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Long Noncoding RNAs That Function in Nutrition: Lnc-ing Nutritional Cues to Metabolic Pathways

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Abstract

Long noncoding RNAs (lncRNAs) are sensitive to changing environments and play key roles in health and disease. Emerging evidence indicates that lncRNAs regulate gene expression to shape metabolic processes in response to changing nutritional cues. Here we review various lncRNAs sensitive to fasting, feeding, and high-fat diet in key metabolic tissues (liver, adipose, and muscle), highlighting regulatory mechanisms that trigger expression changes of lncRNAs themselves, and how these lncRNAs regulate gene expression of key metabolic genes in specific cell types or across tissues. Determining how lncRNAs respond to changes in nutrition is critical for our understanding of the complex downstream cascades following dietary changes and can shape how we treat metabolic disease. Furthermore, investigating sex biases that might influence lncRNA-regulated responses will likely reveal contributions toward the observed disparities between the sexes in metabolic diseases.

Contents

INTRODUCTION

Noncoding RNAs have been demonstrated to play critical roles in every field of biology, with evidence mounting each day for their central role in health and disease. When the first human genome sequence was completed in 2004, it was estimated that the human genome only codes for 20,000–25,000 proteins (38), and this estimate further dropped to 19,000 proteins in subsequent studies (26). While much of the noncoding genome was initially called junk DNA, approximately 60–90% of the genome is transcribed (63). The mammalian genome contains a variety of noncoding RNAs of various lengths, including small RNAs such as microRNAs (miRNAs) and picoRNAs in addition to transfer RNAs and ribosomal RNAs (15). Long noncoding RNAs (lncRNAs) are another class of noncoding RNAs that have rapidly evolved to meet the changing needs of the organism across physiological states and also in response to external stresses and stimuli, such as pathogenic infections (1) or nutritional changes (4). Changing nutritional cues require organisms to rapidly respond via downstream metabolic processes to maintain energy and metabolite homeostasis, necessitating rapid changes to gene expression. This review highlights various lncRNAs that regulate gene expression in response to changes in nutrition across metabolic tissues and examines molecular details of functional mechanisms that contribute to homeostatic maintenance. We focus on lncRNAs whose expression is modulated in response to feeding/fasting in three metabolic tissues that respond to nutritional changes: liver, adipose, and muscle (**Table 1**). This review highlights how these lncRNAs impact metabolic pathways, and we briefly review the molecular mechanism by which each lncRNA influences gene expression. Most of the nutritionally sensitively lncRNAs described in this review are specific to the mouse (**Table 1**). Yet, more than 80% of human lncRNAs are not conserved in the mouse, and there is little overlap of lncRNAs between mice and humans (59, 83). We also include some examples of human-specific lncRNAs that

Table 1 Tissue-specific lncRNAs that function in response to nutritional changes

LncRNA	Metabolic tissue	Response to nutritional change	Impact on downstream metabolic process	LncRNA function and mechanism	Sex of mice	Reference
Gm10768	Liver	↑ with fasting	Positive regulation of gluconeogenic genes (*Pepck*, *G6pase*) in vitro and in vivo	Sequesters miR-214 and relieves negative regulation of Atf4 on gluconeogenic genes	Not specified	21
		↓ with refeeding	Positive regulation of glucose production in vivo			
Bhmt-AS	Liver	↑ with fasting	Positive regulation of gluconeogenic genes (*Pepck*, *G6pase*, *Fbp1*, and *Gys2*) in vitro and in vivo	(?) Positively regulates progluconeogenic antisense transcript Bhmt	Male	73
		↓ with refeeding	Positive regulation of glucose production in vitro and in vivo			
lncLGR	Liver	↑ with fasting	Negative regulation of glucokinase (*Gck*) in vitro and in vivo	Complexes with hnRNPL at the Gck promoter to negatively regulate Gck transcription	Male	69
		↓ with refeeding	Negative regulation of liver glycogen stores in vivo			
lncLSTR	Liver	↓ with fasting	Positive regulation of plasma triglyceride levels	Binds Tdp-43 and relieves its inhibitory effect on Cyp8b1 transcription	Male	47
		↑ with refeeding		Modifies bile acid pools and downstream activation of Apoc2 via Fxr		
				Impacts Apoc2 levels and activity, modulating peripheral tissue activation of Lpl and clearance of plasma triglycerides		
MEG3	Liver	↑ with 6 weeks HFD	Positive regulation of gluconeogenic genes (*Pepck*, *G6pase*) in vitro and in vivo	HFD downregulates Hdac3; MEG promoter is acetylated and upregulated	Male	103
		↓ with 8 weeks HFD	Negative regulation of lipogenic genes	Sequesters miR-21 and prevents Lrp6 from binding and enhancing lipogenic genes	Male	37
lncSHGL	Liver	↑ with 1 month HFD	Negative regulation of blood glucose upon fasting, positive regulation of insulin sensitivity	Binds hnRNPA1 and induces expression of CALM genes	Male	85
		↑ with 2 months HFD	Negative regulation of hepatic triglyceride and cholesterols	CALM genes induce CaM to inhibit mTor/Srebp1c and suppress gluconeogenic and lipogenic genes		
		↓ with 3 months HFD	Negative regulation of lipolysis in peripheral adipose tissue			
			Negative regulation of serum free fatty acids			
			Negative regulation of gluconeogenic and lipogenic genes			
Blnc1	Liver	↑ with 3 months HFD	Positive regulation of lipogenic genes	Recruits Edf1 and Lxr to *Srepb1c* promoter to induce lipogenic program	Not specified	99
Gm16551	Liver	↓ with fasting	Negative regulation of lipogenic genes (*Acly*, *Fas*, *Scd1*)	Negative regulation of Srebp1c	Male	91
		↑ with refeeding	Negative regulation of plasma triglyceride levels			
		↓ with 48 h HFD				
		↓ with 12 weeks HFD				

(*Continued*)

Table 1 (*Continued*)

LncRNA	Metabolic tissue	Response to nutritional change	Impact on downstream metabolic process	LncRNA function and mechanism	Sex of mice	Reference
Gomafu	Liver	↑ with fasting	Positive regulation of glucose production in vivo	Sequesters miR-139 and relieves negative regulation of Foxo1 on gluconeogenic program	Male	90
		↑ with HFD				
Gm15622	Liver	↑ with fasting	Negative regulation of lipogenic genes (*Srebp1*, *Fasn*)	Sequesters miRNA-742-3p and activates transcription of Srepb1c on lipogenic program	Male	55
		↑ with 12 weeks HFD				
hLMR1	Liver	Modified by low-carbohydrate diet (?)	Positive regulation of genes involved in cholesterol synthesis (*SC5D*, *FDPS*, *LSS*, and *HMGCS1*) in vivo	Recruits PTBP1 to the promoters of cholesterol synthesis genes to drive their expression	Not specified	70
		↓ with fasting	Positive regulation of LDL and VLDL levels in vivo			
		↑ with refeeding				
Lnc-leptin	Adipose	↓ with fasting	Positive regulation of adipocyte differentiation	Facilitates enhancer-promoter looping to contact *Lep* and promote its transcription	Male	53
		↑ with HFD	Positive regulation of adipocyte marker genes *Pparg* and *Adipoq*			
SRA	Adipose	↑ with HFD	Positive regulation of lipogenic genes (*Fabp4* and *Adipoq*) and inflammatory genes (*Tnf*, *Il6*, and *Ccl2*)	Not described	Male	52
	Liver	↓ with fasting	Positive regulation of triglyceride and fatty acid accumulation in the liver in response to HFD	Prevents transcriptional induction of *Atlg* by inhibiting Foxo1 and Pparg binding to *Atlg* promoter	Male	17
			Positive regulation of beta-oxidation of free fatty acids via *Atlg*			
Risa	Liver	↑ with fasting	Negative regulation of autophagy	Not described	Male	86
	Muscle		Positive regulation of insulin resistance			

Abbreviations: Fxr, farnesoid X receptor; HFD, high-fat diet; LDL, low-density lipoprotein; lncRNA, long noncoding RNA; VLDL, very-low-density lipoprotein.

respond to nutritional changes and whose mechanisms were elaborated using humanized mouse models. Understanding how lncRNAs function to regulate gene expression in response to nutritional changes across tissues, and how these responses reflect changing diets in the modern world, has important implications for human health and disease.

CHARACTERISTICS OF LONG NONCODING RNAs

LncRNAs are defined as RNAs greater than 200 nucleotides in length that are not translated, and recent estimates suggest that the human genome may contain 16,000–100,000 lncRNAs (27). LncRNAs exhibit tissue-specific expression patterns and can be transcribed by RNA polymerase II and other polymerases (14, 24, 40). Similar to messenger RNAs (mRNAs), lncRNAs are spliced, contain a 5′-end 7-methyl guanosine cap, and are polyadenylated at their 3′ ends. LncRNAs can interact with DNA, RNA, and proteins, and they exhibit a variety of functions including chromatin regulation, nuclear organization, assembly and function of nuclear bodies, mRNA stability or translation, and regulation of signal transduction pathways (44). Because lncRNAs are not well conserved across species, studies that identify lncRNAs in model organisms should specify whether

a particular lncRNA is functional in humans before assuming their broader relevance to human health (59, 83).

LncRNAs can function in *cis* to regulate transcription of neighboring genes or in *trans* at the posttranscriptional, translational, and posttranslational levels. Furthermore, lncRNAs can function in either the nucleus or cytoplasm, and some nuclear RNAs can be exported into the mitochondria or blood exosomes (48, 58). LncRNA expression is highly responsive to environmental cues and also stress signals. This suggests a compelling role for lncRNAs in responding to nutritional cues, which require rapid responses from downstream processes to maintain energy homeostasis. This review focuses on lncRNAs that are responsive to nutritional cues, highlighting recent studies where lncRNA function and molecular mechanisms impact downstream metabolic processes.

lncRNAs RESPOND TO NUTRITIONAL CHANGES IN A TISSUE-SPECIFIC MANNER

Because lncRNAs are highly responsive to environmental stimuli, it follows that the expression and resulting functional mechanisms of specific lncRNAs may change in response to dietary fluctuations. To explore nutritionally sensitive lncRNAs in key metabolic tissues (liver, adipose, and muscle), several studies have profiled lncRNA expression changes in response to experimentally controlled diets in these tissues (65, 91). One study performed microarray analyses of lncRNA transcripts in three metabolically sensitive tissues in mice (liver, adipose, and muscle) in response to controlled diets mimicking different human dietary changes (85). The first experimental diet compared mice with unrestricted access to normal chow (ad libitum), mice that were denied access to chow for 24 h (fasted), and mice that were first denied access to chow for 24 h followed by 4 h of unrestricted access to chow (refed). These metabolic conditions mimic extreme ends of caloric and nutrient availability. Metabolic tissues from fasted mice showed pronounced changes in lncRNA expression in response to fasting (see the left side of **Figure 1*a***). Refeeding had a more pronounced effect on lncRNA expression in the liver, with 566 differentially expressed lncRNAs for a specific nutritional condition. In adipose and muscle tissues, refeeding also changed expression of lncRNAs (see the right side of **Figure 1*a***). Interestingly, there were a number of lncRNAs in the liver with opposite patterns of expression in the fasted group compared with refed mice,

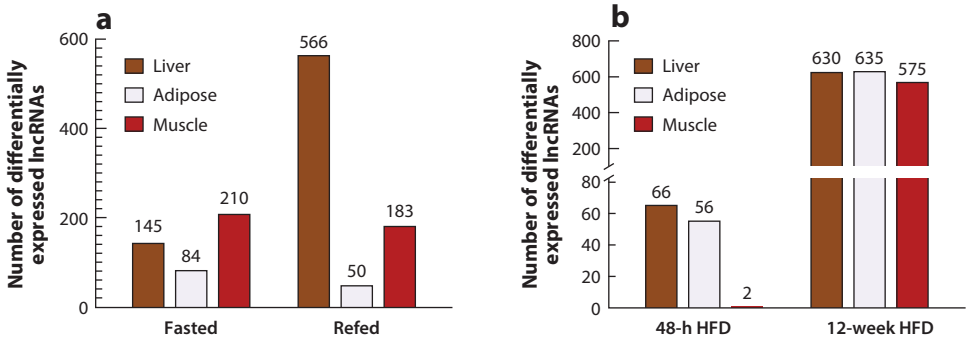

Figure 1

Changes in numbers of lncRNAs expressed in liver, adipose, and muscle tissues in mice after (*a*) fasting or refeeding and (*b*) 48 h or 12 weeks of a high-fat diet. Abbreviations: HFD, high-fat diet; lncRNA, long noncoding RNA.

suggesting that liver lncRNAs are responsive to changing caloric and nutrient availability and are able to be quickly expressed or repressed with changing nutritional states.

Another experimental diet compared mice fed normal chow with mice given a high-fat diet (HFD) for 48 h (48-h HFD), representing the metabolic response to a fatty meal. The final experimental diet compared mice fed normal chow with mice given a high-fat chow for 12 weeks (12-week HFD), mimicking obesity in humans. The 48-h HFD samples displayed similar numbers of lncRNAs differentially expressed in the liver, but only two lncRNAs were differentially expressed in muscle tissue (see the left side of **Figure 1*b***), suggesting that liver and adipose tissues are much more responsive to a short-term HFD or highly fatty meal and that the lncRNA network in muscle tissue is not as responsive to a short duration of an HFD. The 12-week HFD resulted in the highest levels of differentially expressed lncRNAs across the metabolic tissues (see the right side of **Figure 1*b***). Strikingly, 575 lncRNAs were differentially expressed in muscle tissue after 12 weeks of an HFD compared with only two lncRNAs after 48 h of an HFD, suggesting that some lncRNAs respond to either duration or dosage of rich diets. The authors also profiled mRNA expression changes with these diets and found that muscle tissue samples from the 12-week HFD exhibited increased expression of genes involved in RNA metabolism, suggesting that a long duration of an HFD is required to activate a lncRNA response in muscle tissue. Many of the differentially expressed lncRNAs for a specific tissue were shared between different diets, but few overlapped between tissue types, suggesting that certain lncRNAs are likely to respond to different nutritional cues but that lncRNA function is highly tissue specific.

Another study examined hepatic mRNAs and lncRNA expression changes in mice fed various diets (65), finding that different nutrient states often impacted mRNAs more than lncRNAs. For example, the authors observed approximately 50 differentially expressed lncRNAs that were significantly downregulated in response to an HFD, while there was upregulation of many more mRNAs (~580 differentially regulated mRNAs). Conversely, mice that were fasted for 16 h exhibited 92 significantly differentially regulated lncRNAs that were cumulatively upregulated, yet 1,165 mRNAs were predominantly downregulated. Similar trends of regulation were seen following a 6-h refeed, with 59 lncRNAs differentially regulated and 587 mRNAs differentially regulated. The authors hypothesized that these differences may result from variation with transcription factor binding sites within promoters of lncRNAs and mRNAs, resulting in discrete classes of RNA transcription based on nutrient state. In support, this study found that promoters of differentially expressed mRNAs were enriched for E2F transcription factor motifs, while lncRNA promoters were enriched for v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog G (MAFG) and nuclear factor, erythroid 2 like 1 (NFE2L1) motifs. This study identified a novel network of lncRNAs that respond to nutrient changes in the liver, regulated by MAFG binding at promoter regions, and demonstrated that this MAFG–lncRNA axis influenced glucose metabolism.

THE lncRNA LANDSCAPE IN THE LIVER

Liver-specific lncRNAs have been annotated, but little is known about their role in liver development or physiology. Analysis of healthy human tissues from the Genotype-Tissue Expression (GTEx) project identified 224 (40) and 108 (14) lncRNAs expressed in the liver, and approximately half of these lncRNAs are novel and have not been characterized. There are liver-specific lncRNAs that are aberrantly expressed in liver cancer (82) and other liver diseases, including nonalcoholic fatty liver disease (72), and some of these transcripts contribute toward disease progression. There are also liver-specific lncRNAs that function to regulate metabolic processes in healthy livers (56) and are involved in regulation of lipid metabolism and lipid transport. Here, we highlight some liver-specific lncRNAs that respond to nutritional changes and impact physiology and metabolism.

LIVER lncRNAs THAT RESPOND TO FASTING IN MICE

One of the key responses to fasting is an upregulation of gluconeogenic pathways in the liver that generate free glucose during periods of nutrient deprivation. A number of lncRNAs have been identified as mediators of this gluconeogenic response to fasting, acting through distinct downstream pathways to stimulate glucose production. One study compared lncRNAs in the livers of mice that were fed normally, fasted for 16 h, or refed for 8 h following 16 h of fasting (21). The authors filtered their results on the basis of annotated lncRNAs that were upregulated at least threefold during fasting. They identified four lncRNAs that were increased in the livers of fasted mice, compared with those of fed mice, and that were subsequently decreased during refeeding. One of these lncRNAs, Gm10768, was shown by gain- and loss-of-function experiments to positively regulate glucose production in cultured murine hepatocytes (21). The group found that *Gm10768* overexpression increased transcription of gluconeogenic genes, including *phosphoenolpyruvate carboxykinase* (*Pepck*), *glucose 6-phosphatase* (*G6pase*), *acetyl-CoA carboxylase* (*Acc1*), and *fatty acid synthase* (*Fasn*), and also increased protein levels of Pepck and G6Pase. The group recapitulated these findings in vivo by overexpressing *Gm10768* in mice using tail-vein injections with adenovirus constructs containing *Gm10768*. Tail-vein injection of adenoviral vectors is an effective delivery mechanism for lncRNA gain- and loss-of-function experiments [using short hairpin RNAs (shRNAs) or small interfering RNAs (siRNAs)] in the liver, exhibiting lower levels of delivery to the spleen, heart, and kidneys (22). Overexpression of *Gm10768* increased glucose production and upregulation of gluconeogenic genes and protein levels in the liver. Conversely, they found that Gm10768 knockdown in vitro and in vivo decreased glucose production and reduced expression of gluconeogenetic genes.

LncRNAs regulate gene expression in *cis* or *trans*, and modulation of Gm10768 levels had no impact on the neighboring protein coding gene *Abcc2*, suggesting that Gm10768 does not act in *cis*. Furthermore, Gm10768 levels were prominently increased in the cytosol in response to fasting, also supporting a *trans* acting mechanism. Overexpression of *Gm10768* reduced levels of the known gluconeogenetic regulator miR-214 (46), which negatively regulates the transcription factor Atf4 responsible for inducing hepatic gluconeogenesis (46). The authors found that miR-214 inhibition of Atf4 and its gluconeogenic program was rescued by *Gm10768* overexpression. Thus, the lncRNA Gm10768 acts in *trans* to sequester miR-214 and activate a progluconeogenic program in response to fasting (**Figure 2**).

LncRNAs also play a role in glucose intolerance in the setting of diabetes (73). Fasting results in upregulated expression of specific lncRNAs in mouse liver, and one study identified 15 novel lncRNAs that were increased at least threefold after fasting. This list included a novel lncRNA Bhmt-AS, a lncRNA antisense to the protein coding gene *betaine homocysteine S-methyltransferase* (*Bhmt*). Bhmt deficiency can improve glucose tolerance and insulin sensitivity in mice, suggesting that it plays an important role in gluconeogenic processes and the pathophysiology of diabetes (80). Both Bhmt-AS and Bhmt mRNA were increased in the livers of mice after 24 h of fasting, and both transcripts were downregulated 16 h after refeeding (73). Bhmt-AS is most highly expressed in the murine liver compared with numerous other tissues profiled, while Bhmt mRNA is exclusively detected in the liver. Bhmt-AS is localized in the nucleus, consistent with a putative role for regulating Bhmt expression through transcription or as an independent lncRNA.

Knockdown of Bhmt-AS using tail-vein injections of adenoviral shRNA constructs resulted in decreased levels of both Bhmt mRNA and protein in the liver (73), suggesting that Bhmt-AS functions in *cis* to regulate *Bhmt* expression. Bhmt-AS knockdown mice also had reduced expression of gluconeogenic genes *Pepck*, *G6pase*, *fructose-bisphosphatase* (*Fbp1*), and *glycogen synthase* (*Gys2*), decreased protein levels of Pepck and G6Pase, and decreased blood glucose levels. To determine

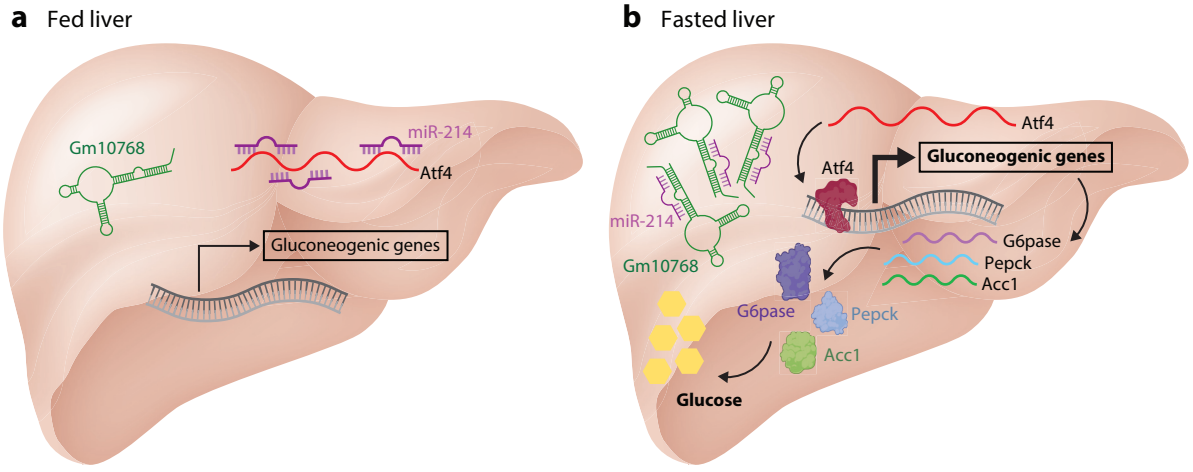

Figure 2

The lncRNA Gm10768 regulates expression of gluconeogenic genes in the liver in fed (*a*) versus fasting (*b*) nutritional states. (*a*) Under normal nutritional diets, there are low levels of Gm10768 in the liver, and miR-214 binds to Atf4 mRNA, which prevents translation and reduces Atf4 protein levels. (*b*) Under fasting conditions, *Gm10768* is upregulated in the liver, and Gm10768 lncRNA sequesters miR-214, which allows translation of Atf4 mRNA. Atf4 protein can function as a transcriptional activator to induce expression of gluconeogenic genes. Abbreviation: lncRNA, long noncoding RNA.

whether Bhmt-AS regulates gluconeogenic pathways by modulating Bhmt levels, the authors overexpressed *Bhmt* in Bhmt-AS knockdown hepatocytes and observed that glucose production was restored. These findings demonstrate a progluconeogenic role for Bhmt that can overcome inhibition of gluconeogenesis in the setting of reduced Bhmt-AS. These findings also suggest that Bhmt-AS might regulate *Bhmt* expression in *cis*, yet it remains possible that Bhmt-AS and Bhmt act independently to regulate gluconeogenesis. The transcription of Bhmt-AS and Bhmt could act synergistically because the genes overlap and their promoters are in the same orientation, which putatively eliminates transcriptional interference as a possible regulatory mechanism. Further work is needed to definitively determine molecular details of a putative *cis*-acting mechanism for Bhmt-AS.

Another response to fasting in the liver is the breakdown of glycogen stores, which increases free glucose levels and inhibits glycogen storage pathways. The lncRNA lncLGR regulates glycogen storage, and *lncLGR* expression is increased in the livers of mice following 24 h of fasting, while *lncLGR* expression decreases with refeeding following fasting (69). Overexpression of *lncLGR* in mice using intravenous injection of adenovirus decreased levels of both liver triglycerides and liver glycogen. LncLGR overexpression also decreased levels and activity of the enzyme glucokinase (Gck), which is a key enzyme regulating glucose storage as glycogen in the liver. Overexpression of both *Gck* and *lncLGR* rescued liver glycogen storage, suggesting that lncLGR regulates liver glycogen stores by regulating Gck levels and/or activity. LncLGR knockdown experiments confirmed that lncLGR negatively regulates Gck and liver glycogen stores, and RNA pull-down experiments identified heterogeneous nuclear ribonucleoprotein L (hnRNPL) as a lncLGR binding protein (69). HnRNPL knockdown in mouse liver increased Gck protein levels, suggesting it plays a lncLGR-independent role in negatively regulating Gck and glycogen metabolism in the liver (69). HnRNPL binds to lncLGR directly, and this complex is found at the *Gck* promoter, cooperating to negatively regulate *Gck* expression (69). While this study did not examine lncLGR–hnRNPL complex enrichment at the *Gck* promoter in the livers of fed and fasted mice, the findings

suggest that fasting induces formation of this complex to negatively regulate *Gck* expression upon nutrient deprivation. LncLGR, through interactions with hnRnpl, functions in response to fasting and regulates glycogen storage in the liver by modulating *Gck* expression.

Liver-mediated responses to fasting can also orchestrate changes in peripheral metabolic tissues including adipose and muscle. The liver-specific lncRNA lncLSTR is nutrient sensitive and impacts other peripheral metabolic tissues where it is not expressed (47). LncLSTR levels are reduced in mouse liver after a 24-h fast, yet lncLSTR expression is restored to fed levels in mice that were refed for 4 h following a 24-h fast (47). LncLSTR knockdown using tail-vein injections of adenoviral shRNAs decreased plasma triglyceride levels across fed, fasted, and refed states. This study also observed that the difference in plasma triglyceride levels was due to enhanced triglyceride clearance by peripheral tissues in the setting of decreased lncLSTR levels in the liver. The authors found that lncLSTR knockdown mice exhibited increased levels of apolipoprotein C2 (Apoc2) and lipoprotein lipase (Lpl) in plasma. Apoc2 is a potent activator of Lpl, which contributes toward triglyceride clearance from the plasma (87).

To understand how downregulation of *lncLSTR* during fasting impacts Apoc2 levels and serum triglycerides, the authors reduced lncLSTR expression in primary hepatocytes and found that Apoc2 levels did not change, indicating a requirement for other cell types. Interestingly, cytochrome P450 family 8 subfamily B member 1 (Cyp8b1), which regulates the bile acid pool through modulation of muricholic acid (MCA) and cholic acid (CA) (23), was highly correlated with *lncLSTR* expression changes in the liver after fasting/refeeding. Knockdown of lncLSTR in both primary hepatocytes and mouse liver also reduced expression of *Cyp8b1* and increased ratios of MCA/CA bile acids, resulting in increased *Apoc2* expression. LncLSTR binds to Tdp-43, an RNA/DNA-binding protein of the hnRNP family that functions as a transcriptional repressor (45), and the lncLSTR-Tdp-43 complex was enriched at the *Cyp8b1* promoter in hepatocytes. LncLSTR knockdown reduced Tdp-43 enrichment at this region, suggesting that lncLSTR functions to recruit Tdp-43 for repressing *Cyp8b1* expression. These findings highlight a complex mechanism by which changing levels of lncLSTR in response to fasting lead to coordinated transcriptional repression, modulation of bile acid pools, and activation of enzymes in peripheral tissues to regulate triglyceride clearance (**Figure 3**).

Collectively, these studies highlight the diverse array of lncRNAs that respond to nutrient deprivation and the unique downstream processes that lncRNAs can regulate to change gene expression in response to fed/fasting states.

LIVER lncRNAs THAT RESPOND TO A HIGH-FAT DIET IN MICE

The HFD mouse model is commonly used to model insulin resistance and type 2 diabetes mellitus (T2DM) (76) as well as nonalcoholic fatty liver disease (NAFLD) (67). Mice fed an HFD exhibit lipid accumulation in the liver that contributes toward the pathogenesis of these diseases.

The lncRNA MEG3 is associated with numerous cancers and NAFLD, and it contributes toward disease through modulating expression of the tumor suppressor gene *p53* (2, 33). In human hepatocellular carcinoma, MEG3 functions as a tumor suppressor through induction of apoptosis (9), and *MEG3* expression is decreased in the livers of patients with NAFLD (74). To examine the role of MEG3 in NAFLD, one study measured Meg3 levels in the livers of mice fed an HFD for 8 weeks (a commonly used model of NAFLD) and found decreased *Meg3* expression (37). They found that lipid accumulation in hepatocytes treated with free fatty acids was reversed by overexpression of *Meg3*, suggestive of an antilipogenic role for Meg3. Accordingly, they also found that *Meg3* overexpression led to decreased expression of prolipogenic genes, including *sterol regulatory element binding protein 1* (*Srebp1*), *liver X receptor alpha* (*Lxralpha*), *carbohydrate-responsive element binding protein* (*Chrebp*), *stearoyl-CoA desaturase 1* (*Scd1*), *Acc1*, and *Fas receptor* (*Fas*).

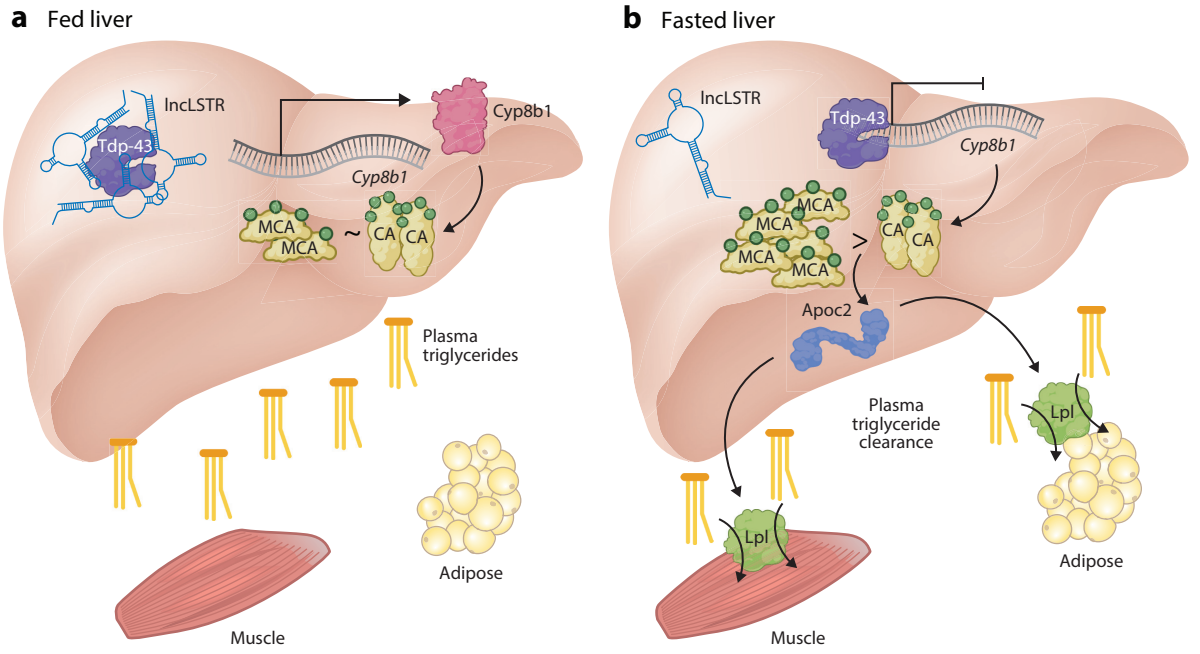

Figure 3

The lncRNA lncLSTR binds Tdp-43 to regulate expression of Cyp8b1 for balancing bile acid pools and facilitating triglyceride clearance from plasma. (*a*) In the fed state, there are high levels of lncLSTR that bind to the repressor Tdp-43 protein and recruit it away from the *Cyp8b1* promoter, promoting *Cyp8b1* expression. When *Cyp8b1* is abundantly expressed, the bile acid pools are balanced, with equal ratios of MA and CA. In the fed state, the triglyceride levels are maintained in plasma. (*b*) LncLSTR is downregulated in the fasting state liver, and Tdp-43 binds to the *Cyp8b1* promoter to inhibit its transcription. Low Cyp8b1 protein increases the MA:CA ratio, altering bile acid pools, which activates Apoc2, increasing Lpl activity in adipose and muscle tissues, resulting in triglyceride clearance from plasma. Abbreviations: CA, cholic acid; lncRNA, long noncoding RNA; Lpl, lipoprotein lipase; MA, muricholic acid.

To investigate the mechanism of Meg3-mediated downregulation of lipogenic genes, the group explored a possible role of miR-21, which had been previously described as a regulator of MEG3 in chronic myeloid leukemia (100). They found that low-density lipoprotein receptor-related protein 6 (Lrp6), which functions in nutrient-sensing pathways (34), is regulated by miR-21 during lipid accumulation. In the setting of an HFD and decreased levels of Meg3, miR-21 binds to Lrp6 mRNA to prevent its translation, promoting a prolipogenic pathway.

LncSHGL is another liver-specific lncRNA whose expression changes with an HFD (85). Interestingly, lncSHGL levels are upregulated after 1 and 2 months of an HFD and then fall at 3 months of an HFD, suggesting that *lncSHGL* expression responds differently to changing metabolic needs with increased duration of an HFD. Induced overexpression of *lncSHGL* in the livers of mice fed an HFD led to decreased blood glucose upon fasting and enhanced sensitivity to insulin, and it also impacted lipogenic programs, leading to reduced hepatic triglyceride and cholesterol levels, repressed lipolysis of peripheral adipose tissue, and reduced levels of serum free fatty acids (85). *LncSHGL* overexpression also repressed gluconeogenic and lipogenic genetic programs. These data suggest a dual role for lncSHGL in modulating gluconeogenic and lipogenic programs in the liver in response to an HFD. Given the pattern of lncSHGL expression during consumption of an HFD, it seems likely that at 1–2 months of an HFD, upregulation of *lncSHGL* works to balance homeostasis by inhibiting gluconeogenesis and lipogenesis in the setting of a diet

with excess fat. However, by 3 months of an HFD, lncSHGL levels decrease, resulting in increased gluconeogenesis and lipogenesis in the liver, potentially contributing to hyperglycemia in T2DM pathogenesis and lipid accumulation, which is observed in NAFLD and modeled by an HFD in mice.

Another lncRNA that responds to an HFD in the liver was initially discovered in adipose tissue. The lncRNA Blnc1 has been shown to play a role in beige and brown adipocyte differentiation and thermogenesis (49, 98) but is also highly expressed in the liver. Blnc1 levels increased in the livers of mice fed an HFD for 3 months (99). Blnc1 overexpression in mice led to increased liver and plasma triglycerides and upregulation of numerous genes involved in lipogenesis and lipid storage, including *Srebp1c*, *Fasn*, *Scd1*, *fat-specific protein 27* (*Fsp27*), *diacylglycerol O-acyltransferase 2* (*Dgat2*), and *fatty acid binding protein 4* (*Fabp4*). Additionally, *Blnc1* expression also increased transcription of various target genes of liver X receptor (Lxr), a central regulator of hepatic lipogenesis (12). Elevations of Lxr can result in the harmful accumulation of lipids in NAFLD, suggesting that Blnc1 may play a role in this pathogenic process.

To determine whether Blnc1 acts in concert with Lxr, a whole-body knockout (KO) of *Blnc1* was generated using CRISPR/Cas9. No gross defects were described in the Blnc1 KO mice. Treatment of mice with an Lxr agonist normally induces a lipogenic gene program and causes hepatic steatosis and hypertriglyceridemia (71), but Blnc1 KO mice were protected against prolipogenic impacts of Lxr agonist treatment. Furthermore, murine hepatocytes overexpressing *Blnc1* treated with an Lxr inverse agonist reversed the induction of a Blnc1-mediated lipogenic program. These findings suggest that Blnc1 and Lxr act in concert to induce lipogenesis (49, 98). Blnc1 lncRNA binds to the endothelial differentiation related factor 1 (Edf1) protein in murine hepatocytes, forming a Blnc1-Lxr-Edf1 complex (99). Knockdown of Edf1, Blnc1 deletion, or removal of Lxr agonism was sufficient to decrease transcriptional activity at the *Srebp1c* promoter. Lxr protein accumulates at the *Srebp1c* promoter by chromatin immunoprecipitation, but additional experiments are needed to confirm that a Blnc1-Lxr-Edf1 complex localizes to the *Srebp1c* promoter. These findings demonstrate a role for Blnc1 in coordinating an RNA/protein complex to induce a lipogenic protein via transcription of *Srebp1c*, potentially driving pathogenesis of diseases such as NAFLD in the setting of an HFD (**Figure 4**).

These studies demonstrate diverse pathways where modulation of lncRNA expression, in response to an HFD, alters gene expression of specific metabolism genes in the liver (**Table 1**), and some of these pathways likely contribute to the pathogenesis of NAFLD.

LIVER lncRNAs MODULATED BY MULTIPLE NUTRITIONAL CUES IN HUMANS AND MICE

Given the ability of lncRNAs to respond to individual nutritional cues, it follows that some lncRNAs may respond to more than one type of nutritional cue. Metabolic organs are poised to respond to ever-changing dietary inputs, and it is likely that lncRNAs may facilitate multiple types of responses.

Gm16551 is a lncRNA robustly expressed in the livers of mice whose expression changes with different nutritional states. *Gm16551* expression in the liver decreased with fasting compared with an ad libitum diet, but *Gm16551* expression increased within 4 h of refeeding (91). Curiously, Gm16551 levels decreased after 48 h of an HFD and were further decreased upon 12 weeks of an HFD. Thus, Gm16551 is a tissue-specific lncRNA whose expression fluctuates upon changes with nutrient availability. Culturing hepatocytes with glucagon or cyclic adenosine monophosphate, which mimics fasting signals, resulted in increased *Gm16551* expression. Forced expression of two transcription factors that regulate gluconeogenesis during fasting, *hepatocyte nuclear*

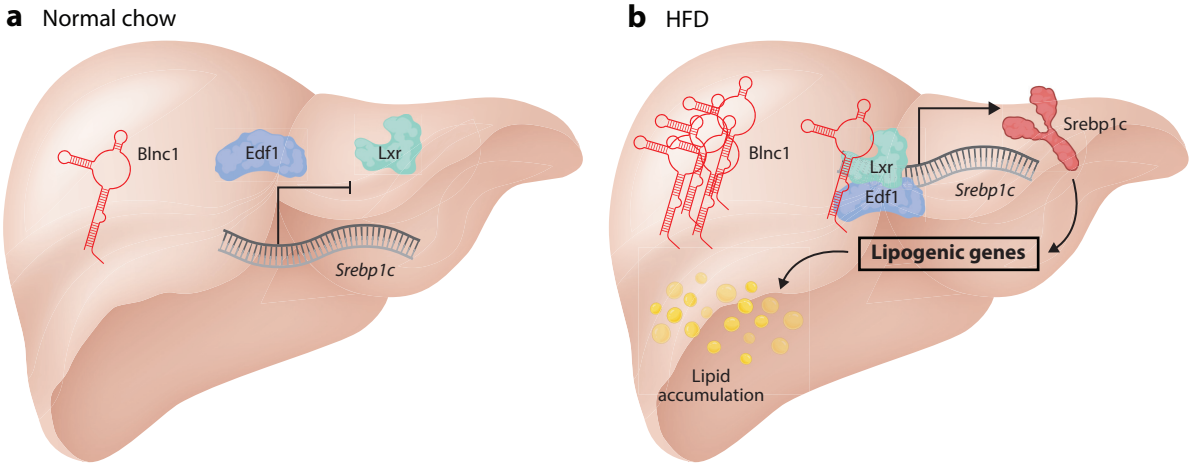

Figure 4

The lncRNA Blnc1 is a molecular scaffold for Lxr and Edf1 activator proteins for activation of *Srebp1c* expression for lipid production in the liver. (*a*) Blnc1 RNA is expressed at low levels in the livers of mice fed a normal diet. Because Blnc1 RNA is limiting, the Edf1/Lxr complex does not form at the *Srebp1c* promoter. (*b*) There are increased levels of Blnc1 lncRNA following an HFD, and Blnc1 acts as a molecular scaffold to bind Lxr and Edf1, recruiting this activation complex to the *Srebp1c* promoter. *Srebp1c* is upregulated and Srebp1c protein activates downstream lipogenic genes, which increase lipid levels in the liver. Abbreviations: Blnc1, brown fat lncRNA1; Edf1, endothelial differentiation related factor 1; lncRNA, long noncoding RNA; Lxr, liver X receptor; Srebp1c, sterol regulatory element binding protein 1c.

factor 4 alpha (*Hnf4a*) and *forkhead box o1* (*Foxo1*), reduced expression of *Gm16551* in murine hepatocytes. However, overexpression of *Srebp1c*, which downregulates lipogenic genes to suppress gluconeogenesis during refeeding (36), resulted in 50-fold increased expression of *Gm16551* in hepatocytes. Knockdown of Gm16551 using shRNAs in mouse liver increased expression levels of lipogenesis pathway genes *ATP citrate lyase* (*Acly*), *Fas*, and *Scd1* and increased plasma triglyceride levels compared with control animals (91). Upregulation of lipogenesis pathway genes following Gm16551 knockdown was abrogated by expression of a dominant negative form of the Srebp1c protein, suggesting that Gm16551 may function by regulating Srebp1c. Overexpression of *Srebp1c* increased expression of lipogenic genes, and the resulting elevations of plasma triglycerides could be rescued with co-overexpression of *Gm16551*. Collectively, these findings highlight a metabolic pathway where multiple nutritional cues are sensed by the lncRNA Gm16551, which then participates in a negative feedback loop with Srebp1c to modulate lipogenesis in response to dietary signals (**Figure 2**). This study highlights the key role of lncRNAs at the intersection of responding to nutritional cues and choreographing downstream metabolic responses to maintain organismal physiology.

The lncRNA Gomafu has been implicated in several aspects of diabetes pathophysiology and functions as a sponge to capture various miRNAs (89, 96, 101). *Gomafu* expression is upregulated in response to fasting and HFD (90). Gomafu has a progluconeogenic role in the liver, and it specifically targets miR-139 in hepatocytes to prevent miR-139-mediated repression of its target gene *Foxo1*, a key gluconeogenic transcription factor. Thus, Gomafu plays a progluconeogenic role in response to fasting, but it remains elusive whether Gomafu also responds to HFD by inducing gluconeogenesis, or whether in the setting of an HFD it induces different downstream metabolic pathways. The lncRNA Gm15622 is another example of a lncRNA that is upregulated in the livers of mice upon fasting or after 12 weeks of an HFD, acting through regulation of miRNAs (55).

In vitro and in silico experiments demonstrated that Gm15622 binds to miRNA-742-3p and prevents negative regulation of *Srebp1c*. Thus, upregulation of Gm15622 induces a lipogenic program in the setting of an HFD, potentially contributing to metabolic disease progression.

There are also human-specific lncRNAs expressed in the liver that respond to dietary changes. One study identified lncRNAs that are differentially expressed across human liver samples and then selected for lncRNAs upregulated in the livers of patients with NAFLD (70). Next, the authors filtered their list by selecting 77 lncRNAs whose expression changed with a low-carbohydrate dietary intervention in a small cohort of NAFLD patients. The authors used a liver-specific humanized mouse model to determine whether these 77 lncRNAs changed expression following fasting/refeeding and identified 20 lncRNAs of the group that met these criteria. From this list of lncRNAs, they discovered hLMR1, which has an important role in regulating cholesterol synthesis. *HLMR1* is downregulated in the liver upon fasting and upregulated upon refeeding. hLMR1 is predominantly expressed in the human liver, and it is not expressed in the mouse liver, highlighting the importance of identifying this lncRNA in human patients and subsequently characterizing it in a humanized mouse model. Knockdown of hLMR1 in the livers of humanized mice decreased expression of multiple cholesterol synthesis pathway genes, including *sterol C5-desaturase* (*SC5D*), *farnesyl diphosphate synthase* (*FDPS*), *lanosterol synthase* (*LSS*), and *3-hydroxy-3-methylglutaryl-CoA synthase 1* (*HMGCS1*), and decreased levels of low-density lipoprotein and very-low-density lipoprotein cholesterol. Overexpression of hLMR1 had the converse effect, upregulating expression of cholesterol synthesis pathway genes *SC5D*, *FDPS*, *LSS*, and *HMGCS1*. Thus, hLMR1 positively regulates cholesterol synthesis upon refeeding in humans. hLMR1 RNA interacts with the protein PTBP1, which is an RNA-binding protein involved in multiple steps of RNA metabolism. PTBP1 is enriched at specific sites upstream of cholesterol synthesis genes whose expression is modulated by hLMR1. hLMR1 functions in *trans* to regulate expression of cholesterol system genes, as knockdown depleted PTBP1 enrichment at the promoters of these genes (70). This work highlights the importance of considering lncRNA conservation between mouse and human models, as nutrient-sensitive lncRNAs may be identified in mice that have no homolog or role in human nutrition and disease. It is critical for many of the lncRNAs identified in mice to be validated in human metabolic tissues before broader implications for human health and disease are suggested.

lncRNAs IN MOUSE ADIPOSE TISSUE: lncRNAs THAT RESPOND TO A HIGH-FAT DIET

LncRNAs also play critical roles in adipogenesis, and various studies have identified between 5 and 368 unique lncRNAs that are expressed in adipose tissue, across the three types of adipocyte cells (14, 40, 75). Comparison of mice fed an HFD for 15 weeks with mice fed normal chow revealed that 93 lncRNAs were upregulated and 31 lncRNAs were downregulated in adipose tissue (93). Next, the authors performed lncRNA-mRNA network analyses to determine whether they could identify putative metabolically regulated lncRNAs that are associated with mRNAs with known metabolic roles, identifying 28 lncRNAs. Of these, 16 lncRNAs had been previously annotated in a lncRNA database and were selected for validation studies by quantitative polymerase chain reaction in mouse adipose tissue after an HFD. Ten of the 16 lncRNAs were significantly enriched in adipose tissue of mice following an HFD compared with a chow diet, while five lncRNAs were significantly depleted. While this study did not perform additional experiments to characterize the mechanism of how these lncRNAs function in adipose tissue in the context of an HFD, these lncRNAs whose expression changes following HFD exposure are intriguing candidates for further study.

ADIPOSE lncRNAs MODULATED BY MULTIPLE NUTRITIONAL CUES IN MICE

Comparison of lncRNA expression profiles across distinct adipose tissues (inguinal white adipose tissue, epididymal white adipose tissue, and interscapular brown adipose tissue) from mice fed an HFD for 16 weeks followed by overnight fasting revealed approximately 10 lncRNAs whose expression changed significantly (53). From this group, nine of the lncRNAs were significantly increased in at least two adipose types after an HFD, and of these, eight lncRNAs were significantly decreased in at least two adipose types after fasting. Because HFD and fasting reflect opposite ends of nutrient availability, it follows that the majority of these lncRNAs are oppositely impacted by HFD and fasting. This study identified the lncRNA Lnc-leptin, which is located 28 kb upstream of the *leptin* (*Lep*) gene. Lnc-leptin increases in adipose tissue following an HFD in mice, and *Lnc-leptin* expression decreases with fasting. Lep is a hormone secreted by adipose that controls satiety and acts to regulate systemic metabolism. *Lnc-leptin* expression changes mirrored that of *Lep* in response to insulin and norepinephrine treatment, suggesting that expression of Lnc-leptin and *Lep* might be coordinated. ShRNA-mediated knockdown of Lnc-leptin in preadipocytes impaired differentiation into adipocytes and reduced levels of *Lep* and the adipocyte markers *peroxisome proliferator-activated receptor gamma* (*Pparg*) and *adiponectin* (*Adipoq*). Knockdown of Lnc-leptin in mature adipocytes also downregulated *Lep* expression and decreased levels of *Pparg* and *Adipoq*, similar to knockdown in preadipocytes. The authors hypothesized that Lnc-leptin and *Lep* may interact by enhancer promoter looping due to their shared regulation and genomic proximity, and they performed chromatin conformation capture experiments. They found that exon 2 of the *Lnc-leptin* gene interacts with the *Lep* promoter and demonstrated that this interaction was disrupted when the lncRNA Lnc-leptin was knocked down using antisense oligonucleotides (ASOs) (53). This observation of ASO-induced reductions of the lncRNA, where the ASOs bind to the lncRNA and disrupt enhancer activity, indicates that the Lnc-leptin transcript itself is a regulatory RNA and discounts the possibility of an RNA-independent mechanism where transcription of the *Lnc-leptin* gene contributes to enhancer regulation. These findings demonstrate that Lnc-leptin likely responds to nutrient cues by modifying chromatin architecture to enact its downstream processes, where chromatin looping with *Lep* occurs during HFD and during fasting, and this looping interaction is disrupted when Lnc-leptin is downregulated. While this study focused on just one lncRNA whose expression decreased after fasting, it is tempting to speculate that the other lncRNAs expressed in adipose tissues following an HFD will target distinct metabolic pathways.

lncRNAs IN MUSCLE TISSUE: AN EMERGING FIELD

LncRNAs have been described to play a role in muscle differentiation and disease states (50). There are approximately 81–176 human muscle-specific lncRNAs that have been identified from human muscle tissue from GTEx data sets (14, 40). Intriguingly, there are various putative lncRNAs that were found to serve as templates for translation of short proteins called micropeptides that play a regulatory role in muscle tissue. One example is *myoregulin*, which codes for a micropeptide that inhibits the function of sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA), a protein that modulates intracellular calcium handling and regulates muscle contraction (3). Another example is DWORF, which enhances SERCA activity by displacing phospholamban, sarcolipin, and myoregulin, which are inhibitors of SERCA (60). Studies of lncRNAs that are differentially regulated in response to changes in diet have identified muscle-specific lncRNAs, but their downstream functions have not yet been investigated (91). Because muscle tissue plays a key role in regulating metabolic responses to changing nutrition, it will be exciting to see how muscle-specific lncRNAs function in a physiological context.

lncRNAs THAT ARE NUTRITIONALLY SENSITIVE IN MULTIPLE METABOLIC TISSUES IN MICE

While most nutritionally sensitive lncRNAs are exclusively expressed in one tissue, there are examples of lncRNAs whose expression changes across multiple tissues in response to dietary changes.

The lncRNA steroid receptor RNA activator (SRA) is a modulator of adipocyte differentiation and adipogenesis that functions to activate *Pparg* expression to induce Pparg-dependent pathways as well as signaling through insulin and tumor necrosis factor alpha (Tnfα) pathways (88). SRA is expressed in both white and brown adipose tissues and at lower levels in the liver, heart, and spleen (52). SRA becomes upregulated in the inguinal white fat after feeding with an HFD for 12 weeks. In the liver, SRA is downregulated following a 16-h fast, yet a 12-week HFD had no significant impact on SRA levels in the liver (17). Interestingly, SRA KO mice were protected from HFD-induced obesity and had decreased white adipose tissue mass and reduced expression of lipogenic genes (*Fabp4*, *Adipoq*) and inflammatory genes including *Tnf*, *interleukin-6* (*Il6*), and the chemokine *Ccl2* in adipose tissue (52). Thus, SRA deletion prevents a lipogenic and inflammatory program in adipose tissue in the context of an HFD. While an HFD does not change expression of SRA in the liver, SRA KO mice on an HFA were protected from lipid accumulation in the liver (52). SRA KO mice had lower levels of triglycerides and free fatty acid in the liver; decreased expression of various lipogenic genes including *Ppara*, *Pparg*, *Fabp4*, and *lipase E*; and increased expression of *adipose triglyceride lipase* (*Atgl*), a hepatic triglyceride lipase that is central to free fatty acid metabolism (62). These observations suggest a *trans* mechanism for SRA regulation in the context of an HFD, yet additional studies are necessary to determine whether SRA itself recruits specific proteins to change gene expression across these pathways.

There is a group of lncRNAs located in the genomic region of sirtuin 1, a key regulator of insulin sensitivity, that could play important roles in insulin sensitivity and the development of diabetes (86). One of these lncRNAs is regulator of insulin sensitivity and autophagy (Risa), which is widely expressed across mouse tissues including liver, adipose, and muscle, and is upregulated in both liver and muscle tissues in response to fasting for 18 h. Overexpression of *Risa* in primary mouse hepatocytes and C2C12 myotubes reduced phosphorylation of insulin signaling pathway proteins, specifically the insulin receptor Akt, and glycogen synthase kinase 3 beta, a regulator of glycogen metabolism, in response to insulin stimulation, reflecting induced insulin resistance (25). Risa also regulates autophagy, a process that has been shown to be critical for the development of insulin resistance. *Risa* overexpression in hepatocytes and myotubes reduced levels of autophagy, determined by reductions in LC3B-II protein and increased levels of phosphorylated Ulk1 (Ser^{757}). Risa knockdown using siRNAs in murine hepatocytes and also in vivo in the liver increased phosphorylation of members of the insulin signaling pathway, and this effect was reversed when autophagy was blocked by knockdown of autophagy regulator *Atg7* or *Atg5* (42, 43). Additional studies are needed to determine how Risa influences phosphorylation of proteins involved in autophagy and insulin regulation, yet it is tempting to speculate that Risa might regulate pathways that are important during the development of diabetes.

These lncRNAs suggest complex regulatory networks at play in metabolic tissues in response to changing nutritional cues and reveal the importance of studying the lncRNA landscape in multiple tissues in response to dietary changes.

THE lncRNA H19 HAS ROLES IN METABOLISM AND NUTRITION

H19 is a lncRNA that exhibits imprinted gene expression, in part due to its location within the *H19/insulin-like growth factor 2* (*Igf2*) gene cluster, where expression is regulated by an imprinting control region (ICR) that is differentially methylated between the maternal and paternal alleles

(95). *H19* is exclusively expressed from the maternal chromosome (6) and *Igf2* is exclusively expressed from the paternal chromosome (5). Loss of allelic-specific methylation of the *H19/Igf2* ICR leads to the congenital overgrowth syndrome Beckwith-Wiedemann syndrome (68). H19 is an important player in many cancer types, exhibiting either oncogenic or tumor-suppressive roles depending on the tissue type (32). Recent work demonstrated that H19 also functions in modulating metabolic processes in the liver and in muscle tissue.

H19 RNA is robustly expressed in both skeletal and cardiac muscle and is low or undetectable in most mature tissues (29). H19 levels in muscle tissue are decreased in patients with T2DM as well as in the muscle tissue of mice fed an HFD, which is often used to model insulin resistance and T2DM (30, 76). H19 can sequester the miRNA let-7 (41), and an HFD reduced the levels of let-7 target genes, suggesting that decreased levels of H19 after an HFD led to increased availability of let-7 for altering downstream regulatory genes. H19 siRNA knockdown in microtubes decreased the expression of the *insulin receptor* gene (*Insr*), a known target of let-7 that is central to insulin signaling (102). Let-7 inhibition using the specific inhibitor iLet-7 in myotubes in addition to H19 siRNA knockdown restored expression of *Insr*. H19 KO in myotubes also impaired glucose uptake in the setting of insulin treatment, suggesting that downregulation of H19 following an HFD may contribute to insulin resistance (30). Intriguingly, in the setting of hyperinsulinemia, let-7 miRNA levels increased yet H19 levels decreased, demonstrating a previously uncharacterized double-negative feedback loop between H19 and let-7 in muscle tissue during the development of insulin resistance. These observations were recapitulated using a full KO of H19, where animals exhibited increased insulin resistance in muscle tissue (31). H19 KO mice fed normal chow also exhibited high levels of let-7 miRNA in muscle tissue, supporting a H19/let-7 double-negative feedback loop. H19 KO mice also exhibited increased insulin resistance in muscle tissue, evidenced by decreased glucose uptake by muscle-specific KO in response to insulin stimulation (31). These studies identified H19 as a nutritionally sensitive lncRNA that responds to an HFD in muscle tissue, and H19 can contribute to the development of insulin resistance and T2DM in the setting of an HFD.

While H19 levels fall in muscle tissues of patients and mouse models with T2DM, patients with T2DM have increased expression of *H19* in the liver compared with healthy controls (61). *H19* is also upregulated in the livers of mice fed an HFD and in mice that were subjected to overnight fasting (97). To determine the impact of *H19* upregulation in the liver after an HFD, *H19* overexpression experiments using adenoviral injections in mice were performed. *H19* overexpression in the liver led to increased hepatic glucose production, hyperglycemia, and increased insulin resistance. Conversely, whole-body KO of H19 in mice led to increased insulin sensitivity in the liver. These results demonstrate that upregulation of *H19* in the setting of T2DM or an HFD model of T2DM may contribute to insulin resistance in the liver.

To determine the mechanism of H19 modulation of insulin resistance, *H19* overexpression experiments were performed in primary murine hepatocytes. *H19* overexpression resulted in increased expression of the gluconeogenic genes *Hnf4a*, *phosphoenolpyruvate carboxykinase 1* (*Pck1*), and *G6pc*, and increased glucose production, reflecting the development of insulin resistance and overactive gluconeogenesis (97).

These studies demonstrate unique roles for the lncRNA H19, present in both humans and mice, in liver and muscle tissues in response to nutritional changes. While *H19* is downregulated in muscle tissue in T2DM and an HFD mouse model of T2DM, *H19* is upregulated in the liver for the same condition in both humans and mice. In both cases, the levels of H19 RNA contribute to disease pathogenesis in a tissue-specific manner. While decreased H19 in muscle tissue contributes to the development of insulin resistance, increased H19 in the liver leads to overactive glucose production.

Collectively, one nutritional cue (HFD) that models human disease (T2DM) can lead to opposite alterations in *H19* expression in two metabolic tissues, with downstream effects that converge on disease pathogenesis.

THE lncRNA XIST: INTERSECTION OF SEX BIASES AND NUTRITION

Dosage compensation involves various lncRNAs, most notably X-inactive specific transcript (Xist), a 17-kb RNA exclusively expressed from the inactive X chromosome (Xi). Female mammals have two X chromosomes, and one X chromosome is transcriptionally silenced during early development to equalize expression of X-linked genes between the sexes (54). This process, called X-chromosome inactivation (XCI), is initiated by upregulation of the lncRNA Xist from the future Xi (8, 10, 11). Xist RNA physically accumulates along the Xi (19, 57, 64) and recruits heterochromatic machinery to deposit repressive histone marks and other silencing features chromosome-wide (**Figure 5*a***) (104). Various heterochromatic modifications including H3K27me3 (16), H2AK119 ubiquitin (35), DNA methylation (20), and H3K9me3 (16) are enriched on the Xi and maintained with each cell division, generating a memory of transcriptional silencing that is inherited with each cell division into adulthood. Not all genes on the Xi are transcriptionally silenced. Some X-linked genes escape XCI (**Figure 5*b***) and exhibit cell- and tissue-specific expression patterns, although expression from the Xi is lower compared with the levels from the active X (7, 13, 81).

Xist RNA is necessary and sufficient for XCI initiation, and recent work indicates that it is also necessary for XCI maintenance in somatic cells (57, 64). Deletion of *Xist* in the hematopoietic compartment results in abnormal overexpression of approximately 200 X-linked genes, and female animals develop a fulminant blood cancer (94). However, *Xist* deletion in the gastrointestinal system has no effect unless female mice are stressed with azoxymethane dextran sulfate, in which case mice with *Xist* deletion in the gastrointestinal epithelium develop larger and more frequent gut polyps compared with wild-type littermates (92).

While Xist RNA is necessary for both XCI initiation and maintenance, recent work suggests that Xist RNA localization to the Xi also plays an important role for XCI regulation in certain somatic cells. Remarkably, XCI is maintained in a unique and dynamic manner in lymphocytes. Naive B and T cells lack Xist RNA and heterochromatin modifications at the Xi, despite abundant Xist RNA transcripts, and in vitro lymphocyte activation stimulates the relocalization of Xist RNA and heterochromatin marks to the Xi (**Figure 5*c*,*d***) (66, 79, 84). Localization of Xist RNA and heterochromatin modifications is perturbed in lymphocytes from mouse models of lupus-like disease and women with lupus (**Figure 5*c*,*d***), along with aberrant X-linked gene expression (77, 78). These findings suggest that perturbations of Xist RNA localization likely contribute to the female bias of lupus (66). While the role of Xist RNA in nutrition and metabolic diseases has not been well explored, there are observations that suggest the lncRNA Xist may also play a role in metabolic health and disease.

The X chromosome is enriched for a number of metabolic genes (39), and there are various metabolic diseases that can result from mutations in the 55 metabolism-related X-linked genes (28). Furthermore, diseases of metabolic tissues, including obesity, cardiovascular disease, and diabetes, are known to exhibit a sex bias (51). Researchers using the four-core-genotypes mouse model discovered that the number of X chromosomes, and not hormones, underlies sex differences in adiposity in mice (18). The researchers found that XX mice, regardless of hormonal status, had increased adiposity and heightened food intake and were predisposed to developing fatty liver on an HFD. They also found a number of genes previously described as escaping silencing on the Xi that were overexpressed in XX mice compared with XY mice, a discovery that may underlie

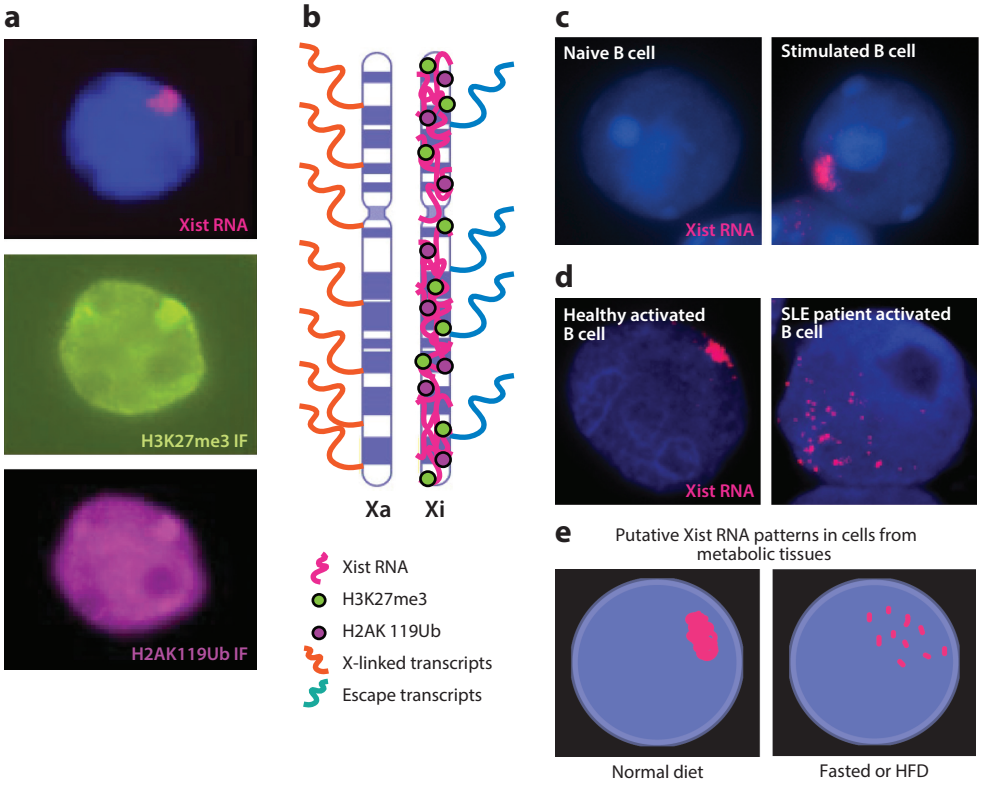

Figure 5

Epigenetic modifications of the Xi for XCI maintenance. (*a*) Xist RNA and heterochromatic modifications H3K27me3 and H2AK119Ub are enriched at the Xi. The same nucleus is shown top to bottom. (*b*) Schematic representation of the Xa and Xi. Repressive modifications H3K27me3 and H2AK119Ub are shown as circles. Transcription of escape genes from the Xi is shown as blue wavy lines. (*c*) Xist RNA FISH for naive B cell (*left*) and in vitro stimulated B cell (*right*). Human and mouse lymphocytes exhibit the same pattern for Xist RNA localization. (*d*) Nuclei from human in vitro activated B cells. (*e*) Putative Xist RNA localization patterns for cells from metabolic tissues (hepatocytes, adipocytes, or muscle cells) under normal (*left*) or fasted or HFD conditions (*right*). Abbreviations: FISH, fluorescent in situ hybridization; H2AK119Ub, H2AK119 ubiquitin; HFD, high-fat diet; IF, immunofluorescence; SLE, systemic lupus erythematosus; Xa, active X chromosome; XCI, X-chromosome inactivation; Xi, inactive X chromosome; Xist, X-inactive specific transcript.

the female bias associated with adiposity. These findings suggest that specific and unidentified X-linked genes are required to maintain metabolic homeostasis and that aberrant gene dosage may contribute toward the female bias of metabolic diseases. The molecular details of how aberrant XCI escape could occur in metabolic tissues have not been examined.

XCI maintenance in metabolic tissues (liver, adipose, and muscle) has not been carefully examined, and epigenetic features of the Xi may not be faithfully maintained across all somatic cells. It is compelling to consider whether some metabolic tissues have a system comparable to that of lymphocytes, where distinct nutritional cues might serve as stimuli to dynamically modulate Xist RNA localization to the Xi (**Figure 5*e***). Furthermore, whether metabolic diseases impact XCI maintenance in these tissues is unknown. Importantly, many of the studies reviewed in this article characterized lncRNAs that are responsive to nutritional cues using only male mice

(**Table 1**), and we did not find any publication that compared nutritionally regulated lncRNAs between male and female mice. Thus, whether lncRNA responses to nutrition exhibit any sex bias is still unclear at this time. It is tempting to speculate that Xist RNA may utilize distinct epigenetic mechanisms in metabolic tissues to facilitate increased X-linked gene escape from the Xi, which would contribute toward sex differences with certain metabolic disorders.

CONCLUSIONS

A new class of lncRNAs is emerging, encompassing lncRNAs that are nutritionally sensitive and regulate responsive metabolic pathways in numerous organs that orchestrate responses to changes in diet, summarized in **Table 1**. The expression of the lncRNAs highlighted in this review is either upregulated or downregulated in response to dietary changes, in a tissue-specific context. These lncRNAs have evolved specific mechanisms that impact various metabolic pathways, spanning gluconeogenesis, lipogenesis, and adipocyte differentiation. Importantly, some of these lncRNAs coordinate physiological responses to nutrient deprivation, and some have roles in the pathogenesis of diseases influenced by nutrition, including T2DM and NAFLD. Importantly, while many lncRNAs have been identified using next-generation sequencing technologies, very few have been functionally characterized, indicating that there are many more lncRNA mechanisms yet to be discovered for metabolic tissues that respond to dietary changes. Some well-characterized lncRNAs, such as H19, have diverse roles in nutritional responses in multiple metabolic tissues, highlighting the complex roles for some lncRNAs beyond the pathways in which they were first discovered. Finally, the studies described here highlight a critical gap in our knowledge of how nutritionally modulated lncRNAs function in male or female tissues. Future studies examining how sex as a biological variable influences the roles of the lncRNAs profiled in this review will be crucial for improving our understanding of sex differences in metabolic and nutritional diseases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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