The Human Fatty Acid Synthase Gene and De Novo Lipogenesis Are Coordinately Regulated in Human Adipose Tissue[1]

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ABSTRACT Despite its potential importance in obesity and related disorders, little is known about regulation of lipogenesis in human adipose tissue. To investigate this area at the molecular and mechanistic levels, we studied lipogenesis and the regulation of 1 of its core enzymes, fatty acid synthase (FAS), in human adipose tissue in response to hormonal and nutritional manipulation. As a paradigm for lipogenic genes, we cloned the upstream region of the human FAS gene, compared its sequence to that of FAS orthologs from other species, and identified important regulatory elements that lie upstream of the FAS coding region. Lipogenesis, as assessed by glucose incorporation into lipids, was increased by insulin and more so by the combination of insulin and dexamethasone (Dex, a potent glucocorticoid analogue). In parallel, FAS expression, activity, and gene transcription rate were also significantly increased by these treatments. We also showed that linoleic acid, a representative PUFA, attenuated the actions of insulin and Dex on fatty acid and lipid synthesis as well as FAS activity and expression. Using reporter assays, we determined that the regions responsible for hormonal regulation of the FAS gene lie in the proximal portion of the gene's 5′-flanking region, within which we identified an insulin response element similar to the E-box sequence we identified previously in the rat FAS gene. In summary, we demonstrated that lipogenesis occurs in human adipose tissue and can be induced by insulin, further enhanced by glucocorticoids, and suppressed by PUFA in a hormone-dependent manner. J. Nutr. 134: 1032–1038, 2004.

KEY WORDS: • *linoleic acid* • *insulin* • *dexamethasone* • *sequence* • *gene transcription*

The ability of adipose tissue to store excess energy in the form of triglycerides is well known. The majority of fatty acids sequestered in adipose tissue derive from dietary intake. However, both liver and adipose tissue are capable of de novo lipogenesis (DNL),[3] primarily from carbohydrate sources. DNL has a relatively high energetic cost and its importance in total triglyceride balance continues to be debated. At most, DNL is thought to contribute minimally to total body lipid stores (1). Nonetheless, the prevalence of obesity and the cumulative benefits of even small reductions in fat storage highlight the need to understand even minor contributions to whole-body lipid balance. In addition, adipose tissue is increasingly recognized for its contributions to comorbid conditions such as diabetes and cardiovascular disease, in part via its endocrine function (2). Indeed, there is growing awareness that adipocytes secrete vasoactive factors such as angiotensin II in proportion to adipose mass, which means that even relatively minor increases in adiposity may negatively affect health (2,3). Furthermore, malonyl-CoA, the product of the first step in DNL, acts as a regulator of several important cellular functions in various tissues, and changes in its levels as a result of DNL regulation may also affect cell and tissue function (4). Collectively, these lines of evidence underscore the potential for alterations in rates of DNL to affect physiology despite a relatively minor contribution to whole-body triglyceride balance.

Studies in rodents, which have intrinsically higher rates of DNL than humans, demonstrated that both dietary and hormonal factors regulate hepatic and adipose DNL (5). Overfeeding, especially with carbohydrates, increases adipose tissue

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[3] Abbreviations used: BSA, bovine serum albumin; Dex, dexamethasone; DNL, de novo lipogenesis; FAS, fatty acid synthase; FIRE, FAS insulin response element; Ins, insulin; IRS, insulin responsive sequence; KRBA, Krebs-Ringer bicarbonate albumin buffer; LA, linoleic acid; PIA, *N*6-phenylisopropyladenosine; SREBP, sterol regulatory element binding protein site.

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lipogenesis and the expression and activity levels of lipogenic enzymes (6). In humans, several studies demonstrated that both energy intake and the macronutrient content of the diet regulate lipogenic rates in liver (7–9). Conclusions about DNL in human adipose tissue, however, continue to be contradictory. Carbohydrate feeding led to increased total fat synthesis that could not be completely attributed to hepatic lipogenesis, suggesting that DNL was also increased in adipose tissue (10). In another study, rates of fatty acid synthesis in human adipose tissue increased ~80-fold when subjects were switched from low- to high-carbohydrate intake (11). Other studies, however, reported no significant effects of dietary manipulations on lipogenesis or lipogenic enzyme activities in human adipose tissue (12,13).

In vitro studies demonstrated that both lipogenesis and key enzymes in the lipogenic pathway are regulated directly by nutrients and hormones in adipocytes (14,15). Fatty acid synthase (FAS) is the central enzyme in DNL, catalyzing all of the enzymatic steps in the conversion of acetyl-CoA and malonyl-CoA to palmitate. FAS activity measurements and FAS mRNA levels are frequently used as markers of DNL, and changes in FAS activity are due primarily to alterations in the transcription rate of the FAS gene. We showed in both murine cell lines and primary human adipocytes that insulin and angiotensin II increase FAS gene transcription (14,15). In cultured adipocytes and hepatocytes as well as in transgenic mice expressing reporter genes under control of the FAS promoter, the *cis*-acting elements necessary for tissue-specific, nutritional, and hormonal regulation of FAS expression are contained primarily in the proximal 2.1 kb of the 5′-flanking DNA (16). We and others have dissected several of the regulatory elements controlling transcription of the rodent FAS genes, but limited analysis has been performed with the orthologous human gene.

Given the potential importance of DNL in adipose tissue, disparate reports about its regulation, and the central role of FAS in this process, the purpose of the present study was 2-fold: *1*) to clone the human FAS gene and its 5′-flanking region and compare its molecular regulation to that of other species, and *2*) to determine whether lipogenesis and FAS enzyme activity and expression levels were coordinately regulated in human adipose tissue by factors known to regulate fatty acid biosynthesis, namely, insulin, glucocorticoid, and fatty acids.

MATERIALS AND METHODS

FAS gene sequence and analysis. A P1 clone containing the human FAS gene was isolated by screening a human P1 genomic library (Genome Systems) using a PCR-amplified human FAS fragment based on conserved primers between the human and rat FAS (5′-GCAGGAGGTGGTGATTGCCG-3′ and 5′-CAGTGCCT-GGGTGGTGAGGA-3′). The amplified fragment used as a probe corresponded to a region in the first exon of the rat FAS cDNA. The P1 clone was subjected to shotgun subcloning, and subclones were confirmed by hybridization and sequencing. The subclones were inserted into pZERO-1 vector (Invitrogen Life Technologies) and subsequently sequenced and subjected to restriction analysis. The assembled sequence of ~4 kb of human FAS contained in the selected P1 clone was compared with the FAS sequences from Mouse (accession #AL 663090), Rat (accession #X 54671), Goose (accession #M 60623), and Chicken (accession #X 77339) obtained through GenBank (17). The sequences were compared using the multiple alignment program, Clustal W (Version 1.81) through Baylor College of Medicine Search Launcher using a gap opening penalty of 10:00 and gap extension penalty of 4:00.

To prove that the new sequence was contiguous and was indeed a part of the 5′ flanking region of the human FAS, 2 different sets of primers were designed and used to amplify specific genomic fragments. Human genomic DNA (100 ng; Clonetech) was used as a template in PCR reactions with 2 sets of primers, ORN 792 (5′GAA TGC AGT GGT GCA ATC TC3′) forward primer and ORN 795 (5′GAG TGA TTC CTC GAA TCG TG3′) reverse primer; ORN 816 (5′GCG GTC AGA AAA GGG AGG3′) forward primer and ORN 819 (5′GGG TTA CTG CCG GTC ATC3′) reverse primer (ORN nomenclature is for primer identification purposes). Reactions were performed using a standard 4-step PCR reaction with annealing at 50°C. The PCR products were subcloned using the TOPO TA cloning kit (Invitrogen Life Technologies) and subsequently sequenced using the Big Dye Terminator Kit (PE Biosystems) and an ABI377 DNA sequencer. These procedures were as described earlier (18).

Sequence analysis. The 1-kb sequences upstream from FAS in 5 species, human, mouse, rat, chicken, and goose (597 base pairs), were aligned in several steps. First, experimentally determined transcription factor binding sites found in all 5 species, including sites for responsiveness to insulin and glucocorticoids, were assumed to be orthologous and served as anchors for the global alignment. The segments between these binding sites were then aligned pairwise using standard algorithms and tools, such as AVID (19), PipMaker (20), and DIALIGN (21). Additional known binding sites not shared by all species, such as the agouti response element found in the rodents, were also used during this phase. Finally, the pairwise alignments were merged into a 5-sequence alignment, which was inspected and adjusted by hand.

For sequence identity comparison, we used Vista, developed at Lawrence Berkeley National Laboratory (22,23), following the default parameters of 100-bp window length and 75% conservation to demarcate conserved regions. All pairwise comparisons were made using 1 kb of sequence immediately upstream from the transcription start except for goose, for which only 597 bp of sequence were available.

Human subjects. Nonobese, nondiabetic women with a mean age of 43 ± 5 y and a BMI < 27 kg/m^2 were used in this study. To our knowledge, these subjects did not exhibit any metabolic disorders or diseases. Subcutaneous fat was obtained from abdominal surgery. No information was available on the medications that the subjects were taking or on their dietary habits or lipid profiles before surgery. The protocol regarding human tissue acquisition and culture was approved by the institutional review board for human subjects and the Committee for Research Protocols of University of Tennessee in Knoxville.

Tissue culture. The procedure described by Fried and Moustaid-Moussa (24) was used for tissue culture. Briefly, in a sterile hood, tissue was minced into 5- to 10-mg pieces and washed with warm sterile saline. Tissue fragments, ~300–500 mg in 15 mL DMEM [25 g/L glucose supplemented with 10 g/L bovine serum albumin (BSA) and 10 mg/L gentamicin], were cultured without hormone (Control), with insulin (10 nmol/L) alone (Ins) or with insulin (10 nmol/L) plus Dexamethasone (250 nmol/L) (Ins + Dex). Cultures were maintained at 37°C under an atmosphere of 50 mL/L CO_2. The medium was changed every other day. After 5 d of culture, some tissue from controls was digested with collagenase to isolate fat cells for transient gene transfections, and tissues were cultured for 2 more days under the same conditions but with or without the addition of linoleic acid (LA; 200 μmol/L). Tissue from each treatment was either frozen in liquid nitrogen and stored at −80°C for total RNA extraction, homogenized in sucrose buffer for FAS activity measurement, or digested with collagenase to isolate fat cells for the glucose incorporation assays. In addition, some tissue from the Ins or the Ins + LA treatment group was used for preparation of nuclei. The experimental design is shown in **Figure 1**.

Adipocyte isolation. Adipocytes were isolated from adipose tissue using the procedure of Rodbell (25) as modified by Honnor et al. (26). Briefly, tissue was washed with warm sterile saline and digested with collagenase (1 g/L) for 30–40 min in modified Krebs-Ringer bicarbonate albumin buffer [KRBA; pH 7.4, containing 40 g/L BSA, 5 mmol/L glucose and 200 nmol/L of the adenosine analog, *N*6-phenylisopropyladenosine (PIA)]. Adipocytes were isolated by gentle centrifugation (<500 × *g* for 1 min). After 3 washes with collage-

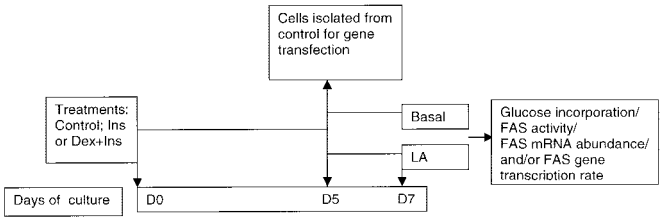

FIGURE 1 Experimental design.

nase-free buffer, the cells were resuspended with fresh buffer (2:8, v:v).

Glucose incorporation. Glucose incorporation was conducted by incubating fat cells with ^{14}C-glucose (27). Briefly, 0.5 mL of cell suspension (1–2 × 10^6 cells) was added to a 15-mL plastic vial with 0.5 mL of KRBA containing 0.25 μCi ^{14}C-glucose. Vials were gassed with 95% O_2 and 5% CO_2, sealed with a cap, and placed in an oscillating 37°C water bath. After 2 h of incubation, total lipid was extracted with heptane for determination of glucose incorporation into total lipid. Incorporation of ^{14}C-glucose into fatty acids and glyceride-glycerol was measured after saponification in ethanolic KOH, acidification, and heptane extraction (28). Cell size was measured by the Multisizer 3 Coulter Counter (Beckman Coulter), and cell number was calculated on the bases of the cell size and lipid content in each condition (29).

FAS activity. FAS activity was measured spectrophotometrically in crude cytosolic extracts of human adipose tissue and normalized per microgram DNA (30).

Northern blot analysis. RNA was isolated from adipose tissue using the guanidium isothiocyanate-cesium chloride density gradient method and analyzed by Northern blot (14). A 2-kb human FAS cDNA, kindly provided by Dr C. F. Semenkovich (Washington University, St. Louis, MO), was used as a probe. Data from autoradiograms were quantified by densitometric scanning and normalized to expression of 18S rRNA.

Preparation of nuclei and nuclear transcription run-on assay. Fat cells were isolated from tissue treated with Ins with or without LA. Nuclei were prepared according to previously described methods (15). Briefly, cells were homogenized and nuclei were pelleted with 2 centrifugations (500 × *g*). Nuclei were then stored in a buffer containing glycerol at −80°C until nuclear run-on assays were performed. These assays were conducted as described previously (15,31) by incubating nuclei with ^{32}P-UTP and unlabeled NTPs. Labeled RNA was purified and hybridized with plasmids containing cDNA encoding human FAS immobilized on nylon membranes. After hybridization and washing, membranes were exposed to film. Autoradiogram results were quantitated by scanning laser densitometry. The FAS transcription rate was normalized to that of β-actin. The human FAS probe was duplicated on the membrane (hFAS1 and hFAS2).

Gene construct. A fragment of the FAS 5′-flanking region spanning −70 to −50 containing a wild-type insulin responsive sequence (IRS) (CATGTG) or mutant IRS (TGTACG) was subcloned into a pGL2 SV promoter-luciferase plasmid (32); a control vector SV-β galactosidase construct was used to account for the transfection efficiency and normalize the luciferase data. A pGL2-luciferase vector without any DNA regulatory sequences was used as an additional control.

Transient transfections. Adipose tissue was cultured without any hormone (Control) for 5 d to eliminate any differences contributed by in vivo circulating factors. Adipocytes were isolated as before but washed twice with DMEM containing 200 nmol/L PIA, then resuspended in DMEM (1:1, v:v). Cells were transfected with gene constructs using electroporation (33). Transfected cells were treated without hormone (Control), with insulin alone (Ins) or with insulin plus Dex (Ins + Dex), in the absence or presence of LA (±LA) for 48 h. A SV-β-galactosidase fusion construct (Promega) was cotransfected to normalize transfection efficiency. PGL2 control, which contains the SV40 promoter linked to the luciferase gene, was used as a control. Luciferase and β-galactosidase activities in cell lysates were measured using a luminometer (Berthold) and a dual-luminescence assay kit (Tropix).

Statistics. Two-factor ANOVA with a randomized block design was used to determine the effects of hormone treatment, LA, and their interaction on glucose incorporation into fatty acids and total lipids, and on FAS activity levels and FAS expression levels. A significant overall *F*-test was followed by multiple comparisons using the least-squares means test to identify pairs of groups that differed significantly from each other. All tests were performed using a significance criterion of $\alpha = 0.05$.

RESULTS

FAS sequence. The alignment of the upstream region of human FAS with that of 4 other species is shown in **Figure 2**. Immediately upstream from the FAS coding region lies a region of ~150 bp that is highly conserved across all 5 species. Further upstream there is a region of little similarity, except between closely related species. Between −400 and −600, another highly conserved region is found. It is in this area that the FAS insulin response element (FIRE)1 binding site is located.

The identities of conserved regions are reported in **Table 1**. Sequence comparison (Fig. 2) clearly showed that ~1 kb upstream the initiation codon, the human FAS gene sequence is highly homologous to that of FAS genes from other species. However, our human FAS sequence extends an additional 3 kb (complete sequence deposited in GenBank, accession number AF 250144). PCR and subsequent sequencing confirmed that the new sequence is indeed a part of the known human FAS gene (data not shown).

Glucose incorporation. ANOVA indicated that glucose incorporation into both total lipid (**Fig. 3***A*) and the fatty acid component (Fig. 3*B*) were significantly affected by the hormonal treatments (Ins or Ins + Dex) and by LA (overall *F*-test, $P < 0.005$), but that the 2 factors did not interact. Irrespective of LA, insulin increased glucose incorporation into total lipids by ~ 54% compared with controls ($P < 0.05$). The addition of the glucocorticoid Dex further enhanced the effects of insulin, with Ins + Dex values ~60% greater than those for Ins alone ($P < 0.001$). Although there was no effect of LA in the control group, the PUFA attenuated the effects of Ins and Ins + Dex by ~ 35% ($P < 0.01$) in each of the 2 treatments. Both Ins and Ins + Dex increased glucose incorporation into fatty acids ($P < 0.05$) and LA diminished these responses ($P < 0.05$). When both LA treatments were combined, insulin tended to have an effect, $P = 0.099$.

FAS gene expression. Similar to glucose incorporation, both FAS activity (**Fig. 4***A*) and mRNA abundance (Fig. 4*B*) were regulated by LA ($P < 0.01$), hormonal treatment ($P < 0.0001$), and their interaction ($P < 0.05$). Both Ins and Ins + Dex increased FAS activity compared with controls ($P < 0.05$ and $P < 0.0001$, respectively), with Dex enhancing Ins effects ($P < 0.0001$); however, there was no effect of Ins with LA present. Again, LA attenuated the effects of Ins alone or in combination with Dex ($P < 0.05$), but did not alter FAS activity levels in untreated control cells.

Densitometric analysis of Northern blot autoradiograms indicated that FAS mRNA levels were increased 14-fold in tissue cultured with Ins compared with the control ($P < 0.01$) and ~100-fold with Ins + Dex ($P < 0.01$). Although LA did not affect the FAS mRNA level in tissue not treated with hormones, it did, however, inhibit FAS expression by 56 ± 8% ($P < 0.01$) and 54 ± 6% ($P < 0.01$) in tissue treated with Ins or Ins + Dex, respectively. Furthermore, in the presence of Ins, LA decreased the FAS transcription rate by 77% compared with Ins alone (Fig. 4C).

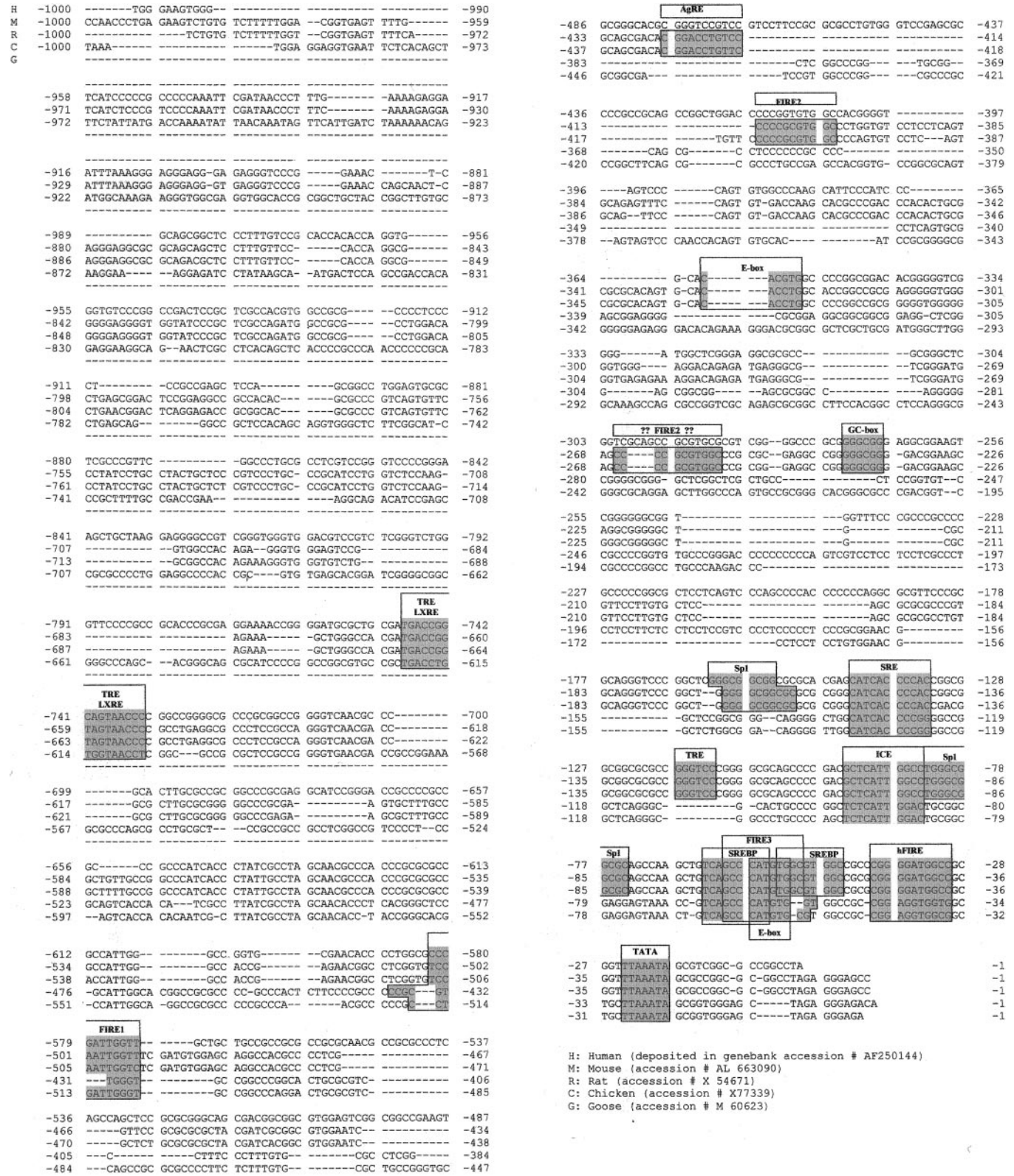

FIGURE 2 Sequence comparison of our human FAS promoter (AF 250144) to mouse, rat, goose, and chicken FAS promoters. Boxed sequences indicate selected regulatory sequences. Abbreviations: AgRE, agouti response element; E-box consensus, CANNTG; GC-box consensus, GGGCGG; hFIRE, hepatic FAS insulin response element; ICE, inverted CAATT element; LXRE, liver X receptor element; Sp1, stimulatory protein 1;SRE, sterol response element; TATA, TATA-box; TRE, thyroid response element.

The effects of insulin were paralleled by activation of the FAS promoter. In the cells transfected with the gene construct containing the wild-type insulin responsive sequences (IRS), both Ins and Ins + Dex increased luciferase activity normalized to galactosidase activity (**Fig. 5**; 178 ± 79 and 265 ± 88%, respectively, $P < 0.05$). Dex did not enhance insulin

TABLE 1

Identity levels (ID) and conserved regions among Human (H), Rat (R), Goose (G), Mouse (M), and Chicken (C) FAS promoters

Species compared	Overall ID, %	Conserved regions; ID, %
H/M	51.52	H-770-558/M-682-473; 72.7 H-182-1/M-188-10; 95.1
M/R	91.95	H-986-1/R-1000-1; 93.2
C/G	41.21	C-161-4/G-161-2; 94.4
H/C	44.5	No regions meeting conserved criteria

action on luciferase activity in gene constructs containing IRS, In cells containing mutant IRS or the control SV promoter alone, there was no difference in luciferase activity among any of the treatments (Fig. 5).

DISCUSSION

This paper addresses the ongoing controversy over regulation of lipogenesis in human adipose tissue. Two main questions have surrounded the issue of DNL in humans, particularly in adipose tissue: *1*) does DNL occur to such a degree that it can potentially contribute to whole-body lipid balance? and *2*) is DNL regulated by diet or energy restriction, as it is in rodents? Several studies determined that the amount of fat contributed by hepatic DNL ranged from 1–2 g/d for lean (34)

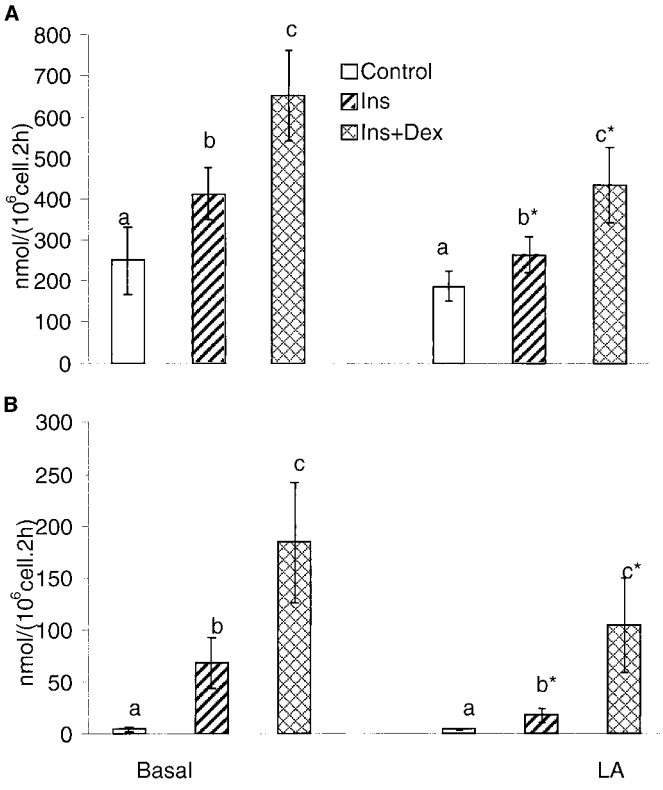

FIGURE 3 Effects of Ins and Ins + Dex on glucose incorporation into total lipid (*A*) and fatty acids (*B*) in human adipose tissue in the presence (*right*) or absence (*left*) of LA. Data are means ± SEM, $n = 6$. Bars in a panel without a common letter differ, $P < 0.05$. *Different from the corresponding basal mean, $P < 0.05$.

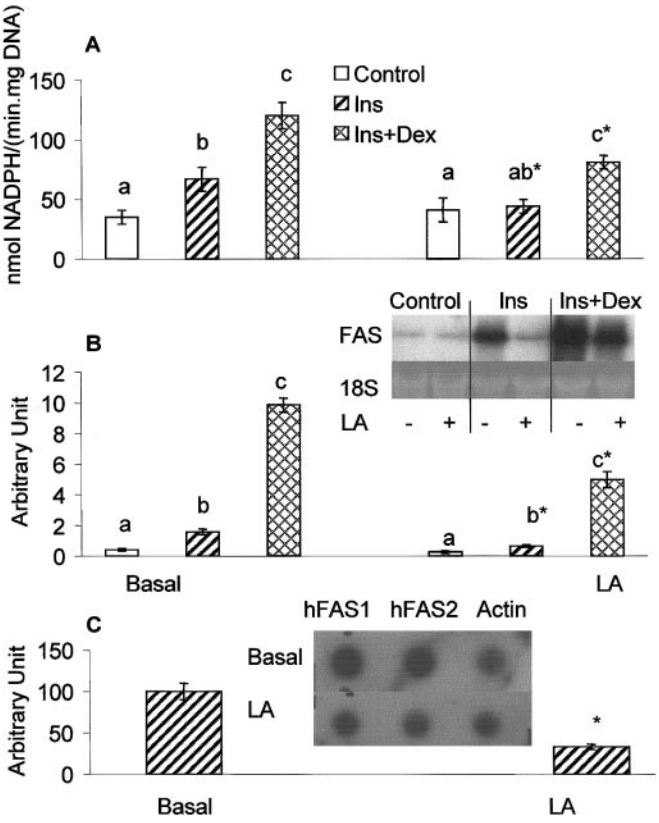

FIGURE 4 Effects of Ins and Ins + Dex on FAS activity (*A*), mRNA levels (*B*), and transcription rate (*C*) in human adipose tissue in the presence (*right*) or absence (*left*) of LA. FAS activity was normalized to per milligram DNA (*A*). Autoradiograms were quantified by densitometric scanning and the FAS signal was normalized to that of 18S rRNA, $n = 6$. Bars in a panel without a common letter differ, $P < 0.05$. *Different from the corresponding basal mean, $P < 0.05$. (*C*) Data are means of FAS transcription/actin transcription from 3 individual experiments; a representative autoradiogram is shown (*B* and *C*). Data are means ± SEM.

and eucaloric men (35), <6 g/d in lean and obese men and women consuming their normal diets (36,37) or fed excessive carbohydrates (7,9), and 7 g/d in former smokers consuming their diet ad libitum (38). Even at the highest measured levels (7 g/d), the amount of fat synthesized is minor relative to the average daily adult consumption of fat, which is 50–150 g (1). These relative lipogenic rates have been accepted to be gen-

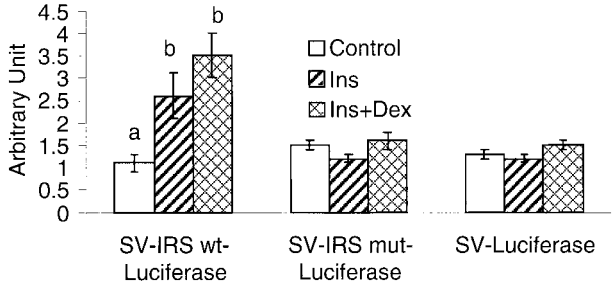

FIGURE 5 Effects of Ins, Dex, and Ins ± Dex, on FAS promoter function. Data for each of the 3 constructs (wild-type IRS, mutant IRS, and SV40 promoter alone) are means ± SEM, $n = 4$, after normalization to galactosidase activity. Bars within each gene construct without a common letter differ, $P < 0.05$.

erally lower than those reported for rodents. The differences have been widely attributed to a much higher fat intake in the typical Western diet (~35% of energy) compared with 8–12% in standard nonpurified diets, and to the corresponding elevation in rodent carbohydrate intake compared with the human diet. In direct support of this long-held belief, Swierczynski et al. (39) controlled for diet by maintaining rats and humans under similar protocols and found that the differences in lipogenic rates were actually moderate when similar amounts of fats and carbohydrates were consumed. We found that human expression of FAS varied among adipose tissue depots, ranging from very low levels to very high levels that equaled or surpassed FAS expression in rodents (unpublished data). The reason for this high variability is not clear and merits further investigation.

Both dietary and hormonal factors modify the rates of DNL in rodents in liver and adipose tissue, with induction by insulin and carbohydrates and suppression by fatty acids (5,6). The majority of human studies in vivo also suggest that diet modulates hepatic rates of DNL. Both overfeeding, particularly with excess energy derived from carbohydrates, and eucaloric elevations in carbohydrates were shown to increase hepatic DNL (7–9,12–13). Two of these same studies, however, did not find an effect of diet on lipogenesis in adipose tissue. Diraison et al. (12) utilized 3 treatment regimens to evaluate the effects of an acute glucose load (12 h) or 2 wk of carbohydrate overfeeding and found that neither treatment significantly altered estimates of adipose DNL rates or the mRNA levels of FAS or sterol regulatory element binding protein (SREBP)-1c. In a follow-up study, this same group examined rats consuming high-carbohydrate or high-fat diets and humans consuming moderately high-fat or moderately high-carbohydrate diets for 3 wk and measured mRNA levels of several lipogenic enzymes (13). They found no significant effect of either diet on expression of FAS, acetyl-CoA carboxylase, or SREBP-1c in subcutaneous or visceral adipose tissue biopsies.

Our findings in this study contrasted somewhat with those few earlier studies that actually examined adipose tissue DNL and concluded that the process is not regulated. Our data obtained in vitro clearly demonstrated that DNL, as measured by glucose incorporation into total lipids and fatty acids, increased significantly in response to insulin, a response that expanded when a glucocorticoid (Dex) was added to the medium. Increased lipogenesis by insulin was paralleled by significant upregulation of FAS enzyme activity and mRNA expression, which were again further enhanced by Dex. These findings are consistent with our previous work demonstrating that insulin increased FAS activity, expression, and transcription rates in human adipocytes (15,30). Beyond the obvious differences between in vivo and in vitro systems and conditions, several factors may explain our clear support of regulated adipose tissue lipogenesis and its absence in the studies reported by Letexier et al. (13) and Diraison et al. (12). In the former study, nondiabetic, nonobese subjects consumed relatively high-carbohydrate or high-fat diets, each for 3 wk, but neither regimen altered insulin or plasma glucose levels. In fact, insulin levels were slightly but not significantly lower in subjects consuming both the high-carbohydrate and high-fat diets. In the study by Diraison (12), 1 group of patients was subjected to a glucose load during the tissue sampling period, thereby raising both plasma glucose and insulin concentrations; however, the duration was only 12 h, at which point FAS mRNA levels were slightly but not significantly increased compared with the initial time point. It is possible that 12 h was too early to observe increases in FAS expression. Indeed, we previously reported that FAS transcription rates and mRNA levels in 3T3-L1 adipocytes were not significantly elevated by angiotensin II until 24 and 48 h, respectively, after treatment initiation (14). Therefore these in vivo studies and our past and current efforts do not yield contradictory findings. Our model, in which adipocytes were exposed to significant elevations in insulin levels over 7 d with constant glucose levels, may more closely parallel that of early insulin resistance, in which elevated insulin secretion is necessary to maintain euglycemia. On this basis, we predict that in vivo studies with hyperinsulinemic-euglycemic clamps and measurements made over a few days would produce results similar to ours. To our knowledge, such studies have not been performed.

The *cis*-acting elements necessary for tissue-specific and nutritional and hormonal regulation of FAS expression in transgenic mice expressing a CAT reporter gene linked to the FAS promoter are contained primarily in the 2.1 kb of the 5′-flanking region of this gene (16). Both ADD1/SREBP1 and an upstream stimulatory factor were identified as major transcription factors regulating FAS gene transcription (40–43). In rodents, several insulin response sequences including an enhancer sequence in the distal promoter of the rat FAS gene (−9700/−4606) were reported (41). We previously identified an insulin response element in the human FAS gene (44) that is similar to the insulin responsive E-box sequence we previously reported in the rat FAS gene in 3T3-L1 adipocytes (32). A partial sequence of the human FAS gene was previously reported (45,46); here, we extended this sequence to >4000 bp of the 5′-flanking region to study the *cis*-acting elements and *trans*-acting factors involved in the nutritional and hormonal regulation of human FAS. The entire sequence has the following accession number in GenBank: AF 250144, and selected regulatory sequences are highlighted in the partial sequence reported in Figure 2. Additional studies are required to address the functional importance of these sequences in human adipose tissue metabolism. It is worth noting that studies by Hsu et al. (46) identified 2 promoters (I and II) in the human FAS gene. In our studies, we focused on promoter I (used in all constructs for our current study), which was reported previously to be a strong promoter (46).

PUFA were shown previously to downregulate lipogenic genes in rodent but not human adipose tissue (46–50). The effects of PUFA on lipogenesis were studied more thoroughly in liver, in which PUFA were shown to suppress several lipogenic genes by decreasing their rates of transcription (48). This downregulation has been attributed to a sterol response element (−278/−131), a nuclear factor-Y site (−99/−93), and a distal PUFA response region (−7382/−6970) (49,50). However, within the promoter sequences tested in luciferase assays, we were unable to identify the PUFA response element. In fact, LA increased luciferase activity in all FAS promoter and SV promoter constructs used (data not shown), in stark contrast to its suppressive effects on FAS activity and transcription rate. We suspect that this effect is due to the absence of additional regulatory elements that are present in the endogenous gene but absent in our constructs, and that are required for FAS downregulation by LA. Additional studies will be required to confirm this possibility and to identify the specific regions of the FAS gene that confer downregulation of the endogenous gene with LA treatment.

Dex did not significantly enhance insulin action on luciferase activity in gene constructs containing IRS, suggesting that Dex may not interact with the IRS found in the FAS gene and that Dex-responsive sequences may be located further upstream or downstream of the FAS promoter. Additional studies will be required to identify the specific regions of the

FAS gene that confer upregulation of the endogenous gene with Dex treatment.

In conclusion, our studies demonstrated the importance of the human adipose tissue culture model for investigating regulation of lipid metabolism in humans. These results emphasized the responsiveness of human adipose tissue lipogenesis to fatty acids, insulin, and glucocorticoid. Additional functional analysis of the human FAS gene 5′-flanking region that we cloned is required to determine mechanisms mediating its regulation by PUFA and Dex, both of which potently but differentially modulate insulin effects. Such studies will provide a better understanding of the regulation of lipid metabolism and adipose tissue mass in humans by nutrients and hormones.

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