Insulin increases fatty acid synthase gene transcription in human adipocytes

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Department of Nutrition and Agricultural Experiment Station, University of Tennessee, Knoxville 37996-1900; and Department of Surgery, Division of Plastic Surgery, University of Tennessee Medical Center, Knoxville, Tennessee 37920

Claycombe, Kate J., Brynn H. Jones, Melissa K. Standridge, Yingshi Guo, Joseph T. Chun, James W. Taylor, and Naima Moustaid-Moussa. Insulin increases fatty acid synthase gene transcription in human adipocytes. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1253–R1259, 1998.—The purpose of this study was to investigate the molecular mechanism whereby insulin increases expression of a key de novo lipogenic gene, fatty acid synthase (FAS), in cultured human adipocytes and hepatoma cells. RNA isolated from cultured adipocytes or from Hep G2 cells treated with or without insulin (20 nM) was analyzed. In addition, run-on transcription assays and measurements of RNA half-life were performed to determine the controlled step in FAS gene regulation by insulin. We demonstrated that FAS mRNA was expressed in both Hep G2 cells and human adipocytes. Insulin induced an approximately five- and three-fold increase in FAS mRNA content in adipocytes and hepatoma cells, respectively. Similar regulation of FAS was observed in adipocytes from lean and obese human subjects. Furthermore, we demonstrated that the induction of human FAS expression by insulin was due to increased transcription rate of the FAS gene in human adipocytes, whereas mRNA stabilization accounted for increased FAS mRNA content in hepatoma cells. In conclusion, we report here for the first time expression of human FAS mRNA and its specific transcriptional induction by insulin in cultured human adipocytes.

human adipose tissue; primary culture; Hep G2; mRNA stability

ADIPOSE TISSUE IS THE MAJOR SITE FOR energy storage and plays an important role in maintaining glucose homeostasis (12). Abnormalities in hormonal and nutritional regulation of this tissue have been implicated in the pathophysiology of obesity, diabetes, and atherosclerosis. It is therefore crucial to investigate regulation of adipose tissue metabolism, in particular that of human adipose tissue.

Although the causes of human obesity are not yet elucidated, this disease is commonly associated with excessive fat storage, leading to adipocyte hypertrophy. In addition, obesity is highly prevalent in type II diabetic patients, and diabetic patients on insulin therapy tend to gain weight. Therefore, the objective of this study was to investigate the role of insulin in regulating fatty acid synthesis in human adipose tissue.

Several studies have demonstrated the presence of the key enzymes for fatty acid synthesis (4, 23) and the importance of human adipose tissue in de novo fatty acid synthesis (2, 5). In addition, recent studies demonstrated that liver plays a minor role in de novo human lipogenesis and suggested that adipose tissue may be the principal lipogenic tissue in humans (1). As an approach toward understanding the role that nutrients and hormones play in regulation of human adipose tissue metabolism, we recently developed a cell culture system in which human adipocytes can be maintained viable and metabolically active for several days (21). We have demonstrated that glucose utilization as well as activities of lipogenic enzymes including fatty acid synthase (FAS) were increased by insulin in a dose-dependent manner in cultured human adipocytes (21).

FAS is a key lipogenic enzyme that catalyzes all of the reactions involved in the synthesis of long-chain saturated fatty acid (palmitate) from acetyl CoA, malonyl CoA, and NADPH. In addition, this enzyme is highly regulated by nutrients and hormones in all species tested. In rodent and murine cell lines, FAS is suppressed by fasting (25), polyunsaturated fatty acids (8), and diabetes (25), whereas it is induced by feeding high-carbohydrate diets (25), glucose (10), obesity (9), and insulin (25). We have recently identified an insulin response sequence in the proximal promoter of the rat FAS gene (20) that mediates regulation of FAS gene transcription by insulin. This element overlaps the CAAT box region and binds upstream stimulatory factor (29). A second insulin response element has been identified within a DNase hypersensitive region (30). Although regulation of human FAS gene serves as a better tool toward our understanding of lipid metabolism disorders in human obesity and diabetes, very limited information is available concerning its regulation. Human FAS has been shown to be expressed in several human tissues (14, 26). Semenkovich and his collaborators (26, 27) have reported that FAS expression was induced by glucose in Hep G2 cells at the posttranscriptional level. However, neither nutritional nor hormonal regulation of FAS gene expression in human adipocytes has been reported before.

Because our previous study showed induction of human lipogenic enzymes, including FAS, by insulin (21), the objective of the present work was to determine the mechanism(s) whereby insulin regulates expression of the FAS gene in cultured human adipocytes from lean and obese patients as well as in Hep G2 human hepatoma cells. We report for the first time that FAS expression was induced by insulin at the transcriptional level in human adipocytes and at the posttranscriptional level in Hep G2 cells.

MATERIALS AND METHODS

Human subjects. Nonobese, nondiabetic [body mass index (BMI) <27] as well as morbidly obese women (BMI >34) with an average age of 37 ± 15 yr were used in this study. To our
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been previously reported. We performed this preparation in assay.
Preparation of nuclei from human adipocytes has not obtained from Clonetech laboratories (Palo Alto, CA). Sequen-
Semenkovich (St. Louis, MO). The 18S rRNA probe was Northern and/or dot blotting (15, 22). A 2-kb human FAS
cesium chloride density gradient method and analyzed by
medication. We maintained adipocytes isolated from these patients in culture for several days to eliminate any differences contributed by in vivo circulating factors. This proposal was approved by the institutional review board for human subjects and the Committee for Research Protocols of the University of Tennessee in Knoxville.

Isolation of human adipocytes and culture conditions. Adipose tissue was obtained from subcutaneous abdominal fat of the above fasted patients and cultured as we recently described (21). Fat was removed at the time of the surgery in a sterile environment and was immersed in Hank's balanced salts solution (GIBCO BRL) supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), and gentamicin (50 µg/ml). Adipose tissue was then washed several times with Hank's balanced salt solution to remove the majority of connective tissue and blood clots. The tissue was minced into small fragments, which were digested with type I collagenase (1 mg/ml, GIBCO BRL) in a shaking water bath at 37°C for 30–60 min in a polypropylene flask. Cells were then filtered through a sterile nylon filter (350 µm mesh). The suspension was centrifuged at 500 g for 1 min to separate the pelleted stromal vascular fraction from the floating adipocyte fraction and was washed three times with Hank's balanced salt solution. Adipocytes were then resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with HEPES (15 mM), glucose (25 mM), bovine serum albumin (1%), 50 mM adenosine, antibiotics, and 1% fetal bovine serum (standard medium). Cells were subsequently cultured in suspension in sterile polypropylene tubes in a humidified incubator at 37°C under 5% CO₂ and 95% air. The culture medium containing the adipocytes (still in suspension) was removed 24 h later, and the adipocytes were cultured in fresh standard medium. The cells were maintained for an additional 3–6 days in this medium and then incubated overnight in serum-free medium before insulin treatment as indicated in figure legends. Media were changed every day during the culture. Trypan blue exclusion test was conducted in all cultures to confirm cell viability.

Culture of Hep G2 cells. Hep G2 cells were purchased from American Type Culture Collection (Rockville, MD). Cells were grown and maintained in DMEM supplemented with 10% fetal bovine serum. Subconfluent cells were incubated overnight in serum-free medium before insulin treatment as indicated in figure legends.

FAS enzyme activity. Adipocytes were washed with phosphate-buffered saline (PBS) and homogenized in sucrose buffer, and then FAS activity was assayed spectrophotometrically as we previously described by measuring the rate of oxidation of NADPH (15). One unit of enzyme activity equals one nanomole of NADPH oxidized per minute per microgram DNA that was assayed fluorometrically (3).

RNA isolation and hybridizations. RNA was isolated by the cesium chloride density gradient method and analyzed by Northern and/or dot blotting (15, 22). A 2-kb human FAS cDNA probe, HFAS (27), was kindly provided by Dr. C. F. Semenkovich (St. Louis, MO). The 18S rRNA probe was obtained from Clontech laboratories (Palo Alto, CA). Sequential hybridizations with FAS, β-actin, and 18S probes were conducted as we previously described (15, 22). Changes in FAS mRNA were normalized to 18S ribosomal RNA or to β-actin.

Preparation of nuclei and nuclear transcription run-on assay. Preparation of nuclei from human adipocytes has not been previously reported. We performed this preparation in various buffer conditions and found that addition of very low concentrations of Nonidet P-40 (NP-40, 0.005%) to the cell lysis buffer containing 5 mM MgCl₂, 10 mM Tris, pH 7.5, 25 mM KCl, 0.1 mM EDTA, and 1 mM dithiothreitol (DTT) yielded satisfactory nuclei recovery. Adipocytes were first rinsed in PBS and resuspended in the above NP-40-supplemented lysis buffer. The homogenate was then centrifuged at 500 g at 4°C for 5 min, and the nuclei pellet was recovered by pipetting through the solid fat layer and transferred to a fresh tube. The suspension was rinsed with the same buffer without NP-40 and then centrifuged as above and resuspended in nuclei storage buffer containing 50 mM Tris, pH 7.8, 5 mM MgCl₂, 0.1 mM EDTA, 0.1 mM DTT, and 40% glycerol. Nuclei were prepared from Hep G2 cells as we previously described (22). Nuclear run on assay and hybridizations were conducted on both human adipocytes and Hep G2 cells as we previously described for 3T3-L1 adipocytes (22). The following cDNA plasmids were used: human FAS plasmid (pHFAS), kindly provided by Dr. C. F. Semenkovich, St. Louis, MO (27); pFos plasmid, kindly provided by Dr. M. S. Miller, University of Tennessee, Knoxville (18); and angiotensin II type 2 receptor (pAR) cDNA, kindly provided by Dr. T. S. Elton, University of Alabama at Birmingham (18).

mRNA stability. Hep G2 cells were cultured as described above. Subconfluent cells were maintained overnight in serum-free medium and then treated with insulin for 24 h. Actinomycin D (5 µg/ml) was then added to cells as we previously described (22). Cells were harvested at different time points after actinomycin D treatment. RNA was then isolated and analyzed by Northern blot. Sequential hybridizations with FAS and 18S probes were conducted as we previously described (15, 22). The relative abundance of FAS mRNA as a function of time was used to determine FAS mRNA half-life.

Data analysis. Autoradiograms from Northern and dot blot analyses and run on assays were quantified by densitometric scanning. Alternatively, membranes were counted using Ambis 4000 direct β-imaging system (Billerica, MA). Post hoc comparisons between groups were made using Student’s t-test. Data are expressed ± SE.

RESULTS AND DISCUSSION

Regulation of expression of human FAS mRNA by insulin in cultured adipocytes from lean and obese subjects. We have recently described a cell culture system of human adipocytes in which glucose consumption and activities of lipogenic enzymes were increased by insulin (21). In these studies, we have also shown that insulin increases FAS activity in a dose-dependent manner, with a maximal induction at <10 nM. Furthermore, FAS activity was increased by insulin within 3 days of treatment (21). In the present work, we investigated the molecular mechanisms whereby this key lipogenic gene was regulated by insulin in cultured adipocytes from lean and obese patients.

In preliminary studies, we first measured FAS mRNA levels in adipose tissue from different patients; these studies indicated expression of a single mRNA species of ~9.3 kb in human adipose tissue (data not shown). This size is ~1 kb larger than that previously reported for other species but comparable to that reported in Hep G2 cells (26, 27). Because adipocytes have been removed from their in vivo environment where patients may exhibit differences in circulating factors, any differences obtained in culture would represent intrinsic
properties of these cells independently of the in vivo conditions. Expression of human FAS mRNA exhibited large variations at basal levels, and this variation may have resulted from differences in dietary intake and other factors such as medications. To alleviate these variations, we cultured human adipocytes for several days to allow for their desensitization to in vivo circulating factors. Previous studies in Zucker rats have shown that the differences observed in adipocytes freshly isolated from lean and obese animals that were fed high-fat diets disappeared when adipocytes were maintained a few days in culture (6).

To investigate effects of insulin on FAS expression, we cultured cells for 4–7 days in standard medium, followed by an overnight incubation in serum-free medium before insulin treatment. Adipocytes were subsequently maintained with or without 20 nM insulin for an additional 2–4 days. We analyzed RNA from adipocytes of 11 nonobese and 6 morbidly obese patients. No statistical difference was observed between data from lean and obese patients. Therefore, we combined data from lean and obese patients and reported for each patient differences in insulin stimulation compared with control (untreated) cells. Preliminary experiments indicated that optimal responsiveness of FAS mRNA to insulin was reached after 48 h of treatment. No further stimulation was obtained at 60 or 72 h. Therefore, we treated cells for RNA analysis for 48 h. Figure 1A is a representative autoradiogram of Northern blot analysis of human adipocyte RNA hybridized with human FAS and 18S RNA probes. Results from this Northern blot analysis indicated low basal level expression of FAS mRNA in human adipocytes and increased mRNA content upon insulin treatment. Densitometric scanning of data obtained from 17 subjects is shown in Fig. 1B and indicates that insulin increased FAS mRNA levels by approximately fivefold in adipocytes from lean and obese patients (P < 0.01). This increase paralleled similar increases in FAS activity (0.073 ± 0.011 units, control, vs. 0.32 ± 0.07 units, insulin; n = 10, P < 0.01). We further investigated whether continuous presence of insulin was required to sustain levels of FAS expression and whether the effect of insulin was reversible. Adipocytes were cultured for 3 days (days 0–3) in insulin-free and serum-free medium then either maintained in the same medium or treated with insulin for 3 additional days (days 3–6). As shown in Fig. 2A, insulin-treated cells (+) exhibited higher FAS activity compared with control cells (−). Deprivation of insulin for 3 days (days 6–9, +/−) from previously insulin-treated cells (+) decreases FAS activity to levels approaching those obtained with control cells. These results indicated that the effect of insulin was reversible and that continuous presence of insulin was required for induction of FAS activity. We also measured total DNA content in insulin-deprived (+/−) and insulin-treated cells (+) and found no significant difference between the two groups, suggesting that insulin at 20 nM induces cell hypertrophy rather than hyperplasia in human adipocytes. In agreement with the enzyme activity data, FAS mRNA levels were lower in insulin-deprived cells (−) compared with insulin-treated cells (+) (Fig. 2B).

Addition of insulin to previously deprived cells (+/−) increases FAS mRNA content, whereas withdrawal of insulin from insulin-treated cells (+/−) decreases FAS mRNA content to levels observed in cells that were never exposed to insulin (−). Insulin is a lipogenic hormone known to increase triglyceride stores; consistent with these effects, we have also recently reported that insulin slightly increases cell size in human adipocytes (21).

Our results thus demonstrate that adipocytes from both lean and obese patients are responsive to insulin, which induces FAS gene expression in these cells when continuously exposed to the hormone. Similar induction of the lipoprotein lipase mRNA by insulin has been
recently reported in cultured human adipose tissue fragments (11). In addition, similar upregulation of the FAS mRNA by insulin has been previously reported in 3T3-L1 adipocytes (24).

Regulation of FAS mRNA by insulin in Hep G2 cells. To determine whether regulation of expression of FAS by insulin was tissue specific, we compared regulation of this gene in adipocytes and Hep G2 cells. Recent studies have reported that FAS gene was posttranscriptionally regulated by glucose in Hep G2 cells (26, 27). However, regulation of FAS gene in these cells by insulin has not been reported. Accordingly, we analyzed RNA isolated from Hep G2 cells treated for 48 h with or without 20 nM insulin. A representative Northern blot is shown in Fig. 3A. Results shown in this figure indicate that human FAS is expressed in Hep G2 cells and that expression of this message is induced upon insulin treatment. Data from densitometric scanning of three independent measurements are shown in Fig. 3B. These results indicate that insulin increases FAS mRNA content approximately threefold in human hepatoma cells. Taken together, the above data indicate that both hepatic and adipocyte FAS mRNA are upregulated by insulin. It is worth noting that angiotensinogen mRNA was not significantly changed by insulin in Hep G2 cells (Fig. 3A). Our findings in Hep G2 cells are supported by other studies in H4 hepatoma cells that demonstrated that glucocorticoid is the only hormone that regulates angiotensinogen expression in hepatoma cells. This is in contrast to previous reports suggesting that insulin is a key regulator of angiotensinogen expression in rodent adipose tissue (7). We have also recently confirmed these findings by demonstrating that insulin increases angiotensinogen gene expression in cultured adipocytes (17). Taken together, these data suggest...
that insulin regulation of angiotensinogen gene may be tissue specific.

Mechanism of insulin regulation of the FAS gene in human adipocytes. To gain insight into mechanisms involved in regulation of FAS by insulin, we next investigated whether human FAS gene was regulated at the transcriptional level. Accordingly, we performed run-on transcription assay in nuclei isolated from control and insulin-treated human adipocytes. These assays are difficult to perform in human adipocytes and require large amounts of cells. To our knowledge, this is the first report on nuclei isolation and measurement of transcription rate in human adipocytes. A representative assay is illustrated in Fig. 4A, and scanning of autoradiograms from independent run-on assays performed in five different patients are presented in Fig. 4B.

Data from Fig. 4B show that the transcription rate of the human FAS gene was increased by approximately fourfold in insulin-treated compared with control untreated adipocytes. Similar results were obtained for lean and obese patients. Time course experiments indicated that insulin had no effect on FAS gene transcription in human adipocytes treated for 6 h, whereas insulin effect at 72 h was lower but not significantly different from 24 or 48 h (data not shown). To demonstrate specificity of the insulin effect, angiotensin II type 2 receptor (AT2) and fos oncogene cDNAs were used as controls. Our results (Fig. 4) demonstrate that AT2 gene transcription was not significantly changed by insulin (range of insulin effect was −23 to +18%). We have recently reported that AT2 antagonist PD-123319 antagonized the lipogenic effect of angiotensin II in murine 3T3-L1 adipocytes (16). However, this is the first report of expression of this receptor in human adipocytes. We also report that fos oncogene transcription (used as a positive control) was also induced by insulin in human adipocytes; this finding is in agreement with previously reported induction of the fos gene transcription by insulin in 3T3-L1 adipocytes (28). Interestingly, when nuclear run-on assays were performed in Hep G2 cells, no significant difference was observed on FAS gene transcription in control compared with insulin-treated cells (data not shown).

Mechanism of insulin regulation of the FAS gene in Hep G2 cells. Previous reports in rodents have demonstrated that the rodent FAS gene was primarily regulated at the transcriptional level in liver and adipose tissue as well as in hepatoma cells and preadipocyte cell lines (9, 20, 25). Interestingly, our study demonstrated that transcriptional regulation of the human FAS gene by insulin is tissue specific because insulin did not affect FAS gene transcription in Hep G2 cells. However, FAS gene transcription was increased by insulin in rat hepatoma H4-II-E cells (20), suggesting species-specific differential regulation of the FAS gene by insulin.

To further support our findings, we used actinomycin D to determine whether FAS mRNA stability was modified by insulin in Hep G2 cells. Because FAS gene transcription accounted for changes in FAS mRNA levels in adipocytes, the message stability was only investigated in Hep G2 cells. The relative abundance of FAS mRNA levels were measured in Hep G2 cells, which were treated with insulin for 24 h before addition of actinomycin D for up to 24 h (n = 3). Results from densitometric scanning analysis of the autoradiograms...
Treatment of these diseases.

Development of new therapeutic approaches for the regulation of these factors may provide rationale for the regulation of FAS by insulin in human adipocytes and emic diabetic patients as well as those patients on (13) recently reported the partial sequence of the identified in murine 3T3-L1 adipocytes (20) mediates insulin response element(s) similar to that previously identified in human FAS upstream promoter is blocked by the genetic effect on fat storage capacity in cultured adipose tissue from Zucker rats. Am. J. Physiol. 267 (Endocrinol. Metab. 30): E439–E446, 1994.


