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New insights into adipocyte-specific leptin gene expression

Christiane D. Wrann1,2 and Evan D. Rosen1,3,*

1Division of Endocrinology, Diabetes, and Metabolism; Beth Israel Deaconess Medical Center; Boston, MA USA; 2Dana-Farber Cancer Institute and Department of Cell Biology; Harvard Medical School; Boston, MA USA; 3Broad Institute; Cambridge, MA USA

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*Correspondence to: Evan D. Rosen; Email: erosen@bidmc.harvard.edu

The adipocyte-derived hormone leptin is a critical regulator of many physiological functions, ranging from satiety to immunity. Surprisingly, very little is known about the transcriptional pathways that regulate adipocyte-specific expression of leptin. In a recent published study, we pursued a strategy integrating BAC transgenic reporter mice, in vitro reporter assays, and chromatin state mapping to locate an adipocyte-specific cis-element upstream of the LEP gene in human fat cells. Quantitative proteomics (stable isotope labeling by amino acids in cell culture, SILAC) with affinity enrichment of protein-DNA complexes identified the transcription factor FOSL2 as a specific binder to the identified region. We confirmed that FOSL2 is an important regulator of LEP gene expression in vitro and in vivo using cell culture models and genetic mouse models. In this commentary, we discuss the transcriptional regulation of LEP gene expression, our strategy to identify an adipocyte-specific cis-regulatory element and the transcription factor(s) responsible for LEP gene expression. We also discuss our data on FOSL2 and leptin levels in physiology and pathophysiology. We speculate on unanswered questions and future directions.

Introduction

Leptin is an adipocyte-derived hormone that regulates food intake and energy expenditure, acting primarily through the central nervous system.1,3 In addition to its well-established role in metabolism, leptin also affects physiological processes as wide-ranging as reproduction, thyroid function, bone density, and immune biology.4,5 Recombinant leptin has been developed as a drug for treating insulin resistance in patients with lipodystrophy.6 Although early studies using leptin as a weight loss agent in human obesity proved disappointing,7 recent trials using leptin in combination with the pancreatic polypeptide amylin show some promise.8 There has also been interest in using leptin to improve insulin action in a weight-loss independent fashion; however, recombinant methionyl human (r-Met hu) leptin did not enhance insulin sensitivity in obese subjects with type 2 diabetes.9

In the two decades since leptin was first identified, over 20,000 papers have been written describing leptin’s actions in physiology and pathophysiology. In stark contrast to the wealth of data regarding the functions of leptin, however, our knowledge about the mechanisms involved in the transcriptional regulation of leptin gene expression, particularly the basis for its adipocyte-specific expression, is rather limited. Leptin is regulated primarily at the transcriptional level, and a wide variety of physiological conditions and pharmacological agents have been shown to affect its expression, including fasting and feeding, insulin, glucocorticoids, thiazolidinediones and even leptin itself.4,10,11 These studies are summarized in Table 1.
chondrocytes and muscle, but the physiological importance of this low level expression, if any, has not been determined.

The proximal promoters of the *LEP* gene in mouse and human have been characterized and a classic TATA box has been identified, as well as binding sites for C/EBP, Sp-1, GR, and CREB, and an E box element that may bind SREBP1c. Although a transgenic reporter driven by a 762 bp leptin proximal promoter cassette was able to drive gene expression in vivo, it showed lower levels of expression in adipocytes than in a variety of non-adipose tissues and was not affected by fasting, suggesting that this region is not responsible for adipose tissue specificity of leptin expression or physiological regulation of leptin gene expression. Recently, the transcription factor activator protein-2β (AP-2β) has been shown to inhibit *LEP* expression by direct binding to the promoter. Various SNPs have been reported in the porcine leptin promoter that correlate with *Lep* expression and demethylation of specific CpG islands occurs during adipogenesis, associated with the onset of *Lep* expression.

All these studies have been limited by the fact that they have focused on the proximal promoter and/or on pre-specified adipocyte transcription factors. In fact, several major obstacles have impeded progress in identifying transcriptional pathways responsible for regulation of adipocyte-specific leptin gene expression, including (1) unreliable methods for unbiased detection of distal cis-regulatory elements, (2) insufficient tools to identify an unknown transcription factor binding to a known cis-regulatory element, and (3) a lack of good cell culture models for studying leptin gene expression in vitro. It is worth noting that the workhorse model of adipocyte research, the 3T3-L1 cell line, expresses extremely low levels of *Lep* and is thus unsuitable for these studies. Recent advances in all of these areas encouraged us to pursue the mechanism of adipocyte-specific leptin gene expression once again.

**Identification of a cis-Regulatory Element for Adipocyte-Specific *LEP* Gene Expression**

We utilized a multi-step approach to finding cis-regulatory elements that direct leptin gene expression. First, we sought to map such regions grossly, using BAC-transgenic reporter mice that express EGFP from the *Lep* translatonal start site. Analyzing multiple transgenic founders carrying BACs with varying lengths of *Lep* flanking sequences allowed us to narrow down the region required for adipose-specific expression and demonstrated that the three *Lep* exons, both introns, and 5.2 kb of 5' flanking sequence were sufficient to drive adipocyte-specific EGFP expression. Next, we performed fine-mapping in vitro, by “tiling” across the upstream flanking region and through both introns with PCR primers, to generate reporter constructs that we could test in cultured human adipocytes. These cells are derived from human adipose stromal cells (hASCs), which can be differentiated into mature adipocytes in vitro and produce high levels of leptin. This effort identified a single 1 kb region approximately 4.5 kb upstream of the *LEP* transcriptional start site (TSS) that acts as enhancer in mature adipocytes. Further deletion and point mutations allowed us to discover a 30 bp region that was required for enhancer activity. Interestingly, we had previously performed chromatin state mapping in human adipocytes in culture, part of which involved mapping histone modifications associated with active and poised enhancers. The region we identified by reporter assays overlay an active enhancer region in our chromatin state maps (the only such place in the vicinity), which provided significant reassurance that we had identified a region of bona fide importance.

**Identification of the Transcription Factor(s) for Adipocyte-Specific *LEP* Gene Expression**

Once a relevant cis-regulatory element is found, it can be very difficult to identify the cognate transcription factor(s) that bind and activate it. The most common approach is to use computational motif finding to find sequences that suggest a particular factor. We tried this and found several interesting motifs for the androgen receptor (AR), peroxisome proliferator-activated receptor α (PPARα) and nuclear factor erythroid 2-related factor 1 (NFE2L1). Unfortunately, functional
Fosl2 global knockout mice
Lep gene expression
Fosl2 expression.

Interestingly, mRNA and feeding increases it.
31 mRNA during the fed
Lep knockout mice can be
mRNA content, with
Fosl2 plays a role in enabling
i Lep are different from the mechanisms
gene expression regardless of depot.
There also appears to be a direct
gene.
To add
levels.
heterozygous LEP
We
Lep a binding site at the 5' end of the
db/db Lep expression observed in
this led to reduced
of FOSL2, we knocked it down with
To demonstrate the functional importance
FOSL2 is an Important Regulator
of LEP Gene Expression In Vitro
and In Vivo
To demonstrate the functional importance
FOSL2, we knocked it down with
RNAi in human adipocytes; as predicted,
this led to reduced LEP gene expression.
Unfortunately, Fosl2 global knockout mice
die shortly after birth and before most
adipose development is complete, so leptin
levels in these animals cannot be inter-
rogated. We did look at Fosl2 heterozygous
mice, which have normal adiposity how-
ever, and they show reduced circulating
leptin, as do mice with adipocyte-specific
deletion of Fosl2. In addition, osteoblastic
precursor cells taken from the skulls of
global Fosl2 knockout mice can be
differentiated into adipocytes in vitro;
these cells display lower Lep gene expres-
sion. Gain-of-function studies were a bit
harder to perform, in that overexpression of
Fosl2 in mature adipocytes did not
affect leptin expression. However, forced
expression of Fosl2 during the develop-
ment process resulted in adipocytes with
normal overall differentiation but elevated
leptin expression. Similarly, osteoblastic
precursors from Fosl2-transgenic mice
showed higher Lep expression when
differentiated into adipocytes in vitro.
Since the Fosl2-transgenic mice suffered
from systemic fibrosis and inflammation,12
metabolic studies of these animals could
not be conducted. It appears that Fosl2 is
both necessary and sufficient for leptin
expression in adipocytes, but it must be
present during the differentiation process
to exert its effects. This implies that Fosl2
has both direct and indirect actions on the
Lep gene.

Roles of FOSL2 in Regulating
Leptin Levels in Physiology
and Pathophysiology
As mentioned above, apart from being
expressed in adipocytes, Lep mRNA
synthesis in adipocytes is regulated by a
variety of physiological and experimental
stimuli. In mice, there are depot-specific
differences in Lep mRNA content, with
inguinal fat expressing higher leptin levels
than retroperitoneal or epididymal adipose
tissue.35 There also appears to be a direct
relationship between adipocyte volume
and Lep expression regardless of depot.34
In our study, a correlation of increased
levels of LEP gene expression with increased
levels of FOSL2 gene expression
was observed during the course of differen-
tiation of human adipocytes and in
different adipose depots in mice. In
addition, we showed that stimulation of
mature human adipocytes with dexam-
ethasone resulted in increased LEP
expression and simultaneous rise in
FOSL2 expression.

Fasting and Feeding Regulation
of Leptin
One of the most important physiological
functions of leptin is the regulation of
fasting-feeding behavior.1 Fasting reduces
Lep mRNA and feeding increases it.35 We
measured Fosl2 mRNA during the fed
state and after 24 h of fasting, but we did
not detect any significant differences. It is
very possible that the mechanisms regu-
lating adipocyte-specific gene expression
of Lep are different from the mechanisms
which regulate other physiological func-
tions of Lep, such as fasting and feeding. It
is worth pointing out that our approach
was geared toward the discovery of
regulators of adipocyte-specific expression
during differentiation, so it is perhaps
unsurprising that Fosl2 might be not a
regulator of the fasting-feeding response.

Roles of FOSL2 in Regulating
Leptin Levels in Obesity
To determine if Fosl2 plays a role in the
elevated Lep expression observed in
obesity, we analyzed Fosl2 expression in
different mouse models of obesity. In mice
with diet-induced obesity as well as in
mice with genetic obesity (db/db), we
found that increased Lep gene expression
was accompanied by increased Fosl2 levels.
A gene expression analysis of samples of
subcutaneous fat from a human cohort
with varying BMIs confirmed this asso-
ciation in humans.

Conclusions and
Unanswered Questions
Several issues, however, remain
unresolved. First of all, we do not yet
understand how FOSL2, which is not
tissue restricted to adipocytes, can regulate
adipose-specific gene expression. There are
several intriguing possible mechanisms,
many of which are known to be employed
by members of the AP-1 transcription
factor family, to which FOSL2 belongs.
These include differential expression,
composition and orientation of the hetero-
dimeric binding partners, post-trans-
lational modifications, and interaction
with ancillary proteins.36 Interestingly,
although we identified JUND as a poten-
tial binding partner on our 30 bp cis-
regulatory element, we could not
demonstrate a specific requirement for
this or any other Jun isoform in Lep
expression. Perhaps there is greater func-
tional redundancy among the Jun proteins
than between FOS, FOSL1 and FOSB,
which apparently cannot compensate for
the loss of FOSL2 in leptin expression.
Other factors may also be acting in concert
with FOSL2. For example, there is a bona
fide PPARY binding site at the 5’ end of the
1 kb cis-regulatory region we discovered.
Perhaps PPARY plays a role in enabling
FOSL2 binding during adipogenesis.
Our results should by no means be taken to exclude a possible role for other cis-regulatory regions important for \( Lep \) expression. Our BAC transgenic results suggest that multiple regions, especially at the 3' end of the \( Lep \) gene, may be involved as well. For example, truncation of the 3' flanking sequence in our BAC transgenic reporter mouse model led to a significant decrease of EGF expression in adipose tissue and increased EGF expression in other tissues. Taken together, these data suggest that many genomic regions and transcription factors will eventually be shown to regulate leptin expression.

Our study provided one of the first clues into the transcriptional regulation of the medically important adipokine leptin. Despite the fact that leptin levels are primarily regulated at the transcriptional level, our understanding of the factors at play has been poorly developed. We utilized an integrated strategy that combined BAC transgenesis, human reporter assays, epigenomics, and unbiased quantitative proteomics to identify FOSL2. We believe that a similar multi-pronged approach will be required to tackle many of the remaining problems in tissue-specific transcriptional regulation.

References


