Feeding and Insulin Increase Leptin Translation

IMPORTANCE OF THE LEPTIN mRNA UNTRANSLATED REGIONS

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The post-transcriptional mechanisms by which feeding and insulin increase leptin production are poorly understood. Starvation of 6–7-week-old rats for 14 h decreased leptin mRNA level by only 22% but decreased plasma levels, adipose tissue leptin content, and release by over 75%. The decreased leptin with starvation was explained by >85% decrease in relative rates of leptin biosynthesis measured by metabolic labeling and immunoprecipitation. In vitro insulin treatment of adipose tissue from fed or starved rats for 2 h increased relative rates of leptin biosynthesis by 2–3-fold, and the effect was blocked by inhibition of phosphatidylinositol 3-kinase or mammalian target of rapamycin. Consistent with the hypothesis that feeding/insulin increases leptin translation, more leptin mRNA was associated with polysomes in adipose tissue of fed than starved rats, and in vitro incubation of adipose tissue of starved rats with insulin shifted leptin mRNA into polysomes. To assess the mechanisms regulating leptin translation, chimeric human leptin untranslated region (UTR) reporter constructs were transiently transfected into differentiated 3T3-L1 adipocytes. The 5′-UTR of leptin mRNA increased luciferase reporter activity 2–3-fold, whereas the full-length 3′-UTR (nucleotides 1–2804) was inhibitory (~65%). Sequences between nucleotides 462 and 1130 of the leptin 3′-UTR conferred most of the inhibitory effect. Insulin stimulated the expression of constructs that included both the full-length 5′-UTR and the inhibitory 3′-UTR, and the effect was blocked by inhibition of phosphatidylinositol 3-kinase or mammalian target of rapamycin. Our data suggest that insulin derepresses leptin translation by a mechanism that requires both the 5′-UTR and the 3′-UTR and may contribute to the increase in leptin production with feeding.

Serum leptin levels decrease with starvation and increase in response to feeding. These changes in serum leptin are not entirely accounted for by variations in leptin mRNA levels (1–4). In vitro, insulin increases leptin secretion from rat adipocytes 2-fold without affecting leptin mRNA levels, and the insulin effect was not blocked by inhibition of transcription with actinomycin D (5, 6), suggesting that insulin increases leptin release through post-transcriptional mechanisms. No previous studies have directly assessed the regulation of leptin by feeding or insulin at the translational step. Thus, in the current study, we used metabolic labeling and immunoprecipitation to measure basal and insulin-stimulated leptin biosynthesis in adipose tissue from fed and starved rats. To provide further evidence for translational regulation of leptin mRNA, we compared polysome profiles in adipose tissue from fed and starved rats and the effects of in vitro insulin treatment.

The 5′-untranslated region (UTR) of mouse and human leptin mRNA has potential for secondary structure that is implicated in translational regulation (5). Additionally, the 3′-UTR of both mouse and human leptin is relatively long (~3 kb) and possesses multiple conserved motifs with high potential for secondary structure (7). We noted potential AU-rich elements in the 3′-leptin UTR including a UUAUUUAUU nonamer (nucleotides 487–495, where 1 is the first nucleotide after stop codon, see Fig. 6A) and several AUUU(U)A (starting at nucleotides 499, 785, 1765, and 1951) that are implicated in controlling mRNA stability and translation (8). We therefore tested the effects of the 5′- and 3′-UTRs of human leptin mRNA on expression of a reporter gene after transfection into differentiated 3T3-L1 adipocytes. Overall, our data show that insulin increases leptin translation through mechanisms involving both the 5′- and the 3′-leptin UTRs. The stimulatory effect of insulin on leptin synthesis may contribute to the increase in leptin production with feeding.

EXPERIMENTAL PROCEDURES

Animals—Male Wistar rats (5 weeks old) were purchased (Charles River Laboratory, Wilmington, MA) and housed with free access to laboratory chow and water until they reached the age of 6–7 weeks. The dark-light cycle was maintained at 12 h of dark and 12 h of light. After 14 h of starvation or ad libitum feeding with water available, animals were anesthetized using CO2 and killed by decapitation, and trunk blood was collected.

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‡‡ The abbreviations used are: UTR, untranslated region; LPL, lipoprotein lipase; LUC, luciferase; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; RT, reverse transcription.

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Epididymal and retroperitoneal fat pads were dissected, pooled, minced into 5–10 mg fragments, and then used for experiments. After washing with 0.9% NaCl on a 250-μm nylon mesh, an aliquot of tissue was frozen immediately in liquid nitrogen to measure tissue leptin content and leptin mRNA levels. The protocol was approved by the Institutional Animal Care and Use Committee of the University of Maryland at Baltimore.

Measurement of Leptin Secretion and Tissue Leptin Content—Leptin release and tissue leptin content were measured as described previously (9). Leptin concentrations were measured by radioimmunoassay (Linco, St Charles, MO) with inclusion of a leptin standard (0.25 ng/ml).

Determination of Fat Cell Size and Fat Cell Number—Mean fat cell weight was determined by Coulter counting of freshly isolated adipocytes (10). Fat cell number was determined by dividing the weight of lipid in tissue (measured with Folch extraction (11)) by mean fat cell weight.

Measurement of Relative Rates of Leptin Biosynthesis—After preincubation of adipose tissue fragments in minimal essential medium (without methionine and cysteine, Sigma) in the absence (basal) or presence of insulin (6 nM) for 2 h using the modified method of Chomczynski and Sacchi (15). Leptin mRNA levels were detected as described previously (16).

RNA Extraction and Northern Blotting—Total RNA was extracted from adipose tissue (frozen immediately (0 minutes)) or after incubation in the absence (basal) or in the presence of insulin (6 nM) for 2 h using the modified method of Chomczynski and Sacchi (15). Leptin mRNA levels were detected as described previously (16).

Polysome Profile and RT-PCR—Polysome profiles were obtained as described previously (13). Post-mitochondrial supernatant was prepared from adipose tissue from overnight starved or ad libitum fed rats and after insulin treatment by homogenizing tissue in Buffer I (10 mM Tris, 150 mM KCl, 10 mM MgCl₂, 0.1% Triton X-100, 20 mM dithiothreitol, 150 μg/ml cycloheximide, 250 μg/ml heparin, 100 units/ml RNasin) and centrifugation. The tissue lysate was layered on the top of a 10–50% continuous sucrose gradient prepared in Buffer II (20

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**Translational Regulation of Leptin**

**TABLE 1**

Sequences of oligomers used for generation of reporter constructs, sequencing, and RT-PCR

| Construct     | Oligonucleotide name | Sequence                                      |
|---------------|----------------------|-----------------------------------------------|
| 5'-UTRpGL3    | Hob5'-UTR F          | 5'-CACCAAAACCTCTAGAAGATGCAGCCAACGCCAAGGCTGCAAGCGCCATCGG-3' |
|               | Hob 5'-UTR R         | 5'-ATGAGAAAACCTGACCTGATGC-3'                  |
| 3'-UTRpGL3-full| HOB 3'UTR: 1-20 EcoRV F | 5'-GAGATTCCGCTTGAAGTCATCCTTCC-3'              |
| 3'-UTRpGL3-1.1kb| HOB 3'UTR: 2804-2782 Fse1 R | 5'-GGCCGCGACAGCCGATCCTGCTG-3'                 |
| 3'-UTRpGL3-0.5kb| HOB 3'UTR: 462 Fse1-442 R | 5'-TGCGCCGCGCTTGAAGTCATCCTGCTG-3'              |

**Sequencing**

| Vector | oligonucleotide name | Sequence |
|--------|----------------------|----------|
| T3 primer | T3 primer primer | 5'-TAATACACCTCACTAAAAGG-3' |
| T7 primer | T7 primer primer | 5'-GTAGAAGACCACTTACTAGGG-3' |
| pGL3 control | pGL3 control primer | 5'-CTAGACAAATAGCGTTCCTCC-3' |
| PGL3 control 1981-1908 | PGL3 control primer | 5'-ATCCCTCAATTGAGGCAAG-3' |
| PGL3 control 1981-1908 | PGL3 control primer | 5'-TTTTGACACATTCTAACAT-3' |
| Leptin 3'-UTR | Leptin 3'-UTR | 5'-TTTTGACACATTCTAACAT-3' |
| Leptin 3'-UTR | Leptin 3'-UTR | 5'-TTTTGACACATTCTAACAT-3' |
| Leptin 3'-UTR | Leptin 3'-UTR | 5'-TTTTGACACATTCTAACAT-3' |
| Leptin 3'-UTR | Leptin 3'-UTR | 5'-TTTTGACACATTCTAACAT-3' |
| Leptin 3'-UTR | Leptin 3'-UTR | 5'-TTTTGACACATTCTAACAT-3' |
| Leptin 3'-UTR | Leptin 3'-UTR | 5'-TTTTGACACATTCTAACAT-3' |
| Leptin 3'-UTR | Leptin 3'-UTR | 5'-TTTTGACACATTCTAACAT-3' |
| Leptin 3'-UTR | Leptin 3'-UTR | 5'-TTTTGACACATTCTAACAT-3' |

**RT-PCR**

| Oligonucleotide name | Sequence |
|----------------------|----------|
| Leptin               | 5'-CACCAAAACCTCTAGAAGATGCAGCCAACGCCAAGGCTGCAAGCGCCATCGG-3' |
| Leptin R             | 5'-AGCTCTTAGAAGATGCAGCCAACGCCAAGGCTGCAAGCGCCATCGG-3' |
| Cyclophilin A F      | 5'-ACAGGTGCAATGACGCTATGAGGCATACAGGGGACACAG-3' |
| Cyclophilin A R      | 5'-GCTTCTCCGACCTACAGAGG-3' |
| Luciferase F         | 5'-GAATCC TCCTCCGACCTACAGAGG-3' |
| Luciferase R         | 5'-CTAGACAAATAGCGTTCCTCC-3' |

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mRNA sequence (GenBank™ accession number, gi) UTR PCR transcripts were designed using human leptin primers and oligomers (Table 1) used to generate the leptin between the second and the fifth subculture.

dexamethasone (1/10,000) and insulin (167 nM), and 3T3-L1 adipocytes were transfected with 1

tiated 3T3-L1 adipocytes and Luciferase Assay were generated by PCR using a full-length human leptin (where 1 is the first nucleotide after stop codon, see Fig. 5) was then cloned into the downstream of the luciferase. Leptin 3'UTR truncations left 462, 1130, or 2804 nucleotides and NcoI restriction sites) was cloned into the upstream of the luciferase coding region. The entire (or truncated) 3'UTR-LUC) were treated for 16 h with or without 500 nM rapamycin and release of leptin during a 3-h acute incubation is indicated by black bar.**, \( p < 0.001 \) fed versus starved.

**Chimeric Leptin UTR-Luciferase Reporter Constructs**—The primers and oligomers (Table 1) used to generate the leptin UTR PCR transcripts were designed using human leptin mRNA sequence (GenBank™ accession number, gi 6981147). The leptin UTRs were cloned into the pGL3 control vector (Promega, Madison, WI) that contains SV40 promoter and enhancer sequences to drive a high and stable expression of luciferase (LUC). The entire 5'UTR of human leptin mRNA (56 bp, synthesized as oligomers with HindIII and NcoI restriction sites) was cloned into the upstream of luciferase coding region. The entire (or truncated) 3'UTR was then cloned into the downstream of the luciferase. Leptin 3'-UTR truncations left 462, 1130, or 2804 nucleotides (where 1 is the first nucleotide after stop codon, see Fig. 5) were generated by PCR using a full-length human leptin cDNA plasmid as template (17).

**Transient Transfection of Reporter Constructs into Differentiated 3T3-L1 adipocytes and Luciferase Assay**—On day 4 of differentiation, 3T3-L1 adipocytes were transfected with 1 \( \mu \)g of DNA using Lipofectamine Plus reagents (Invitrogen). Briefly, cells were washed with phosphate-buffered saline and incubated with reduced serum medium (Opti-MEM®, modification of Eagle’s minimal essential medium, Invitrogen) for 45 min at 37 °C. Pre-complex DNA-Plus-Lipofectamine™ reagent was prepared according to the manufacturer’s protocol and added into each well for transfection. After overnight transfection, cells were replenished with 10% fetal bovine serum Dulbecco’s modified Eagle’s medium and incubated for another 36 – 48 h for expression. For monitoring transfection efficiency, pRL-TK vector (containing Renilla luciferase, Promega) was co-transfected. Cells cotransfected with various UTR-pGL3 constructs and pRL-TK vectors were harvested in lysis buffer, and activities of firefly and Renilla luciferase were measured by using the Dual-Luciferase assay kit (Promega) in a luminometer (Turner Designs, Sunnyvale, CA). The reporter activity was expressed as arbitrary LUC units (firefly/Renilla).

To determine whether insulin increases the activity of the leptin-UTR-luciferase constructs, cells were incubated with serum-free Dulbecco’s modified Eagle’s medium with 5 mM glucose and 3% bovine serum albumin, with or without insulin (100 nM), for an additional 3 h prior to harvesting. To assess the signaling pathways by which insulin increased luciferase activity, cells transfected with constructs containing full-length 5'- and 3'-UTR (3'5'-UTR-LUC) were treated for 16 h with or without insulin in the absence or presence of phosphatidylinositol 3-kinase (PI3K) (LY294002, 50 \( \mu \)g/ml or wortmannin, 100 nM) or the mTOR inhibitor (rapamycin, 100 nM).

**RNA Extraction and RT-PCR**—Parallel wells were transfected and used for RNA extraction using TRIzol reagent (Invitrogen). LUC mRNA levels were determined by performing RT-PCR using a DuraScript™ RT-PCR kit (Sigma). Cyclophilin A mRNA levels were used as a control. Products were run

### Table 2

| Body weight | Fat cell size | Serum leptin |
|-------------|--------------|--------------|
| Fed         | 173.1 ± 4.8  | 0.044 ± 0.006|
| Starved     | 169.4 ± 3.7  | 0.048 ± 0.0007|

* ND, not detectable (<0.25 ng/ml)
on 1% agarose gels, visualized by staining with ethidium bromide, and quantified using the Bio-Rad imaging system.

Statistical Analyses—All data are mean ± S.E. Prior to statistical analysis, data were log-transformed to normalize variance. One-way or two-way analysis of variance was used to determine treatment effects versus the appropriate control group at a specific time point. When significant main effects and/or interactions were found, post hoc comparisons between treatments were made with a Bonferroni t test. Significance was set at the \( p < 0.05 \) level.

RESULTS

Changes in Leptin mRNA Levels Do Not Explain the Decreased Leptin Release or Adipose and Serum Leptin Levels after Short Term Starvation—Body weight and fat cell size of overnight-starved (14 h) rats did not differ from those of \textit{ad libitum} fed animals (Table 2, NS, \( n = 4 \) each). Overnight starvation decreased leptin mRNA levels in adipose tissue by 22% (see Fig. 2A, \( p = 0.034, n = 5 \)), yet decreased serum leptin by more than 85% (1.7 ng/ml \textit{versus} not detectable <0.25 ng/ml, \( p < 0.01, n = 4 \)). In addition, overnight starvation decreased tissue leptin content by 74 ± 9% (fed; 22 ± 5 \textit{versus} starved; 7 ± 2 ng of leptin/10^7 cells, \( p < 0.01, n = 5 \)) (Fig. 1). Leptin release during an acute 3-h incubation of tissue fragments was 90% lower in adipose tissue from starved as compared with fed rats (fed: 33.9 ± 10.7 \textit{versus} starved; 3.6 ± 2.1 ng of leptin/10^7 cells \times 3 h, \( p < 0.01, n = 5 \)). Interestingly, adipose tissue from fed animals secreted more leptin than their initial stores (160 ± 8%), suggesting that new synthesis contributes to leptin release over 3 h. In contrast, adipose tissue from starved animals secreted less than their initial leptin stores (60 ± 6% of their initial level).

Starvation Decreased and Insulin Stimulated Leptin Biosynthesis—To determine whether changes in leptin biosynthesis can explain starvation-induced changes in serum leptin levels, relative rates of leptin biosynthesis in adipose tissue of fed and starved rats were assessed with metabolic labeling. Starvation decreased the general protein synthesis in adipose tissue by ~25% (fed; 10.8 ± 2.0 \textit{versus} starved; 8.0 ± 2.5 \times 10^6 cpm/10^6 cells, \( n = 3 \), as expected (14)). Even after correcting for its effect on general protein synthesis, starvation over 3 h decreased relative rates of leptin biosynthesis by more than 85% (Fig. 2B, \( p < 0.01, n = 4 \)).

In accordance with previous studies on adipocytes from fed rats (5, 6), insulin treatment \textit{in vitro} for 2 h did not affect leptin mRNA levels in either starved or fed adipose tissue. However, insulin increased total protein synthesis (starved: basal; 8.0 ± 2.5 \textit{versus} insulin; 9.2 ± 2.9 \times 10^6 trichloroacetic acid-precipitable cpm/10^6 cells, \( n = 3 \); fed: basal; 10.8 ± 2.0 \textit{versus} insulin; 14.4 ± 2.3 \times 10^6 cpm/10^6 cells, \( n = 3 \)) as well as relative rates of leptin biosynthesis. After correcting for the difference in total protein synthesis, insulin stimulated relative rates of leptin biosynthesis in adipose tissue of starved rats by 3-fold and \textit{ad libitum} fed rats by 2-fold (each \( p < 0.01, n = 4 \); Fig. 2B). As a result, insulin-stimulated rates of leptin biosynthesis were ~4-fold greater in the fed as compared with the starved groups (\( p < 0.05, n = 4 \)). The marked increase in leptin biosynthesis without a change in leptin mRNA levels indicates that insulin increased leptin biosynthesis by increasing the translation of leptin mRNA.

The Effects of Starvation and Insulin on Leptin Biosynthesis Are Specific, Comparison with LPL—In agreement with previous studies (14), overnight starvation did not change the rates of basal LPL biosynthesis in adipose tissue (Fig. 2C, each \( n = 3 \)). These data show the decrease in leptin biosynthesis after starvation was specific and not a result of a general decline in the biosynthesis of adipose-specific genes or due to an artifact related to alterations in the specific activity of the [35S]Met/Cys precursor pool. Additionally, insulin increased the relative rates of LPL biosynthesis both in starved (+72 ± 13%, \( p < 0.05, n = 3 \)) and in fed tissue (+66 ± 21%, \( p < 0.05, n = 3 \)) but to a lesser extent than leptin.

Signaling Pathways in Insulin-induced Leptin Biosynthesis—To investigate the signaling mechanisms by which insulin stim-
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ulates leptin biosynthesis, we tested the effect of pharmacological inhibitors of the PI3K and mTOR pathways, wortmannin or LY294002 and rapamycin, respectively. Neither inhibitor affected basal leptin synthesis or release, but both blocked the insulin stimulation of leptin biosynthesis and release (Fig. 3, A and B). The inhibitors did not affect basal but decreased insulin-stimulated general protein synthesis by 5–10% (data not shown), much less than their effects on leptin biosynthesis (over 90% inhibition).

Overnight Starvation Decreased the Association of Leptin mRNA with Polyribosomes—To provide further evidence that feeding increases leptin mRNA translation, we compared polysome profiles in adipose tissue from starved and fed rats. The UV absorbance at A260 and/or the distribution of ribosomal RNA in the different gradient fractions did not differ in the fed and starved groups (Fig. 4, A and B). In adipose tissue of starved rats, ~90% of leptin mRNA was associated with monosome/free ribosome fractions (fractions 8–12) (Fig. 4, C and D). In contrast, in adipose tissue from fed rats, 60% of leptin mRNA was associated with monosomes, and 40% of the leptin mRNA was found in the heavier fractions (fractions 1–7) that contained polyribosomes, suggesting that feeding increased the translational initiation of leptin mRNA. In contrast, starvation did not affect the association of the control mRNA cyclophilin A with polysomes, indicating that leptin is among the subset of mRNAs whose translation is specifically regulated by feeding (Data not shown). Similar results were obtained in four independent experiments.

Insulin Increased the Association of Leptin mRNA with Polyribosomes—To determine whether the greater association of leptin mRNA in the fed state was mediated by insulin, the effects of insulin on the polysome profiles of leptin mRNA were analyzed after in vitro insulin treatment (6 nm, 3 h) of adipose tissue from starved animals. Insulin did not affect the UV absorbance at A260, distribution of ribosomal RNA in the different gradient fractions (Fig. 5, A and B), or the polysome profile of cyclophilin A mRNA (data not shown). Insulin shifted leptin mRNA from monosomes into polysomes fractions (Fig. 5B).

The Leptin 5′-UTR Stimulated and the 3′-UTR Suppressed Expression of a Reporter Construct—To study the molecular mechanism(s) involved in the regulation of leptin translation, we tested the effects of the leptin 5′- and 3′-UTRs on the translational efficiency using chimeric luciferase reporter constructs (Fig. 6, A and B) transiently transfected to 3T3L1 adipocytes. The strong promoter and enhancer sequences in the constructs drove similar levels of luciferase mRNA expression under all conditions, as illustrated by the typical gel in Fig. 6E. Thus, the changes in luciferase activity observed with the leptin UTR-luciferase constructs were due to the altered levels of translation of the reporter. Insertion of 5′-UTR of leptin upstream of luciferase coding region (5′-UTR/pGL3) increased luciferase activity 2–3-fold over the pGL3 control (n = 5, p < 0.001, Fig. 6C). The 5′-UTR was also stimulatory when inserted to a vector containing the leptin 3′-UTR in the downstream of luciferase coding region (Fig. 6D). Taken together, these data indicate that the leptin 5′-UTR includes positive cis element(s).

Insertion of the entire leptin 3′-UTR (1–2804) downstream of the luciferase in the control vector (3′-UTR/pGL3) and in a construct with the leptin 5′-UTR (5′-3′-UTR/pGL3) decreased luciferase activity by 50% (n = 5, p < 0.01) and 65%, respectively (p < 0.01, n = 4), as compared with appropriate controls (Fig. 6, C and D). Thus, negative element(s) in 3′-UTR suppressed luciferase activity independent of elements in the leptin 5′-UTR. To determine the location of these negative elements, 3′-UTR constructs that deleted AU-rich regions (Fig. 6A) with predicted secondary structure were tested. Removal of sequences in the leptin 3′-UTR distal to nucleotide 1130, (1–1130, 5′-3′-ΔUTR/pGL3) significantly decreased the magnitude of the inhibitory effect from 65% (full-length 3′-UTR) to only 35%. A construct with only the proximal 1–462 of the leptin 3′-UTR (5′-3′-ΔUTR/pGL3) was ineffective in suppressing luciferase activity. Thus, we concluded that elements between nucleotides 462 and 1130 were largely responsible for the suppressive effect of the leptin 3′-UTR on reporter activity but that more distal elements also played a role. Similar negative effects on lucif-
Erase activity were obtained when we used the truncated 3′-UTR in constructs that did not include the leptin 5′-UTR upstream of LUC coding region (data not shown).

**Insulin Increased the Luciferase Activity of the 3′-UTR Inhibitory Constructs**—To determine whether the 5′-UTR and/or 3′-UTR was important for the insulin stimulation of leptin translation, the transfected cells were serum deprived for 1 h and then treated with or without insulin for 3 h before harvesting. Insulin increased LUC activity of the reporter constructs 5′-3′-UTR/pGL3 (+82%, n = 4, p < 0.001) and 5′-3′ΔUTR/pGL3 (+30%, n = 4, p < 0.05). In contrast, insulin did not affect the LUC activity driven by the control vector, 5′-UTR/pGL3, 3′-UTR/pGL3, and 5′-3′ΔUTR/pGL3.

**Insulin Effects on Luciferase Activity Were Blocked by Inhibition of PI3K or mTOR**—To provide direct evidence that the insulin effect on leptin translation involved activation of PI3K and mTOR, 3T3L-1 adipocytes were transfected with the full-length (3′-5′-UTR-LUC) and treated with or without insulin in the absence or presence of inhibitors of these pathways overnight (16 h). Insulin increased the expression of LUC activity by 2.5-fold, and these effects were blocked with the inhibitor treatments (Fig. 7).

**DISCUSSION**

We provide several lines of evidence that increases in leptin production with feeding are due to increased rates of translation of leptin mRNA and that insulin is an important regulator of leptin mRNA translation. First, despite only modestly higher (+22%) levels of leptin mRNA in adipose tissue of fed as compared with starved rats, relative rates of leptin biosynthesis, leptin content, and leptin release were 4–5-fold higher. The effect of starvation on leptin biosynthesis was specific as rates of LPL biosynthesis were unaffected (14). Furthermore, feeding shifted leptin
mRNA into the polysome fractions, indicating an increase in translational initiation. Finally, studies of chimeric constructs established that the insulin stimulation of leptin translation occurs by derepression and requires the presence of both 5'-UTRs and 3'-UTRs.

Consistent with the hypothesis that increases in insulin levels with feeding contribute to the higher leptin synthesis, we found that in vitro treatment of adipose tissue with insulin increased relative rates of leptin biosynthesis without altering leptin mRNA levels. Our metabolic labeling results show that the insulin-induced increase in leptin biosynthesis was specific as the magnitude of the effect far exceeded its small effect on overall protein synthesis. Furthermore, we found that pharmacologic inhibitors of PI3K-mTOR signaling blocked the insulin effect. Bradley and Cheatham (6) reached a similar conclusion with studies of leptin release from isolated adipocytes, but they did not directly measure leptin biosynthesis. We also noted that the ability of insulin to stimulate relative rates of leptin biosynthesis was quite robust (200–300%) as compared with its effect on LPL biosynthesis (only 60–70%), suggesting that the mechanisms involved differ. Since inhibition of PI3K also blocks the insulin effect on LPL synthesis (18), further work is needed to establish the mechanisms involved in the nutritional regulation of the synthesis of these two key adipocyte secretory proteins.

FIGURE 6. Translational regulation of leptin by cis elements in its mRNA. A, schematic presentation of 3'-UTR of human leptin mRNA. The UUAUUAUU A U nonamer is conserved both in human (H) and mouse (M) 3'-UTR of leptin. B, chimeric UTR-LUC constructs carrying the 5'-UTR and/or various lengths of 3'-UTR of leptin mRNA. The pGL3 control contained no leptin UTR sequences and was used as control. C–E, differentiated 3T3-L1 cells were transiently co-transfected with the chimeric leptin UTR-LUC constructs and the pRL-TK (Renilla luciferase vector) as described under “Experimental Procedures.” Cells were harvested 36 h after transfection, and luciferase activity was assessed using the Dual-Luciferase assay system. Transfection efficiency for each well was normalized by dividing firefly luciferase by Renilla luciferase activity. Data were presented as mean ± S.E. of ≈ three independent experiments performed in duplicate. Assessment of luciferase mRNA levels was assessed by RT-PCR with RNA extracted from parallel transfections. PCR products were run on agarose gels and stained with ethidium bromide, and a representative scan is displayed in E.
The 5′-UTR of leptin mRNA is relatively short (56 bp) but is conserved between species (74% between mouse and human, 93% between mouse and rats). Recently, Kandror and colleagues (5) suggested a potential secondary structure in 5′-UTR of mouse and human leptin mRNAs. We also confirmed with the KineFold program that there is a potential pseudoknot in the 5′-UTR of human leptin mRNA. Although in many cases, 5′-UTR secondary structure inhibits translation (19), we observed that the 5′-UTR of leptin increased LUC activity 2–3-fold without any apparent effect on luciferase mRNA levels. A stimulatory effect of secondary structure in 5′-UTR on translational efficiencies of apoB (20) and heat shock protein 70 (21) has been previously observed, but the mechanisms involved are not understood. We speculate that the potential secondary structure or cis element in 5′-UTR of leptin may provide binding sites for eukaryotic translation initiation factors or other RNA-binding proteins that promote formation of translation initiation complexes.

In contrast to the activating effect of 5′-UTR, the 3′-UTR significantly inhibited translational efficiency. The 3′-UTR of leptin mRNA extends 3 kb from the stop codon and has multiple potential structured elements (7). Analysis with the KineFold program extends 3 kb from the stop codon and has multiple negative elements. Although the 3′-UTR of leptin contains several negative elements. Although the 3′-UTR of leptin does not contain clustered AUUUA sequences as in the 3′-UTR of many other cytokine genes (22), we noted several individual AUUUA motifs and one AUAAUUAAU nonamer that are conserved between human and mouse in the 3′-UTR of leptin (Fig. 6A). Although the leptin 3′-UTR does not include typical strong AU-rich elements, studies of AU-rich elements in the COX2 3′-UTR indicate that such elements are nevertheless capable of modulating translational efficiency and mRNA stability (23).

Overall, our data suggest that feeding, at least in part through the action of insulin, increases the translation of leptin mRNA. Insulin may increase the translational efficiency of leptin mRNA by post-translational modification or de novo synthesis of a factor, or factors, that interact with the leptin UTRs. Further work is needed to define the cis elements that modulate leptin mRNA translation in response to alterations in nutritional state and to identify any RNA binding proteins involved.

In summary, metabolic labeling and polysome profile studies demonstrate that feeding, at least in part through insulin, causes a specific increase in leptin translation. Studies of the expression of chimeric constructs showed the essential roles of the leptin 5′- and 3′-UTRs in the control leptin mRNA translation and in mediating the stimulation by insulin. Although insulin also increases leptin secretion per se (4–6), the magnitude of the effect of insulin on translation that we observed suggests that it is the main mechanism responsible for the higher tissue leptin content and release in adipose tissue from the young, insulin-sensitive rats that we studied herein.

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