol/L beraprost, the release of leptin over 48 hours was reduced by −23% ± 9% as the means ± SEM of the percent change over control in 4 paired experiments. We have measured the formation of 6-keto $PGF_1\alpha$, which is the stable metabolite of prostacyclin, and found that over a 48-hour incubation of adipose tissue, its accumulation averaged 35% of that for PGE_2 in 23 paired experiments. These data indicate that there is appreciable formation of prostacyclin over a 48-hour incubation of human adipose tissue explants, and that exogenous addition of stable analogs of prostacyclin does not stimulate leptin release.

Another approach to eliminate possible effects of the other cells present in adipose tissue and the possible increases in preadipocyte differentiation over a 48-hour incubation of human adipose tissue explants is to incubate isolated human adipocytes for 48 hours. In experiments using adipocytes from 9 different individuals, an average of 470 mg of isolated human adipocytes was incubated in 5 mL of medium for 48 hours, and the basal release of leptin was 36 ± 6 ng/g of fat cells in contrast to 121 ng/g (Fig 3) or 155 ng/g (Fig 2) for leptin release by intact adipose tissue. The percentage increase in leptin release due to dexamethasone (200 nmol/L) in the experiments using adipocytes was 100% as compared to 180% in the studies shown in Fig 3 using tissue explants. The addition of 50 μmol/L arachidonic acid resulted in a +29% ± 9% increase in leptin release as the mean ± SEM of 9 paired experiments ($P < .01$). These data indicate that both dexamethasone and arachidonic acid stimulate leptin release by isolated adipocytes, but the percentage increase is about half that seen in tissue explants.

We also examined the effects of NS-398 (5 μmol/L) on leptin release by isolated adipocytes over a 48-hour incubation in primary culture. We found effects of NS-398 that were comparable to those seen in intact tissue (Fig 3) in that there was no effect of NS-398 on leptin release in the absence of dexamethasone (+12% ± 11% as the mean ± SEM of 5 paired experiments). However, in the presence of 200 nmol/L dexamethasone, there was a −21% ± 7% decrease in leptin release (mean ± SEM of 5 paired experiments, $P < .05$). This decrease was comparable to the −25% decrease due to NS-398 seen in explants of intact adipose tissue incubated in the presence of dexamethasone (Fig 3).

DISCUSSION

Our data indicate that there is a stimulation of leptin release after addition of exogenous arachidonic acid or PGE_2 to explants of human adipose tissue incubated in primary culture for 48 hours. Furthermore, the stimulation by arachidonic acid of leptin release is probably secondary to conversion to a prostanoid. The possibility that the increase in leptin release due to arachidonic acid is due to a direct effect or conversion to lipooxygenase or other products, such as epoxides, is unlikely because the increase in leptin release due to arachidonic acid was abolished in the presence of NS-398 a specific COX-2 inhibitor (Table 1).

The ability of PGE_2 to stimulate leptin release by human adipose tissue in primary culture confirms similar findings using mouse[22] and rat[23] adipose tissue incubated for 24 hours. The administration of PGE_2 to rats 6 hours prior to killing resulted in an increase in serum leptin.[23] The effects of NS-398 on inhibition of PGE_2 formation were comparable in all species except that at 0.05 μmol/L NS-398 the formation of PGE_2 was reduced by 70% in rat fat,[23] while in either mouse[22] or human fat (Fig 3), the inhibition was around 40%.

There was no effect of NS-398 on lipolysis by rat adipose tissue,[23] while lipolysis was enhanced by NS-398 in the absence of dexamethasone in both mouse[22] and human adipose tissue (Fig 3). Effects of NS-398 on leptin release were comparable to what was seen with respect to lipolysis. Leptin release was unaffected by NS-398 in explants of rat adipose tissue[23] while leptin release by mouse adipose tissue in the presence of dexamethasone was inhibited by 0.05 μmol/L NS-398.[22] These data suggest that the role of endogenous prostanoids produced by COX-2 is minor in rat adipose tissue as compared with the effects on mouse and human adipose tissue.

Endogenous PGE_2 or a closely related prostanoid may play a role in the stimulation of leptin release seen in the presence of 200 nmol/L dexamethasone since leptin release was significantly reduced by 1 or 5 μmol/L NS-398, which completely blocked PGE_2 formation (Fig 3). The complication is that total PGE_2 formation was also abolished in the presence of 1 or 5 μmol/L NS-398, yet only in the presence of dexamethasone was leptin release inhibited by NS-398. It may be that NS-398 has side effects that account for its failure to reduce leptin release in the absence of dexamethasone. The negative effect of

5 μmol/L NS-398 on basal leptin release could also be interpreted as evidence against a role for endogenous prostanoids in the regulation of leptin release. However, the data for NS-398 effects on lipolysis are as expected if endogenous prostanoids are physiologic regulators of lipolysis. One question is why dexamethasone did not stimulate lipolysis, as did NS-398, but dexamethasone may also affect other enzymes besides COX-2. In conclusion, the effects of NS-398 suggest that the role of endogenous prostanoids as regulators of leptin release is complex, and a simple explanation is not possible due to possible side effects of the drug and/or the presence of both stimulatory and inhibitory prostanoids.

In murine 3T3L1 cells, there is a low release of leptin that is inhibited by $dPGEJ_2$, which is an eicosanoid derived from PGJ_2 an alternative product of the metabolism of PGH_2.[24,25] PGJ_2 and thiazolidinediones are ligands for peroxisome proliferator-activated receptor γ ($PPAR_\gamma$).[26,27] However, we found that 1 μmol/L dPGJ2 or 3.3 μmol/L troglitazone enhanced leptin release by human adipose tissue incubated for 48 hours in the absence of dexamethasone.[28] Furthermore, troglitazone enhanced leptin release in the absence, but not in the presence, of 200 nmol/L dexamethasone.[28] These data suggest that over a 48-hour incubation of subcutaneous tissue from obese humans known activators of $PPAR_\gamma$ can enhance leptin release under appropriate conditions.

The studies on lipolysis by human adipose tissue indicate that a very low concentration of NS-398 (0.05 μmol/L) enhanced basal lipolysis in the absence, but not in the presence, of dexamethasone (Fig 3A). These data suggest that products of COX-2 are endogenous regulators of lipolysis in human fat. Prior studies using rat adipose tissue and indomethacin, which is a nonspecific inhibitor of both COX-1 and COX-2, were inconclusive.[6] Richelsen[6] suggested that the use of indomethacin might obscure the role of endogenous eicosanoids in regulation of adipocyte lipolysis, but the problem was the use of rat adipose tissue, because NS-398 does not affect lipolysis by rat adipose tissue.[23]

PGE_2 reacts with EP_3 receptors on the surface of adipocytes, which through activation of the inhibitory guanine nucleotide binding protein $G_{i\alpha 2}$, inhibits lipolysis.[6,29] In human adipose tissue explants, lipolysis was low over a 48-hour incubation, and only a nonsignificant 10% ± 7% inhibition by PGE_2 (mean ± SEM of 12 paired experiments in the presence of 200 nmol/L dexamethasone using human fat) was seen of lipolysis (unpublished experiments). Therefore, the ability of PGE_2 to stimulate leptin release does not appear to be secondary to effects on lipolysis. However, it remains to be established whether activation of the $G_{i\alpha 2}$ protein is involved in regulation of leptin release. In rats, the administration of N^6-cyclopentyladenosine (CPA) results in an elevation in plasma leptin[30] as does the administration of PGE_2.[23] CPA also reacts with receptors that activate $G_{i\alpha 2}$ and inhibit lipolysis.[31] These data suggest that both CPA and PGE_2 receptor activation results in leptin release, as well as $G_{i\alpha 2}$ activation, but whether there is a causal relationship remains to be established.

The factors that are responsible for the marked elevation of plasma leptin in obese individuals remain to be established. It appears that the larger the fat cell the greater the amount of leptin release, but this cannot, as yet, be linked to any known hormone or paracrine signal. The present results indicate that if there is enhanced turnover of triacylglycerol stores in fat cells from obese individuals, this would result in elevated levels of arachidonic acid and possibly enhanced leptin release due to increased formation of prostanoids. It remains to be established whether there is enhanced formation of prostanoids in adipose tissue from obese individuals, and if so, whether prostanoids are physiologic regulators of lipolysis and leptin release. Our data indicate that endogenous prostanoids can inhibit lipolysis in adipose tissue from obese individuals, and this might promote lipid deposition and hence obesity by reducing mobilization of stored lipids. Inhibition of lipolysis by endogenous prostanoids may well be more important than regulation of leptin release by adipocytes.

Carbaprostacyclin, a stable analog of prostacyclin, has been shown to promote terminal differentiation of murine preadipocyte cells lines, but this process is slow and requires a 12-day incuation.[18,19] However, carbaprostacylin also has an early stimulatory effect on the accumulation of the mRNA for adipocyte fatty acid binding protein in these cells lines that occurs by a mechanism distinct from classical effects mediated via the prostacyclin receptor.[17] This effect may be mediated via activation of $PPAR_\gamma$ which is readily activated by carbaprostacyclin in transactivation assays using extract of NIH fibroblasts.[32] However, we have reported that troglitazone, another known of activator of $PPAR_\gamma$, actually stimulated leptin release by explants of human adipose tissue,[28] so it is unclear whether the effects of troglitazone or of carbaprostacylin are due to activation of $PPAR_\gamma$ in human adipose tissue. Beraprost is another stable analog of prostacyclin, which is used clinically and may be a more specific activator of prostacyclin receptor.[20,21] However, in contrast to PGE_2, neither analog of prostacyclin stimulated leptin release by human adipose tissue explants. Our data indicate that endogenous prostacyclin is more likely to be an inhibitor of leptin release than a stimulator as is the case with PGE_2.

We were able to show that dexamethasone and arachidonic acid stimulate leptin release by isolated human adipocytes over a 48-hour incubation in primary culture, but the effects were less than in in intact tissue, as was also the case with unstimulated leptin release. It may well be that the collagenase digestion has deleterious effects on the subsequent rate of leptin release by adipocytes and/or that cells when incubated in the absence of the usual tissue matrix are less responsive to agents. However, the ability of both arachidonic acid and dexamethasone to act on isolated adipocytes clearly indicates that factors released by the connective tissue and blood vessels cells of adipose tissue are not required for demonstration of stimulatory effects of these agents on leptin release. Furthermore, because adipocytes are terminally differentiated cells, the effects of agents on these cells cannot be secondary to differentiation of preadipocyte cells.

In conclusion, our data indicate that arachidonic acid stimulates leptin release by human adipose tissue and adipocytes incubated in primary culture for 48 hours. The effect of arachidonic acid is probably secondary to formation of prostanoids. The effect of arachidonic acid can be mimicked by PGE_2, which stimulates leptin release and leptin mRNA

accumulation by human adipose tissue in the presence of dexamethasone. In human adipose tissue incubated for 48 hours, there is a marked upregulation of COX-2 activity, and inhibition of this process by NS-398 results in an enhancement of basal lipolysis. These data are compatible with the hypothesis that under appropriate conditions endogenous prostanoids regulate lipolysis and possibly leptin release by human adipose tissue.

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