



REVIEW ARTICLE

Critical roles of FTO-mediated mRNA m⁶A demethylation in regulating adipogenesis and lipid metabolism: Implications in lipid metabolic disorders

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Abstract The goal this review is to clarify the effects of the fat mass and obesity-associated protein (FTO) in lipid metabolism regulation and related underlying mechanisms through the FTO-mediated demethylation of m⁶A modification. FTO catalyzes the demethylation of m⁶A to alter the processing, maturation and translation of the mRNAs of lipid-related genes. FTO overexpression in the liver promotes lipogenesis and lipid droplet (LD) enlargement and suppresses CPT-1-mediated fatty acid oxidation via the SREBP1c pathway, promoting excessive lipid storage and nonalcoholic fatty liver diseases (NAFLD). FTO enhances preadipocyte differentiation through the C/EBP β pathway, and facilitates adipogenesis and fat deposition by altering the alternative splicing of RUNX1T1, the expression of PPAR γ and ANGPTL4, and the phosphorylation of PLIN1, whereas it inhibits lipolysis by inhibiting IRX3 expression and the leptin pathway, causing the occurrence and development of obesity. Suppression of the PPAR β/δ and AMPK pathways by FTO-mediated m⁶A demethylation damages lipid utilization in skeletal muscles, leading to the occurrence of diabetic hyperlipidemia. m⁶A demethylation by FTO inhibits macrophage lipid influx by downregulating PPAR γ protein expression and accelerates cholesterol efflux by phosphorylating AMPK, thereby impeding foam cell formation and atherosclerosis development. In summary, FTO-mediated m⁶A demethylation modulates the

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expression of lipid-related genes to regulate lipid metabolism and lipid disorder diseases.
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Abbreviations

FTO	the fat amount and obesity-related protein
SREBP1C	sterol regulatory element binding protein-1c
FAS	fatty acid synthase
SCD	stearoyl-CoA desaturase
ACC1	acetyl-CoA carboxylase 1
DGAT	diacylglycerol acyltransferase 2
CPT1	carnitine palmitoyltransferase 1
ROS	reactive oxygen species
CIDEc	cell death-inducing
DFFA	(DNA fragmentation factor- α)-like effector C
C/EBP β	CCAAT/enhancer-binding protein β
UCP-1	uncoupling protein
RUNX1T1	Runt-related transcription factor 1
SRSF2	serine/arginine-rich splicing factors 2
PPAR γ	peroxisome proliferators-activated receptors γ
CTSB	cathepsin B
ANGPTL4	angiopoietin-like protein 4
LPL	lipoprotein lipase
IRX3	Iroquois-class homeobox protein
CUX1	cut-like homeobox 1
RPGRIP1L	regulatory factor interaction protein-1-like.

Introduction

The prevalence of lipid disorders in the Western world has become increasingly alarming,¹ and up to 58% of the adult population worldwide is predicted to be overweight or obese by 2030.² Accumulating evidence indicates that obesity is closely associated with an increased risk of lipid disorder diseases such as NAFLD, type 2 diabetes mellitus (T2DM), dyslipidemia and atherosclerosis.^{1,3} The results of recent studies have shown that the N6 methyladenosine (m⁶A) modification is implicated in lipid metabolism and the progression of lipid disorders in the human body.⁴ As the most abundant epigenetic modification of eukaryotic messenger RNA (mRNA), m⁶A plays key roles in modulating of substrate mRNA metabolism, including that of some lipid-related genes.⁵

The fat mass and obesity-associated gene (*FTO*) was first discovered in a genome-wide association study (GWAS) of T2DM patients in Europe.⁶ The discovery of *FTO* as the first m⁶A mRNA demethylase established the concept of reversible RNA modifications.⁷ *FTO* is the primary m⁶A eraser that belongs to the ALKB family of Fe(II)/ α -keto-glutarate-dependent dioxygenases, which preferentially

bind to pre-mRNA strands in intronic regions, in the proximity of alternatively spliced exons and poly(A) sites.⁸ *FTO* has powerful demethylase activity toward multiple methylated RNA substrates, which affects mRNA splicing, stability, decay and translation. *FTO* is primarily localized in the nucleus and cytoplasm⁹ and controls the expression of lipid-related genes to regulate multiple processes associated with lipid metabolism.^{10–12}

The tissue expression profile of *FTO* showed that it is distributed in adult and fetal livers, adipose tissue, the hypothalamus, islets, skeletal muscle and macrophages.¹³ The expression of hepatic *FTO* is regulated by metabolic signals, such as nutrients and hormones, which affect the metabolism of glucose and lipids in the liver. *FTO* overexpression was shown to lead a dose-dependent increase in body weight and fat mass, regardless of whether mice were fed a standard or high-fat diet.¹⁴ *FTO* was observed to alter changed the expression of genes related to glucose metabolism, resulting in the development of hyperlipidemia in T2DM patients.¹⁵ However, the regulatory roles and underlying mechanism of *FTO* in lipid metabolism are not well understood, which hinders the discovery of effective tactics to control *FTO*. Thus, additional investigations are also required to evaluate the role *FTO* in lipid disorders to prevent or treat certain lipid disorder diseases.

Due to the increasing importance of the demethylase *FTO* in lipid metabolism, we first summarized general information on *FTO*, including the gene locus, protein structure and catalytic activity before discussing the role of *FTO* in lipid synthesis and decomposition in the liver. Subsequently, we discuss the effect of *FTO* on the differentiation, transformation and lipid metabolism of adipose tissue. Finally, we summarize the role of *FTO* regulation in the occurrence of diabetic hyperlipidemia and macrophage lipid flux. The goal of this review is to clarify the exact roles and underlying mechanisms of the demethylase *FTO* in lipid metabolism to better understand and evaluate the *FTO*-driven pathogenesis of lipid disorder diseases.

FTO biology

The *FTO* gene was initially confirmed as an obesity-susceptibility gene that predispose individuals to obesity by independent GWASs in obese cohorts of Belgian Caucasian and European American individuals.^{16,17} The mouse *FTO* gene is located on chromosome 8, while the human *FTO* gene is located on 16q12.2. Human *FTO* is approximately 400 kb in length, comprises 8 introns and 9 exons and encodes multiple protein products. The *FTO* gene is highly homologous among mammalian species such as mice, pigs and other mammals.¹⁸ To date, only a few SNPs have been identified in the first intron of the *FTO* gene.¹⁹ The approximately 3.4-kb region upstream of the human *FTO*

gene contains a transcriptional initiation site for the retinitis pigmentosa GTPase regulator interacting protein 1-like (*RPGRIP1L*) gene. The first intron of *FTO* harbors a binding site for the transcription factor cut-like homeobox 1 (*CUX1*), which promotes the expression of *RPGRIP1L* after binding to this site.²⁰ The region downstream of the *FTO* gene is adjacent to *IRX3*, *IRX5* and *IRX6* which belong to the Iroquois gene family (Fig. 1A). The first intron of the *FTO* gene possesses an enhancer sequence for the *IRX3* gene, which binds to the promoter of *IRX3* to boost its expression.^{19,20}

The FTO protein is a member of the non-heme dioxygenase [Fe(II)- and 2-oxoglutarate-dependent dioxygenases] superfamily.^{21,22} The FTO protein contains an amino-terminal AlkB-like domain (also referred to as amino-terminal domain; NTD) and a carboxy-terminal domain (CTD) with novel fold (Fig. 1B). The conserved sequence of the AlkB-like domain is located at amino acid positions 57–324 of the N-terminus of the FTO protein. The catalytic core of NTD consists of a double-stranded β -helix motif (DSBH; also referred to as the jelly roll motif) with the highly conserved residues His231, Asp233 and His307 coordinated with Fe(II), and Arg316 and Arg322 forming a salt bond with N-oxalylglycine (NOG). One side of the DSBH motif is buttressed by two α -helices ($\alpha 3$ and $\alpha 4$), while the other side is covered by a long loop (called an L1 ring) linking $\beta 5$ and $\beta 6$. The interaction of the L1 loop with the jelly-roll motif primarily occurs through Trp230-mediated hydrophobic contacts. Moreover, the L1 loop completely covers this region and makes it less positively charged. Hydrophobic interactions also stem from the packing of Leu109 and Val228 against the sugar ring of 3-methylthymidine (3-meT) in the single-stranded DNA, indicating that FTO has a strong preference for 3-meT or 3-methyluracil (3-meU) in single-stranded DNA. The 31-residue truncated N-terminal region of FTO (FTO Δ 31) was shown to bind to the single nucleotide 3-meT to participate in substrate sorting, and NOG was used for the formation of a catalytically inert FTO-substrate complex. The CTD of the FTO protein does not possess any known structure that is significantly homologous to the other members of the non-heme dioxygenase superfamily and represents a new fold. The CTD contains approximately 170 residues and primarily forms α -helices, of which $\alpha 7$, $\alpha 8$ and $\alpha 10$ form a triple helical bundle. One end of the helical bundle has extensive interactions with the NTD, suggesting that the CTD primarily plays an important role in stabilizing the conformation of the NTD.²³ Therefore, the catalytic activity of FTO is achieved by the interaction between the NTD and CTD.²²

FTO, the first identified RNA m⁶A eraser, has been shown to have strong catalytic activities toward multiple RNA methylation substrates, including m⁶Am and m⁶A at the 5' cap of mRNAs and snRNAs.²⁴ The RNA m⁶A modification involves the catalytic transfer of methyl groups from reactive methyl compounds (such as S-adenosyl-L-methionine) onto the sixth position nitrogen atom (N) of adenine (A) on the RNA strand by the m⁶A writers METTL3, METTL14 and WTAP.²⁵ RNA m⁶A modification can be reversibly removed by the m⁶A erasers FTO and ALKBH5 from the methylated mRNA strand (Fig. 2). As an m⁶A eraser, FTO reversibly catalyzes the demethylation of m⁶A. First, FTO oxidizes m⁶A to the intermediate N⁶-hydroxymethyl adenosine (hm⁶

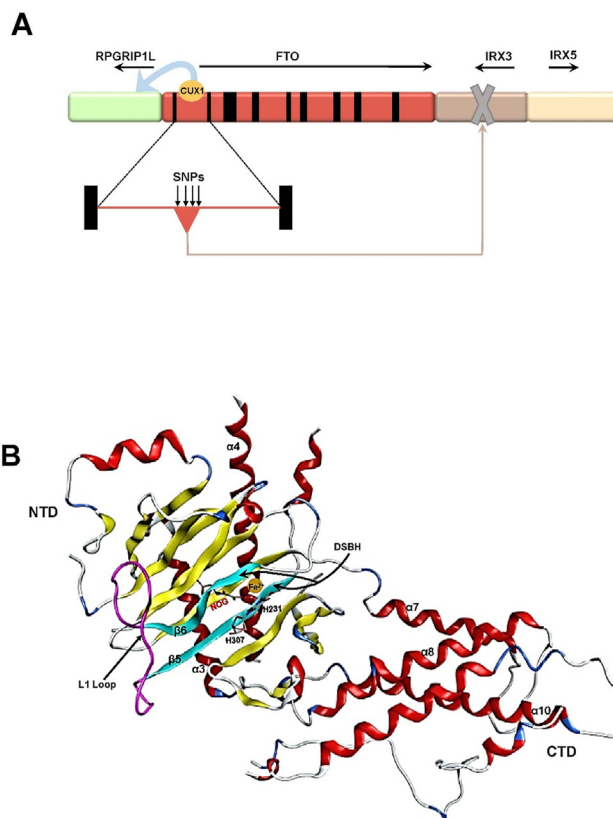


Figure 1 The structure of human FTO. (A) Human FTO is approximately 400 kb in length, comprises 8 introns and 9 exons and encodes multiple protein products. The approximately 3.4-kb region upstream of the human FTO gene contains a transcriptional initiation site for the *RPGRIP1L* gene. The first intron of FTO harbors a binding site for the transcription factor *CUX1* which promotes the expression of *RPGRIP1L* after binding to this site. The region downstream of the FTO gene is adjacent to *IRX3* and *IRX5* which belong to the Iroquois gene family. The first intron of the FTO gene possesses an enhancer sequence for the *IRX3* gene, which binds to the promoter of *IRX3* to promote its expression. (B) The conformation of FTO protein. The FTO protein contains an NTD and a CTD with a novel fold. The catalytic core of NTD consists of a DSBH with the highly conserved residues: His231, Asp233 and His307, which are coordinated with Fe(II), and Arg316 and Arg322, which form a salt bridge with NOG. One side of the DSBH motif is buttressed by two α -helices $\alpha 3$ and $\alpha 4$, whereas the other side is covered by a long loop linking $\beta 5$ and $\beta 6$. The CTD contains approximately 170 residues and primarily forms α -helices, of which $\alpha 7$, $\alpha 8$ and $\alpha 10$ form a triple helical bundle.

A) by intermediate modification of ferrous ions and 2-oxoglutarate-dependent dioxygenase. Second, the FTO protein further oxidizes metastable hm⁶A in the same manner to form N⁶-formyladenosine (f⁶A). It is possible that additional protein factors are involved in the FTO-mediated demethylation of m⁶A by catalyzing the hydrolysis of hm⁶A and f⁶A. Finally, hm⁶A and f⁶A naturally decompose into adenine (A) in aqueous solution.²⁶ After the m⁶A modification is removed by FTO, the original sites of m⁶A modification cannot be recognized by “reader” proteins (such as YTH or non-YTH proteins), and some RNA binding proteins

cannot interact with these sites, which affects the splicing, maturation, translation or degradation of target RNAs.

In vivo and *in vitro* studies have shown that m⁶A is closely associated with lipid metabolism. For instance the m⁶A modification was shown to be increased in patients with fatty livers.⁹ In addition, an increase in m⁶A levels was confirmed to cause obesity in both children and adult populations.²² These findings suggest that the abnormal expression and function of FTO is implicated in the development of lipid disorder diseases. An analysis of FTO protein expression profile showed that it is primarily distributed in the liver, hypothalamus, pancreas and adipose tissue, but little is currently known regarding the regulation and underlying mechanism of FTO in lipid metabolism.²⁷ Therefore, our review primarily focuses on the role and regulatory mechanism of FTO in lipid metabolism and related lipid disorder diseases in these tissues.

FTO and lipid metabolism in the liver

FTO and FTO-dependent m⁶A demethylation intimately participate in various aspects of hepatic lipid metabolism. The expression and function of FTO is modulated in response to alterations in hepatic lipid metabolism.²⁸ Significant increases in FTO mRNA and protein levels has been observed in the livers of in NAFLD patients,²⁹ where FTO expression was shown to be significantly increased and large amounts of fat were accumulated in the liver in NASH patients. *In vitro* FTO depletion reduces palmitic acid-induced lipotoxicity in HepG2 cells by alleviating endoplasmic reticulum (ER) stress and restoring mitochondrial function.³⁰ FTO overexpression leads to decreased m⁶A levels and contributes to fat deposition in HepG2 cells.³¹ Furthermore, FTO overexpression causes triacylglycerol (TG) accumulation and the upregulation of lipogenic genes in human hepatocytes, aligning with the abnormal characteristics of lipid metabolism observed in the context of NAFLD.^{29,31} Interestingly, a mutant FTO lacking demethylase activity fails to induce lipid deposition in HepG2 cells.³¹ These

findings support that aberrant levels of FTO expression and FTO-catalyzed m⁶A demethylation results in excessive lipid deposition in hepatocytes.

FTO enhances hepatocellular lipid synthesis and lipid droplet (LD) enlargement through the sterol regulatory element binding protein-1c (SREBP1c) pathway (Fig. 3). FTO-dependent m⁶A demethylation promotes the expression of these key genes including SREBP cleavage-activating protein (SCAP), site-1 proteases (S1P), and site-2 proteases (S2P), which are involved in the processing and nuclear translocation of SREBP1c mRNA in HepG2 cells, and indirectly elevates SREBP1c expression. SREBP1c is a key transcription factor that initiates the transcription of downstream genes involved in lipid synthesis. SREBP1c overexpression was reported to facilitate the development of extensive hepatic steatosis in mice.³² Mature SREBP1c binds to the promoter and increases the expression of target lipogenic genes, including fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD), acetyl-CoA carboxylase 1 (ACC1) and diacylglycerol acyltransferase 2 (DGAT2).³³ FAS and SCD1 are known to participate in the *de novo* synthesis, esterification and desaturation of fatty acids, and DGAT2 catalyzes the final step in TG generation, greatly accelerating hepatic lipid synthesis.³¹ SREBP1c also binds with the promoter sequence of the cell death-inducing DFFA (DNA fragmentation factor α)-like effector C (CIDE) gene to enhance its transcription.³⁴ CIDE often localizes on the surface of LDs and accelerates LD enlargement. The CIDE-mediated LD growth process requires a net directional transfer of lipids from smaller to larger LDs at LD-LD contact sites (LDCs), which is conducive to excessive lipid storage in HepG2 cells.²⁸

In addition, FTO overexpression damages hepatocellular mitochondria and inhibits the decomposition of lipids, resulting in lipid accumulation in the liver (Fig. 3). FTO-upregulated SREBP1c increases ACC1 expression, which subsequently inhibits carnitine palmitoyltransferase 1 (CPT-1)-mediated fatty acid oxidation. ACC1 catalyzes the synthesis of malonyl coenzyme A, which is an allosteric inhibitor of CPT1. CPT1 is a rate limiting enzyme localized onto the outer mitochondrial membrane and conveys long chain fatty acids into the mitochondrial compartment for β -oxidation. The failure of mitochondrial fatty acid β -oxidation induces the generation of reactive oxygen species (ROS) under CPT1 deficiency, which triggers oxidative stress in hepatocytes.³⁵ FTO overexpression was confirmed to lead to the accumulation of fatty acids and increased levels of oxidative stress in HepG2 cells.³¹ Interestingly, the levels of the oxidative stress biomarker malondialdehyde (MAD) were shown to be dramatically increased, while those of the antioxidant enzyme superoxide dismutase (SOD) were significantly inhibited in the context of hepatocellular overexpression of FTO in humans.³⁵ Oxidative stress further exacerbates mitochondrial dysfunction and hinders the decomposition of TG. Moreover, FTO overexpression has been shown to reduce the biogenesis and quantity of hepatocellular mitochondria by upregulating specific genes implicated in mitochondrial fusion while downregulating that of genes involved in mitochondrial biogenesis and fission, ultimately weakening mitochondrial function and impeding hepatic lipid decomposition.³¹

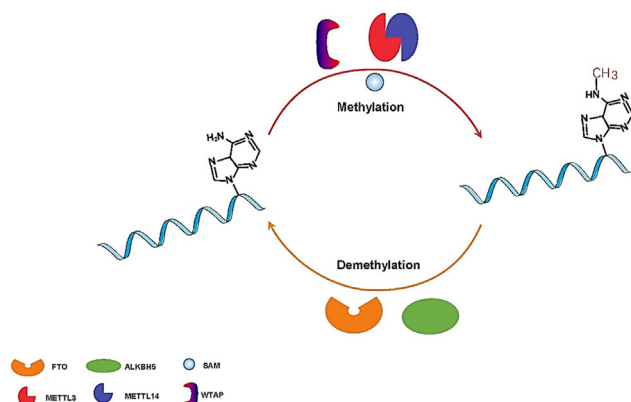


Figure 2 The reversible processes of methylation and demethylation of m⁶A modification. METTL3, METTL 4 and WTAP act as methylases to transfer the methyl-group onto the sixth carbon atom of substrate mRNA from the reactive methyl compound SAM. The demethylase FTO and ALKBH5 erase the m⁶A modification from the methylated mRNA strand.

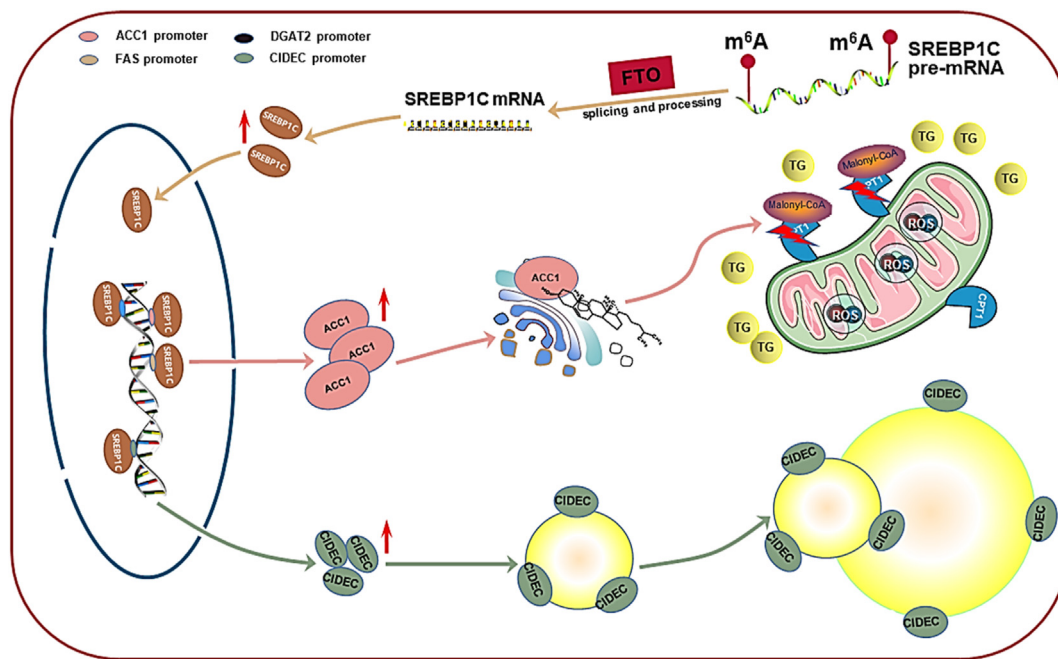


Figure 3 The roles and underlying mechanisms of FTO in hepatocellular lipid metabolism. FTO-dependent m⁶A demethylation promotes the mRNA processing, translation and nuclear translocation of SREBP1C. SREBP1C binds to the promoters of lipogenic genes, including FAS, SCD, ACC1 and DGAT, to enhance their expression and facilitate lipid synthesis in the liver. SREBP1C-upregulated ACC1 catalyzes the synthesis of malonyl CoA which inactivates CPT1 activity and suppresses CPT1-mediated β -oxidation of long-chain fatty acids. The failure of fatty acid oxidation in mitochondria triggers oxidative stress and leads to high ROS generation in hepatocytes, which further aggravates mitochondrial dysfunction and hinders TG decomposition. SREBP1C also promotes the transcription and expression of CIDEA which often resides on the surface of LDs to accelerate the expansion of hepatocellular LDs.

FTO and lipid metabolism in adipose tissue

FTO is closely associated with adipogenesis and increased fat mass. The results of an epidemiological investigation demonstrated that FTO mRNA levels in adipose tissue are positively correlated with body mass index (BMI).³⁶ FTO mRNA levels were higher in the adipose tissue of obese individuals than in that of subjects with normal BMI.³⁷ In C57BL/6N mice, FTO deficiency was shown to cause a significant reduction in body weight and fat mass, especially a marked decrease in white but not brown adipose tissue. FTO deficiency was also observed to promote the transformation of white adipocytes into brown or beige adipocytes.³⁸ Impaired FTO expression in the hypothalamus has been demonstrated to result in a decrease in overall energy consumption in obese populations.^{39,40} In another study, the systemic knockout or a limited inhibition of hypothalamic FTO led to a lean phenotype in C57BL/6N mice.⁴¹ These findings indicate that FTO ubiquitously participates in fat metabolism and accelerates adipogenesis.

FTO-dependent m⁶A demethylation promotes the differentiation and transformation of adipose tissue (Fig. 4).⁴² The early differentiation of preadipocytes into white or brown adipocytes is promoted by CCAAT/enhancer-binding protein β (C/EBP β), which is typically suppressed by miRNA130 and miRNA155. FTO was shown to downregulate the expression of miRNA130 and miRNA155 expression via m⁶A demethylation, resulting in an upregulation of C/EBP β

in 3T3-L1 preadipocytes and enhanced preadipocyte differentiation. FTO inhibits the expression of uncoupling protein (UCP)-1 in brown adipocytes, inhibiting the transformation of adipose tissue into brown adipose tissue. C/EBP β was also observed to reduce adipocyte UCP-1 expression and inhibit the UCP-1-mediated transformation to the brown adipocyte phenotype.⁴³ In another study, FTO was shown to upregulate C/EBP β levels and inhibit the differentiation of adipose tissue into brown adipose tissue, which was proven to heighten the susceptibility of individuals to being obese and overweight by inhibiting the browning of white adipose tissue.⁴⁴

FTO-dependent m⁶A demethylation promotes lipogenesis and fat deposition in adipose tissue. FTO controls the exogenous splicing of Runt-related transcription factor 1 (RUNX1T1) by reducing the number of m⁶A modifications around the splicing sites and reducing the binding affinity of the RUNX1T1 RNA strand with the splicing factor serine/arginine-rich splicing factor 2 (SRSF2). RUNX1T1 is an adipogenesis-related transcription factor that has two splice variants, including long (L) and short (S) isoforms. FTO increases the S isoform levels of RUNX1T1 and stimulates lipogenesis in 3T3-L1 preadipocytes, resulting in an increase in fat mass.⁴⁵ FTO-dependent demethylation suppresses miRNA130 expression, increasing the level of peroxisome proliferator-activated receptor γ (PPAR γ) and fat production in 3T3-L1 preadipocytes, with PPAR γ being a key effector of miRNA130 that supports lipogenesis.⁴⁶ FTO

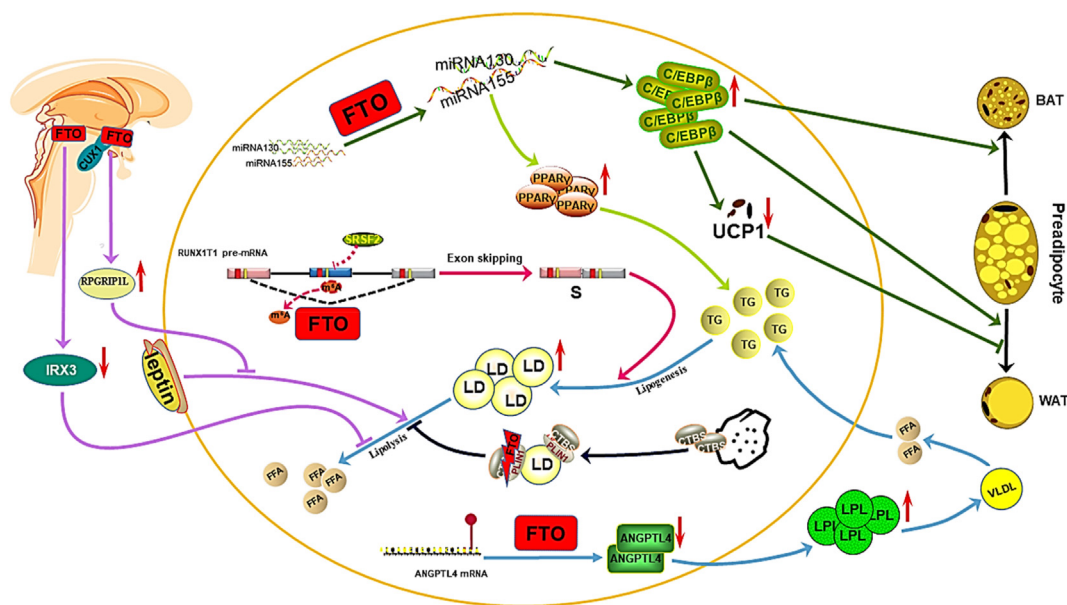


Figure 4 The roles and underlying mechanisms of FTO in lipid metabolism in adipose tissue. FTO-dependent m⁶A demethylation downregulates the expression of miRNA130 and miRNA155, causing the upregulation of C/EBPβ and the induced differentiation of preadipocytes. FTO-upregulated C/EBPβ expression reduces UCP-1 expression and inhibits the yield of brown adipocytes. FTO-dependent m⁶A demethylation alters the alternative splicing of RUNX1T1 and increases the production of the spliceosome sub-type, which stimulates adipogenesis in preadipocytes and increases body fat mass. FTO-downregulated miRNA130 expression enhances PPAR_γ expression and adipogenesis in preadipocytes. FTO disrupts the binding of CTSB with LDs and inhibits phosphorylation of plin1 by CTSB, resulting in the failure of TG hydrolysis in preadipocytes. FTO-dependent m⁶A demethylation reduces ANGPTL4 protein levels, accelerating LPL release and extracellular TG hydrolysis which provides the material for the synthesis of TGs and LDs in adipocytes. The long-range enhancer of the *FTO* gene downregulates hypothalamic IRX3 and inhibits lipolysis in peripheral adipocytes. In specific subsets of hypothalamic neurons, the binding site in *FTO* intron 1 interacts with the transcription factor CUX1 to increase RPGRIP1L expression, which reduces leptin signal transduction and leptin-induced lipolysis in adipocytes.

augments PLIN1 expression by inhibiting cathepsin B (CTSB)-mediated phosphorylation. CTSB is secreted by the lysosome and contacts the PLIN1 protein on the surface of LDs. PLIN1 localizes to the surface of mature LDs and promotes the accumulation and fusion of LDs in adipocytes. FTO interrupts the binding of CTSB with LDs and inhibits PLIN1 phosphorylation by CTSB, resulting in an inability of TG in LDs to be hydrolyzed in 3T3-L1 preadipocytes.⁴⁷ FTO binds to angiopoietin-like protein 4 (ANGPTL4) mRNA in a m⁶A modification-mediated manner and reduces ANGPTL4 protein levels.^{48,49} ANGPTL4 is an endocrine signal in white adipose tissue that is involved in lipid metabolism, and FTO promotes lipoprotein lipase (LPL) expression by reducing the level of ANGPTL4, resulting in the hydrolysis of extracellular TG.⁵⁰ Subsequently, free fatty acids enter adipocytes and become adipogenic substrates for the synthesis of TG and LD,⁴⁹ which promotes the accumulation of intracellular lipids. Therefore, plin1 promotes fat deposition by distributing excessive fatty acids to the TG library.⁵¹

FTO inhibits the lipolysis of adipocytes (Fig. 4) and downregulates the expression of UCP-1, leading a decreased mitochondrial uncoupling and lipolysis in 3T3-L1 preadipocytes.⁴³ The mediobasal hypothalamus is an important brain region involved in homeostatic control of caloric intake and lipolysis.⁵² Iroquois-class homeobox protein 3 (IRX3), which belongs to the homologous frame gene family Iroquois, is highly expressed in the hypothalamus and inhibits the occurrence of obesity.⁴¹

The long-range enhancer of the *FTO* gene downregulates hypothalamic IRX3 levels and inhibits lipolysis in peripheral adipocytes.⁵³ Inhibition of hypothalamic IRX3 was shown to reduce thermogenesis and increase body weight and white adipose tissue (WAT) mass in mice and humans.⁴¹ Specific subsets of hypothalamic neurons also inhibit lipolysis by regulating FTO expression.⁵⁴ *In vitro* and *in vivo* experimental results suggest that the binding site of *FTO* intron 1 interacts with the transcription factor CUX1, upregulating the adjacent RPGRIP1L to inhibit leptin receptor transport and leptin signaling.^{18,55,56} RPGRIP1L is located >100 bp in the opposite transcriptional orientation of *FTO* and is involved in anchoring the complex to promote leptin receptor aggregation to control food intake and regulate leptin sensitivity at the cell level.⁵⁵ The increased activity of FTO in the hypothalamus results in a progressive resistance to leptin in the hypothalamus, resulting in increased caloric intake and reduced steatolysis.⁵⁷ Thus, CUX1 upregulates RPGRIP1L by binding to *FTO* intron 1, thereby reducing leptin sensitivity and lipolysis, and promoting the occurrence and development of obesity.

FTO and lipid metabolism in other tissues

FTO participates in lipid disorders in diabetic patients and lipid metabolism in skeletal muscle and macrophages. The

FTO locus was shown to be closely involved in hyperlipidemia in patients with T2DM in a GWAS.^{58,59} FTO expression has been observed to be elevated and accompanied by increased VLDL-TG secretion from the liver to the plasma in T2DM patients, causing mild to moderate hypertriglyceridemia.⁶⁰ In another study, FTO mRNA and protein levels were shown to be significantly higher in the lateral femur in T2DM patients than in normal weight individuals.³⁵ FTO overexpression leads to excessive lipid storage in the skeletal muscles of T2DM patients.⁶¹ In addition, FTO has been confirmed to inhibit macrophage uptake of extracellular lipid and facilitate intracellular lipid efflux, suppressing macrophage lipid accumulation and foam cell formation.⁶²

FTO overexpression leads to the dysfunction of islet cells, the inhibition of insulin secretion and the occurrence of diabetic hyperlipidemia. FTO overexpression was shown to cause a cascade of ROS production in C57BL/6 mice, activating the NF- κ B pathway and triggering the inflammatory response and ER stress in pancreatic β cells,⁶³ reducing insulin secretion.³⁵ FTO overexpression was observed to delay the dephosphorylation of cAMP response element binding protein (CREB) and repress the CREB signaling pathway, resulting in an increase in hepatic secretion of VLDL-TG into the circulation in diabetic patients.⁶⁴ The CREB pathway promotes the expression of fatty acid oxidation-related genes and accelerates the hydrolysis and clearance of circular VLDL-TG. FTO inhibits the CREB pathway, increases VLDL-TG secretion from the liver and postpones VLDL-TG clearance, eventually causing hypertriglyceridemia in T2DM patients. Furthermore, the decrease in insulin secretion contributes to disturbances in lipid metabolism in the periphery, especially in skeletal muscles. FTO was shown to impair lipid uptake in skeletal muscles by suppressing PPAR β/δ expression.⁶⁵ In another study, FTO-dependent m⁶A demethylation was shown to inhibit lipid utilization by skeletal muscles by inhibiting the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) pathway.⁶⁶

Skeletal muscles are the primary peripheral organs associated with lipid utilization under physiological conditions. When insulin deficiency or resistance occurs, there are obstacles to skeletal muscles taking up and utilizing plasma lipids. PPAR β/δ overexpression was shown to enhance lipid uptake in skeletal muscles in mouse myogenic C2C12 cells.⁶⁵ Interestingly, FTO inhibited the expression of PPAR β/δ , leading to impaired lipid uptake and metabolism in skeletal muscle and the occurrence of hyperlipidemia. AMPK is considered to be a key energy sensor and plays a crucial role in lipid metabolism in skeletal muscles, and FTO-dependent m⁶A demethylation has been demonstrated to be closely involved in these regulatory processes. AMPK normally prevents excessive lipid storage in skeletal muscle fibers by upregulating the m⁶A methylation of FTO mRNA.^{66,67} During insulin deficiency, AMPK activity is evidently inhibited, which contributes to the upregulation of FTO expression, and leading to the suppression of adipose TG lipase (ATGL), hormone-sensitive triglyceride lipase (HSL) and PGC1 α expression C2C12 cells. ATGL and HSL are two key enzymes in lipolysis and lipid utilization in skeletal muscles.⁶⁸ PGC1 α promotes mitochondrial biosynthesis,

promoting lipid oxidation in skeletal muscles.⁶⁹ FTO expression also inhibits the CPT1- and CPT2-mediated transfer of fatty acids from the cytoplasm to the mitochondrial matrix for β -oxidation in C2C12 cells.⁷⁰ The downregulation of these lipid oxidation-related factors severely disrupts lipid utilization in skeletal muscle. In addition, the increase in FTO expression promotes the expression of C/EBP α and lipid synthesis in skeletal muscle when AMPK activity is inhibited.

FTO inhibits macrophage lipid uptake and accelerates cholesterol efflux. The scavenger receptor CD36 is the primary transporter that mediates the uptake of extracellular lipids by macrophage and is directly targeted by PPAR γ . FTO-dependent m⁶A demethylation has been observed to decrease PPAR γ protein expression, resulting in the downregulation of CD36 expression and a decrease in lipid uptake in RAW264.7 cells. FTO overexpression was shown to decrease CD36 mRNA levels and dramatically lessen the rate of lipid uptake by mouse peritoneal macrophages.⁷¹ In addition, FTO promotes AMPK phosphorylation and upregulated ATP-binding cassette transporter A1 (ABCA1) in mouse macrophages. ABCA1 consumes ATP to mediate intracellular cholesterol efflux, which strongly prevents excessive lipid accumulation in macrophages.⁷² Decreased AMPK activity was shown to block FTO-induced ABCA1 upregulation.^{71,73,74} Therefore, FTO increases ABCA1 expression in an AMPK activity-dependent manner, inhibiting lipid ingestion and enhancing cholesterol efflux, suppressing foam cell formation and atherosclerosis development.

Conclusion and prospective

As an m⁶A eraser, FTO catalyzes the demethylation of m⁶A and abrogates its subsequent binding to with reader proteins, which alters the splicing, maturation, translation or degradation of substrate mRNAs, including those of lipid-related genes. FTO overexpression promotes lipogenesis and lipid storage and suppresses lipolysis via the SREBP1c pathway, promoting the accumulation of excessive lipids in the liver. FTO enhances preadipocyte differentiation through the C/EBP β pathway. Moreover, FTO not only facilitates adipogenesis and LD formation, but also inhibits lipolysis in adipose tissue. FTO upregulation reduces insulin secretion via the inflammatory NF- κ B pathway and disrupts lipid utilization in skeletal muscles by suppressing the PPAR β/δ and AMPK pathways, leading to the occurrence of diabetic hyperlipidemia. FTO-catalyzed m⁶A demethylation inhibits macrophage lipid influx by downregulating PPAR γ , and accelerates cholesterol efflux through the phosphorylation of AMPK, thereby impeding foam cell formation and atherogenesis (Table 1). These findings suggest that FTO-catalyzed m⁶A demethylation controls the expression of lipid-related genes, participating in lipid metabolism and lipid disorder diseases.

Although research on the role of FTO in lipid metabolism has made excellent progress, there are still many problems worthy of further study. One substrate mRNA stand typically possesses multiple sites for m⁶A modification, and binds various reader proteins to produce distinct biological effects. However, it remains unclear how FTO selects

Table 1 Summary of the role of FTO in lipid metabolism.

FTO	Tissue/Cell	m ⁶ A of substrate RNA	expression of substrate mRNA	Effectors	Lipid metabolism	Reference
↑	HepG2 cells	↓	SREBP1C↑	FAS, SCD, ACC1↑	lipid synthesis↑	Conte et al (2010) ³³
↑	HepG2 cells	↓	SREBP1C↑	DGAT2, CIDEC↑	lipid store↑	Yabe et al (2003) ³⁴
↑	HepG2 cells	↓	SREBP1C↑	CPT1↓	fatty acid oxidation↑	Bravard et al (2011) ³⁵
↑	3T3-L1 preadipocytes	↓	MiRNA130↓	PPAR _γ , C/EBPβ↑	pre-adipocytes differentiation↑	Lee et al (2011) ⁴⁶
↑	3T3-L1 preadipocytes	↓	MiRNA130↓	UCP1↓	brown adipocytes↑	Kajimura et al (2009) ⁴³
↑	3T3-L1 preadipocytes	↓	—	RUNX1T1↑	fat mass↑	Zhao et al (2014) ⁴⁵
↑	3T3-L1 preadipocytes	↓	CTSB↓	PLIN1↑	triglyceride hydrolysis↓	Marcinkiewicz et al (2006) ⁴⁷
↑	3T3-L1 preadipocytes	↓	—	ANGPTL4↓	lipid accumulation↑	Wang et al (2015) ⁴⁹
↑	Mice and human hypothalamus	↓	IRX3↓	—	lipolysis↓	de Araujo et al (2020) ⁵³
↑	Mice and human hypothalamus	↓	RPGRIP1L↑	Leptin↓	lipolysis↓	Tung et al (2015) ⁵⁷
↑	C57BL/6 mice	↓	CREB↓	VLDL-TG↑	Lipid circulation clearance↓	Lin et al (2014) ⁶⁴
↑	C2C12 cell	↓	AMPK↓	ATGL, HSL, PGC1↓	lipid oxidation↓	Haemmerle et al (2002) ⁶⁸
↑	C2C12 cell	↓	PPARβ/δ↓	—	lipid uptake and metabolism↓	Dressel et al (2003) ⁶⁵
↑	C2C12 cell	↓	—	CPT1, CPT2↓	β-oxidation↓	Bonnefont et al (2004) ⁷⁰
↑	RAW264.7 cells	↓	PPAR _γ ↓	CD36↓	lipid uptake↓	Mo et al (2017) ⁷¹
↑	J774.A1 cells	↓	AMPK↑	ABCA1↑	cholesterol efflux↑	Wu et al (2019) ⁷²

Remarks: SREBP1C, sterol regulatory element binding protein 1c; FAS, fatty acid synthase; SCD, stearoyl-CoA desaturase; ACC1, acetyl-CoA carboxylase 1; DGAT2, diacylglycerol acyltransferase 2; CIDEC, cell death-inducing DFFA (DNA fragmentation factor-α)-like effector C; CPT1, carnitine palmitoyltransferase1; PPAR_γ, peroxisome proliferators-activated receptors γ; C/EBPβ, CCAAT/enhancer-binding protein β; UCP1, uncoupling protein1; RUNX1T1, Runt-related transcription factor 1; CTSB, cathepsin B; ANGPTL4, angiopoietin-like protein 4; IRX3, Iroquois-related homeobox 3; RPGRIP1L, regulatory factor interaction protein-1-like; CREB, cAMP response element binding; AMPK, adenosine 5'-monophosphate (AMP)-activated protein kinase; ATGL, adipose triglyceride lipase (ATGL); HSL, hormone-sensitive triglyceride lipase; PGC1, PPAR_γ coactivator 1α; CPT1, carnitine palmitoyltransferase 1; ABCA1, ATP-binding cassette transporter A1. ↑, increased; ↓, decreased; —, non-observed result.

specific sites with m⁶A modifications and regulates the expression of substrate mRNA. The m⁶A erasers includes FTO, ALKBH5, and ALKBH3 as well as and novel unknown demethylases awaiting discovery in the future. The function or expression of FTO may be altered under medical intervention, and the side effects on other m⁶A demethylases or whether other demethylases will compensate for FTO intervention is not known. The upstream mechanism manipulating FTO expression and downstream molecules targeted by FTO requires further investigation, which will be helpful in identifying several strategies to effectively manipulate the expression and function of FTO. Although FTO protein expression is distributed in the hypothalamus and peripheral tissues, the results of multiple studies have shown that hypothalamic FTO appears to exhibit a powerful and extensive effect on intracorporeal lipid metabolism and energy consumption.^{54,75} Therefore, the modulation of

hypothalamic FTO would be valuable in preventing imbalances in lipid metabolism and lipid disorder diseases. The modulation of peripheral FTO is a seemingly suitable therapeutic approach for specific lipid disorder diseases.

The aberrant expression and function of FTO protein causes abnormalities in lipid metabolism and the development of lipid disorder diseases, including obesity, NAFLD, T2DM hyperlipidemia, and atherosclerotic cardiovascular diseases. As an essential factor in the developmental process, FTO knockout leads to death at a young age in mice. Thus, it is necessary to identify a new means of thoroughly and comprehensively evaluating the regulatory roles and underlying mechanisms of FTO in intracorporeal lipid metabolism, and to obtain a theoretical basis for FTO-mediated regulation of lipid metabolism. Further studies are warranted to identify promising upstream or downstream target molecules and feasible treatments to

effectively alter the expression and activity of FTO, which will correct FTO regulation in lipid metabolism and suppress the progression of lipid disorder diseases in pathogenic circumstances.

Conflict of interests

The author declares no conflict of interest.

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