Transcriptional regulation of N⁶-methyladenosine orchestrates sex-dimorphic metabolic traits

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Males and females exhibit striking differences in the prevalence of metabolic traits including hepatic steatosis, a key driver of cardiometabolic morbidity and mortality. RNA methylation is a widespread regulatory mechanism of transcript turnover. Here, we show that presence of the RNA modification N⁶-methyladenosine (m⁶A) triages lipogenic transcripts for degradation and guards against hepatic triglyceride accumulation. In male but not female mice, this protective checkpoint stalls under lipid-rich conditions. Loss of m⁶A control in male livers increases hepatic triglyceride stores, leading to a more 'feminized' hepatic lipid composition. Crucially, liver-specific deletion of the m⁶A complex protein Mettl14 from male and female mice significantly diminishes sex-specific differences in steatosis. We further surmise that the m⁶A installing machinery is subject to transcriptional control by the sex-responsive BCL6–STAT5 axis in response to dietary conditions. These data show that m⁶A is essential for precise and synchronized control of lipogenic enzyme activity and provide insights into the molecular basis for the existence of sex-specific differences in hepatic lipid traits.

The liver is the central tissue orchestrating rapid adaptations in gene regulation to maintain metabolic homeostasis. Control of mRNA biogenesis is a key target of multiple regulatory pathways in liver and its disruption can result in metabolic disturbances including fatty liver disease. For example, chronic high-fat/high-carbohydrate feeding stimulates the master lipogenic transcription factor SREBP1C, transcriptional induction of lipogenic genes and increased hepatic triglyceride stores. Reciprocally, under fasting conditions, activity of SREBP1C and its downstream targets Scd1, Dgat2 and Fasn is reduced. Fasting is characterized by low insulin, almost absent nuclear SREBP1C and low demand for fatty acid biosynthesis. Mysteriously, despite lower levels of lipogenic mRNA and protein compared with those in the fed state in liver, the absolute expression of lipogenic genes during fasting is remarkably high (top 0.1–2% by percentile rank). Others have reported sex-specific discordance in lipogenic mRNAs, their protein levels and/or triglyceride content. These observations hint at the existence of a post-transcriptional axis contributing to lipogenesis; however, the mechanisms underlying synchronized control of lipogenic mRNA metabolism and protein function remain poorly understood.

Recent evidence suggests that selective m⁶A methylation of RNA can affect the stability, translation and/or localization of mRNA. The installation of m⁶A occurs at predictable motifs through the collaborative activities of a 'writer' complex made up of the methyltransferases METTL14 and METTL3. Although several elegant studies have shown that m⁶A plays a critical role in diverse biological processes, understating the molecular basis of temporal/spatial variation in m⁶A levels and how the activity of m⁶A installing machinery may be fine-tuned in response to environmental cues is still emerging. Here, we demonstrate that m⁶A modifications potently alter the fate of lipogenic mRNA and hepatic triglyceride stores. In addition, we show that m⁶A modifications are dynamically altered in response to dietary conditions, and we provide mechanistic evidence that the m⁶A installing machinery is subject to tight transcriptional control by sex-dimorphic dietary regulators to sustain metabolic control. These data identify a new pathway for lipid degradation and, at least in part, explain the molecular basis for the existence of sex-specific differences in hepatic triglyceride composition.

Results
RNA methylation strongly enriches lipogenic transcripts and is dynamically regulated with diet. Chemical modifications on RNA are critical for the ability of cells to execute development programmes and to respond to environmental challenges. To further investigate the contributions of RNA modifications in metabolic control, we performed unbiased interrogation of mRNA, lipids and RNA methylation patterns by using m⁶A sequencing (m⁶A-seq) on livers from mice (C57BL/6) fed a chow diet, a western diet (WD) or a high-fat diet (HFD; Fig. 1a). As expected, HFD or WD feeding led to increased weight gain, fat composition and serum lipids at 2 weeks or 4 weeks (Extended Data Fig. 1a–i). Principal component analysis (PCA) of genome-wide RNA abundance segregated sample

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**Fig. 1** | RNA methylation strongly enriches lipogenic transcripts and is dynamically regulated with diet. **a**, Schematic of experimental design. **b**, PCA of gene expression for mouse liver samples under chow diet (n = 5 females, 3 males), WD (n = 4 females, 5 males) and HFD (n = 5 females, 4 males). The first two components (PC1 and PC2) are shown along with the percentage of gene expression variance explained. Clustering was obtained with data from all detected genes without additional filters. Results are representative of two independent experiments. **c**, Major hepatic lipid species analysed by lipidomics in liver collected from male and female mice fed a chow diet, WD or HFD (n = 5 per group). Results are representative of two independent experiments. CE, cholesterol ester; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TAG, triacylglycerol. **d**, Pie chart illustrating the relative position of m6A on immunoprecipitated transcripts and motif enrichment analysis of m6A-containing RNAs. E values were computed as the enrichment value (Fisher’s exact test for enrichment of the motif in the positive sequences) times the number of candidate motifs tested. **e**, PCA plot for m6A-seq in mouse liver under different conditions. Both input and m6A-immunoprecipitated samples are displayed. **f**, Scatterplot comparing m6A enrichment between Chow-fed and WD-fed male livers. Hypermethylated peaks with higher m6A enrichment (log(m6A/0)) in Chow compared to WD are noted with orange dots, and hypomethylated peaks with lower m6A enrichment (log(m6A/0)) in Chow compared to WD are noted with blue dots. Genes with no significant difference in m6A enrichment between chow diet and WD are shown in orange (hypermethylated in chow diet versus WD) and purple (hypomethylated in chow versus WD). **g**, DAVID functional annotation of the top 500 genes with highest m6A enrichment in male mice fed chow versus WD. Gene Ontology analysis was performed with \(-\log(P\text{ value})\) plotted (x axis) as a function of classification meeting a \(P\text{ value} < 0.001\). **h**, University of California, Santa Cruz (UCSC) browser screenshots and enrichment of m6A modification on lipogenic transcripts in male liver as determined by m6A-IP-qPCR (n = 5 per group). The relative enrichment of m6A in each sample was calculated by normalizing to tenfold input. Results are representative of two independent experiments. All mice were fed the indicated diet for 4 weeks and fasted for 4 h before euthanization. Values are the mean ± s.e.m. of five biological replicates (c and h). **P** values were calculated using one-way analysis of variance (ANOVA) followed by a multigroup comparison test (Fisher’s) in **c** and **h** or an unpaired two-tailed \(t\)-test in **g**, \(*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001\). The precise n values, \(P\) values and details of the statistical testing are provided as source data.
groups, showing that sex is the dominant factor influencing gene expression, with diet composition playing a critical but secondary role (Fig. 1b). Length of diet feeding did not have a strong impact on gene expression. Congruently, PCA of unbiased lipidomics showed a similar trend, with sex and diet being the strongest variables influencing hepatic lipid composition (Extended Data Fig. 2a).

We observed minimal shifts in most lipid species across groups (Extended Data Fig. 2b–d), but striking differences were noted for a number of neutral lipids including triglycerides (Fig. 1c). In line with PCA results, sex had a strong influence on fasting triglyceride content irrespective of diet composition, with females consistently having higher levels than males (Fig. 1c).

Examination of the RNA methylation landscape in liver showed the m6A modifications strongly clustered near 3′ untranslated regions (UTRs) or last exons in line with previous reports (Fig. 1d)\(^1\). In addition, we found that the RNA methylome was enriched at consensus motifs known to be bound by the METTL3–METTL14 complex (Fig. 1d)\(^2\). Intriguingly, PCA based on global m6A patterns led to stronger segregation of samples than use of gene expression alone (Fig. 1e and Extended Data Fig. 2e). We examined dynamic changes in m6A profiles with dietary conditions using several unbiased approaches, including comparing fold enrichment of m6A with diet, as well as examining the contributions of individual genes to variance. Regardless of approach, consistent enrichment of lipogenic genes was observed. PCA showed that m6A peaks (Fig. 1f). Comparison of chow and WD showed that differentially regulated m6A peaks preferentially enriched lipogenic pathways with many lipogenic genes containing m6A consensus motifs (Fig. 1g and Extended Data Fig. 2f,g). In agreement with these findings, m6A profiles were reduced on lipogenic transcripts during WD feeding in comparison with chow (Fig. 1h). We confirmed our findings using m6A immunoprecipitation and reverse transcription coupled with quantitative PCR (m6A-IP–RT–qPCR; Fig. 1h). A similar pattern was observed when comparing HFD versus chow, where HFD feeding led to a reduction in m6A on mRNAs involved in fatty acid and triglyceride biosynthesis (Fig. 1i and Extended Data Fig. 2h). Taken together, our data suggest that m6A peaks are strongly enriched on lipogenic mRNAs in liver and that their deposition is highly sensitive to dietary conditions.

Liver-specific deletion of the m6A methylase METTL14 increases lipogenesis. The RNA modification m6A is installed by a multi-protein complex containing METTL3 and METTL14 (refs. \(^6\)\(^,\)\(^1\)\(^,\)\(^2\)). Disruptions of the catalytic enzyme METTL3 or its allosteric activator METTL14 disrupt m6A modifications on target RNAs\(^3\)\(^,\)\(^4\). To directly investigate the contributions of m6A in hepatic lipid metabolism, we generated mice with liver-specific deletion of Mettl14 (hereafter referred to as L-KO) by administering an adenov-associated virus for Cre expression under the control of the TBG promoter (AAV8-TBG-Cre)\(^5\)\(^,\)\(^6\) or AAV8-TBG-control (referred to as control or wild type (WT) for paired experiments) to Mettl14 floxed (Mettl14\(^\text{fl/fl}\)) mice. We confirmed that METTL14 was readily deleted in liver but not in other tissues after 4 weeks on a Chow diet (Fig. 2a and Extended Data Fig. 3a). Unbiased lipidomics showed a dramatic increase in triglyceride content in Mettl14 L-KO livers compared with controls (Fig. 2b). Most other lipid classes were unchanged comparing WT or Mettl14 L-KO mice (Fig. 2b and Extended Data Fig. 3b,c).

We did not observe changes in food intake or body weight including fat mass in the setting of loss of METTL14, suggesting that altered caloric balance is not a contributor to triglyceride accumulation (Extended Data Fig. 3d,e). Because changes in hepatic fatty acid oxidation can reciprocally affect triglyceride stores, we measured mitochondrial fatty acid metabolism in whole liver. Our results showed no change in fatty acid oxidation in Mettl14 L-KO livers compared with controls (Extended Data Fig. 3f). Measurement of genes involved in beta-oxidation showed no change or a slight increase, suggesting that the observed increase in hepatic triglyceride stores cannot be explained by changes in beta-oxidation or altered mitochondrial function (Extended Data Fig. 3g). Similarly, serum triglycerides, hepatic ApoB and lipid export and lipolysis genes were minimally altered, suggesting that impaired lipid secretion or lipid droplet breakdown is not a cause of triglyceride accumulation (Extended Data Fig. 3h,i).

Given that m6A modifications are known to predominantly affect gene regulation by impacting mRNA stability, which can then result in corresponding changes in translation\(^7\)\(^–\)\(^10\),\(^17\),\(^18\), we measured protein levels of major lipogenic genes. We observed a significant increase in protein levels of lipogenic genes in Mettl14 L-KO mice compared with controls (Fig. 2c and Extended Data Fig. 3j). We confirmed this critical finding in Mettl14 L-KO mice generated using crosses with Cre\(^\text{alo}^\text{b}\) transgenic mice, suggesting that the effects of hepatic Mettl14 deletion are sustained in both acute and chronic perturbations (Extended Data Fig. 3k,l). Taken together, these results suggest that enhanced fatty acid and triglyceride biosynthesis are the major contributors to triglyceride accumulation in Mettl14 L-KO mice. Notably, we observed an increase in polyunsaturated fatty acids (PUFAs) and SCD1 index or desaturation index in Mettl14 L-KO mice, in line with the observed increase in fatty acid biosynthetic enzymes (Fig. 2d)\(^2\). Protein levels and gene expression of other genes involved in triglyceride metabolism such as Apoe, Apob and Mitf were not increased (Extended Data Fig. 3i,j)). In addition, we confirmed reduced m6A enrichment of lipogenic transcripts in Mettl14 L-KO mice by using m6A-IP–RT–qPCR (Extended Data Fig. 3m,n). Collectively, our findings are consistent with the idea that RNA modifications act to repress lipogenic proteins in the liver and are congruous with the observed changes in the m6A landscape in response to diet.

It is well established that SREBP signalling is the dominant mechanism that influences lipogenic gene transcription and feedback regulation\(^11\). To more thoroughly investigate how m6A modifications affect Srebp1c and lipogenesis, we measured mRNA levels of fatty acid biosynthetic genes. Despite the observed increase in lipogenic proteins and triglycerides levels, lipogenic mRNAs and pre-mRNA were reduced (Extended Data Fig. 4a,b). These results are in line with multiple previous studies showing that accumulation of PUFAs suppresses SREBP1C processing and potently feeds back on lipogenic gene transcription\(^12\). In addition, we found that loss of Mettl14 L-KO mice led to a marked reduction in Srebp1c mRNA and nuclear SREBP1C protein (Extended Data Fig. 4a–c). Measurement of gene expression at an earlier time point (72 h versus 4 weeks), when PUFAs accumulation is reduced, showed an increase in lipogenic mRNAs (Extended Data Fig. 4d). Taken together, these results imply that the discordance between RNA and protein levels may be a late effect due to rampant accumulation of PUFAs. More importantly, these data suggest that a primary perturbation in fatty acid biosynthetic gene transcription is unlikely to explain the observed phenotype. We therefore reasoned that altered mRNA stability and/or protein translation may be at play, consistent with known roles of m6A. To explore this further, we performed ribosome profiling from control or Mettl14 L-KO livers, which showed that loss of METTL14 enhances the abundance of lipogenic mRNAs but not all transcripts in the translating pool compared with controls (Fig. 2e and Extended Data Fig. 4e,f). To test whether METTL14 regulates lipogenic genes through modulating their mRNA stability, we performed mRNA stability assays using the transcription inhibitor actinomycin D in primary hepatocytes isolated from control and Mettl14 L-KO mice (Fig. 2f,g, Extended Data Fig. 4g and equations (1) and (2)). In the setting of transcriptional inhibition, we observed enhanced
Liver-specific deletion of METTL14 increases de novo lipogenesis and hepatic triglyceride content. a, Western blot from liver of WT and Mettl14 L-KO chow-fed male mice. Equal amounts of protein were pooled from eight animals and run in triplicate. The experiment was repeated three times with similar results. b, Quantification of major lipid species detected in livers of chow-fed male WT and Mettl14 L-KO mice by unbiased lipidomics (n = 8 per group). DAG, diacylglycerol; LPC, lysophosphatidylcholine. c, Western blot comparing lipogenic protein levels in WT versus Mettl14 L-KO chow-fed male mice livers (n = 8 per group). d, Unbiased lipidomic measurement from liver of PUFAs (measured by lipidomics) and SCID index for WT and Mettl14 L-KO chow-fed male mice (n = 8 per group). e, qPCR analysis of transcript abundance by qPCR at 2 h and 4 h after transcription inhibition (TI) in WT and Mettl14 L-KO chow-fed male mice (n = 8 per group). f, mRNA lifetime of lipogenic transcripts in primary hepatocytes collected from WT and Mettl14 L-KO chow-fed male mice (n = 4). The experiment was repeated twice with similar results. g, qPCR analysis of lipogenic gene expression in Mettl14 knockout acute myeloid leukaemia (AML) cells. Images are representative of three independent experiments. h, qPCR analysis of lipogenic gene expression in Mettl14 knockout acute myeloid leukaemia (AML) cells. Images are representative of three independent experiments.
these results, we observed increased Scd1 mRNA abundance in the setting of loss of Mettl14 by fluorescence in situ hybridization (FISH; Fig. 2h and Extended Data Fig. 4h). Interestingly, knockdown of Mettl14 decreased localization of Scd1 with DCP1a, a key decapping enzyme involved in mRNA degradation pathways and a marker of processing bodies (Fig. 2h). To reinforce the idea that post-transcriptional mechanisms are more critical factors in the effects of m’A, we observed that knockdown of Mettl14 enhances mRNA levels of lipogenic transcripts in the setting of functional SREBP deficiency (Scap−/−)2,21 (Fig. 2i). Collectively, our findings suggest that increased biosynthesis of lipogenic mRNAs does not contribute to the observed increase in fatty acid and triglyceride levels in Mettl14 L-KO mice and that post-transcriptional mechanisms are more critical drivers of the observed change in hepatic lipid composition.

**Mettl14 expression is inversely correlated with human fatty liver disease.** To investigate the potential contribution of m’A in human fatty liver disease, we examined the expression of Mettl14 in liver from the Mexican Obesity Surgery (MOBES) cohort, which includes non-related Mexican mestizo participants aged 18–59 years old. Unique strengths of this study include using a gold-standard liver biopsy to confirm the presence and grading of non-alcoholic fatty liver disease (NAFLD) by Kleiner’s scoring method3. Participants were classified into three groups: controls (normal histology), simple steatosis (steatosis grade ranging from 1 to 2) and non-alcoholic steatohepatitis (NASH; NAFLD activity score >5) based on independent scores from two experience pathologists. Clinical, metabolic and biochemical data were collected from all participants. Mettl14 expression was negatively correlated with human steatosis and NASH (Fig. 3a,b). Although triglyceride content is one of many factors determining progression to chronic liver disease, these findings are consistent with our observed animal studies and hint at functional conservation of our proposed mechanisms.

Our results hint that modulation of m’A levels can influence pathological fat storage in liver, which led to testing an m’A-based gene therapy strategy overexpressing the Mettl3–Mettl14–Wtap ‘writer’ complex in a mouse model of NAFLD (Fig. 3c). In vivo administration of m’A writers using an AAV8 vector with a liver-specific promoter enhanced gene expression of MTA complex components (Fig. 3d) and m’A enrichment on lipogenic mRNA (Fig. 3e). In addition, overexpression of m’A writers reduced lipogenic proteins (Fig. 3f) and resulted in a modest decrease in Oil Red O staining, and hepatic triglyceride content, although the results did not reach significance (Fig. 3g and Extended Data Fig. 4i). We observed a significant reduction in genes associated with inflammation and fibrosis (Fig. 4h), key drivers of NASH progression. It should be noted that chronic overexpression of METTL14 alone was not sufficient to alter lipogenic proteins (data not shown), probably because its catalytic partner METTL3 is required to efficiently enhance m’A deposition. Recent evidence suggests that site-specific programmable RNA modification is readily achievable with minimal off-target effects using a guide RNA (gRNA) and a Cas13-tethered writer complex4,5,22. To test the influence of programmable RNA methylation of lipogenesis, we introduced an RNA methylation modification complex with individual RNA guides targeting Scd1 m’A sites (Fig. 3i). Targeted RNA methylation enhanced Scd1 m’A levels measured by m’A-IP–RT–qPCR and resulted in lower protein levels of SCD1 (Fig. 3j,k). Altogether, our findings hint at strong relevance of m’A in the regulation of human fatty liver and that modulation of RNA methylation through multiple approaches, including a platform approved by the Food and Drug Administration, is technically feasible and efficacious in reducing important markers of chronic liver disease.

m’A machinery undergoes transcriptional regulation by sex-biased transcription factors. Our global methylene analysis revealed a reduction in m’A signatures with WD or HFD feeding in male mice along with a potent decrease in lipogenic RNA modification (Fig. 1). In line with these results, we found that chronic WD feeding resulted in a significant increase in hepatic triglycerides in male Mettl14 L-KO mice compared to controls (Fig. 4a,b), but the effect size was diminished in comparison with Chow diet (Figs. 4a and 2b). Similarly, loss of Mettl14 resulted in only a mild increase in lipogenic enzymes under WD as opposed to the dramatic effect observed under chow conditions (Fig. 4c). Consistent with chow feeding results, we did not observe major changes in other lipid species (Extended Data Fig. 5a). Interestingly, the fed state was associated with a reduction in Mettl14 protein, and loss of Mettl14 resulted in no observable change in triglycerides or lipogenic proteins under re-fed conditions, when lipogenesis is known to be highest (Fig. 4d,e). These findings imply that m’A modifications appear to be more relevant under basal fasting conditions where there is minimal demand for de novo fatty acid biosynthetic enzymes and that contributions of m’A are diminished under WD or re-feeding conditions. Thus, m’A modifications act in a coordinate fashion with transcriptional control mechanisms driven by SREBP1C, becoming less important when SREBP1C-driven lipogenesis is at play.

To better explore the mechanism of reduction in m’A with dietary challenge, we investigated the regulation of Mettl14. In line with the findings in Fig. 4c, we observed a decrease in the...
expression of the m^6A writer Mettl14 with either HFD or WD (Fig. 4f). We confirmed that METTL14 protein is reduced with diet feeding (Fig. 4g). Intriguingly, the pattern of regulation in male mice was opposite from that observed in female mice, in which WD or HFD feeding did not reduce Mettl14 mRNA and protein levels and in fact trended towards increased levels (Fig. 4f,g). We measured m^6A levels in the liver with mass spectrometry, which closely mirrored the Mettl14 regulation pattern (Fig. 4h). To further explore

The basis of the sex-specific differences in Mettl14, we used a genetic model that segregates gonadal sex from chromosomal sex effects by generating XX and XY mice with ovaries, and XX and XY mice with testes on a hyperlipidaemic background. We found that XX animals had higher Mettl14 levels than XY animals (Fig. 5a), consistent with the observed regulation of Mettl14 under lipid-rich conditions (Fig. 4f-g). These results imply that Mettl14 levels are strongly influenced by chromosomal sex composition. In addition, examination

| NAFLD trait                  | Pearson r | P value |
|------------------------------|-----------|---------|
| Steatosis stage              | −0.27     | 0.001   |
| Lobular inflammation         | −0.14     | 0.078   |
| Hepatocellular ballooning    | −0.25     | 0.002   |
| NAS score                    | −0.26     | 0.002   |

For non-NASH and NASH groups, the co-expression analysis of Mettl14 expression and NASH-related traits is presented. The Pearson's correlation coefficient (r) and associated P values are provided. A significant correlation indicates a positive or negative relationship between Mettl14 expression and the NASH-related traits. The MOBES cohort data also show a significant correlation between Mettl14 expression and NASH stages (P < 0.0006 and P < 0.0005).
of publicly available histone marks in liver under different dietary conditions led us to hypothesize that Mettl14 may be subject to tight transcriptional regulation, as H3K27ac, a mark of transcriptional activation, was reduced with HDF feeding (Extended Data Fig. 5b). To more thoroughly explore the molecular basis of alteration in METTL14 and m^6^A with dietary feeding, we performed the assay for transposase-accessible chromatin by sequencing (ATAC-seq) in liver under chow, WD or HFD feeding (Fig. 5b). This technique may be critical for docking of canonical regulatory factors or recruiting additional factors with dietary perturbations within this region. Motif interrogation of accessible regions revealed binding of a number of factors known to be involved in metabolic control including the sexually dimorphic STAT5A and BCL6 complex. Chromatin immunoprecipitation–sequencing studies confirmed the binding of STAT5a and BCL6 at METTL14 in both mice and humans (Extended Data Fig. 5c,d). Consistent with previous studies, we observed an increase in Bcl6 levels with diet and feeding state (Fig. 5c and Extended Data Fig. 5e). Levels of m^6^A on lipogenic transcripts were altered in a comparison of fasted and re-fed states (Extended Data Fig. 5f). In addition, other MTA complex proteins were also differentially regulated with diet composition or feeding state (Extended Data Fig. 5g). Levels of m^6^A on lipogenic transcripts were altered in a comparison of fasted and re-fed states (Extended Data Fig. 5e). Interestingly, binding of BCL6 has been shown to repress STAT5 targets in male but not female livers. In addition, hepatic loss of Bcl6 has been shown to have the opposite phenotype of the Mettl14 L-KO whereby it reduces triglyceride stores. These results hint that the observed reduction in METTL4 enzyme levels in male but not female mice with dietary challenge may be mediated by BCL6-repressive effects at the Mettl14 promoter region. To directly test this theory, we generated Bcl6 liver-specific knockout mice. When fed a WD, L-Bcl6^−/−^ mice showed no differences in Mettl14 mRNA and protein levels between the sexes, in
had higher m6A levels than females on lipogenic mRNAs (Fig. 6e). In line with our findings (Fig. 6a), transcriptome-wide fasting liver triglyceride levels than males but not necessarily all lipid species5,36, suggesting that m6A installing machinery undergoes transcriptional regulation by sex-biased transcription factors. a, qPCR analysis of Mettl14 expression in livers of male and female Apoe<sup>−/−</sup> mice (n = 3) with core four genotypes. Bcl6 expression in male mouse livers under different diets and transcription factor motifs identified at this site. Screenshots are representative of five independent biological replicates. c, qPCR analysis of Bcl6 expression in male mouse livers under different diets (n = 4 WD, n = 5 chow, n = 5 HFD). The experiment was repeated three times with similar results. d, qPCR analysis of Mettl14 expression in livers of male and female WD-fed WT and Bcl6 L-KO mice (n = 5 WT mice, n = 6 Bcl6 KO mice). The experiment was repeated three times with similar results. NS, not significant. e, Western blot comparing Mettl14 protein levels in livers of male and female WD-fed Bcl6 KO mice compared to WD-fed WT mice. Equal amounts of protein were pooled from five animals per group and run in triplicate. The experiment was repeated two times with similar results. All mice were fed the indicated diet for 4 weeks and were fasted for 4 h before euthanization. Values are the mean ± s.e.m. of three (a), four (c), five (c and d) or six (d) independent biological replicates. P values were calculated using one-way ANOVA followed with multigroup comparison (Fisher’s) in c or two-way ANOVA followed by multigroup comparison (Tukey) in a and d. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. The precise n values, P values and details of the statistical testing are provided as source data.

**Fig. 5 | m6A machinery undergoes transcriptional regulation by sex-biased transcription factors.** a, qPCR analysis of Mettl14 expression in livers of male and female Apoe<sup>−/−</sup> mice (n = 3) with core four genotypes. b, UCSC browser screenshot of ATAC–seq data for the Mettl14 promoter region from male mouse liver under different diets and transcription factor motifs identified at this site. Screenshots are representative of five independent biological replicates. c, qPCR analysis of Bcl6 expression in male mouse livers under different diets (n = 4 WD, n = 5 chow, n = 5 HFD). The experiment was repeated three times with similar results. d, qPCR analysis of Mettl14 expression in livers of male and female WD-fed WT and Bcl6 L-KO mice (n = 5 WT mice, n = 6 Bcl6 KO mice). The experiment was repeated three times with similar results. NS, not significant. e, Western blot comparing Mettl14 protein levels in livers of male and female WD-fed Bcl6 KO mice compared to WD-fed WT mice. Equal amounts of protein were pooled from five animals per group and run in triplicate. The experiment was repeated two times with similar results. All mice were fed the indicated diet for 4 weeks and were fasted for 4 h before euthanization. Values are the mean ± s.e.m. of three (a), four (c), five (c and d) or six (d) independent biological replicates. P values were calculated using one-way ANOVA followed with multigroup comparison (Fisher’s) in c or two-way ANOVA followed by multigroup comparison (Tukey) in a and d. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. The precise n values, P values and details of the statistical testing are provided as source data.

### Stark contrast to the differences observed in WT mice (Fig. 5d,e). Overall, these results strongly suggest the existence of sex-specific differences in RNA methylation and that m6A installing machinery is subject to tight transcriptional control.

**Loss of m6A diminishes sex-specific differences in hepatic lipid composition and sex-biased gene expression.** Multiple studies reported on sex-specific differences in lipogenic mRNAs, their protein abundance and fatty liver traits across species, although the direction of these differences varied depending on species, genetic background and context5,35–37. Female mice were noted to have higher fasting liver triglyceride levels than males but not necessarily all lipid species5,36, in line with our findings (Fig. 6a). Transcriptome-wide gene expression analysis showed minimal differences in lipogenic mRNAs between the sexes regardless of diet (Fig. 6b). We confirmed by RT–qPCR that differences in lipogenic RNAs did not explain the observed sex differences in fasting hepatic triglyceride content (Fig. 6c), although, surprisingly, females were noted to have markedly higher protein levels of fatty acid and triglyceride synthesis enzymes compared with males (Fig. 6d). Scd1, for example, showed higher RNA levels in males despite having higher protein levels in females (Fig. 6c,d). A similar trend was observed for other genes involved in triglyceride biosynthesis where strikingly higher protein levels were noted in females despite minimal differences in gene expression. These results imply that post-transcriptional mechanisms may be contributing to sex-specific variation in lipogenic proteins and triglyceride content. Remarkably, the protein levels of lipogenic genes were discordant with sex differences in m6A, where males had higher m6A levels than females on lipogenic mRNAs (Fig. 6e).

These findings are in line with the above results (Figs. 2 and 3) and reinforce the idea that m6A modifications on lipogenic transcripts may be a factor contributing to sex differences in lipogenic protein levels and triglyceride content. To test this hypothesis more directly, we compared the effect of Mettl14 deletion between the sexes and found that liver-specific deletion of Mettl14 diminished the striking differences in lipogenic protein levels between male and female mice (Fig. 6f). In addition, we note that under chow conditions liver-specific deletion of Mettl14 in male mice increased triglyceride levels, almost matching the levels seen in WT female mice, suggesting that m6A activity leads to suppression of triglyceride levels and its loss leads to a more ‘feminized’ lipid composition (Fig. 6g). Loss of m6A control in females under chow conditions led to only a modest increase in triglyceride content (Fig. 6g). Reciprocally, loss of Mettl14 in female mice, compared with male mice, was associated with greater differences in hepatic triglyceride under WD conditions (Fig. 6g). These results are consistent with the sex-dimorphic regulation of Mettl14 (Fig. 5). Taken together, our findings strongly imply that sex differences in fasting triglyceride stores are predominantly dictated by differences in RNA modifications.

To more thoroughly dissect the contributions of m6A in hepatic sex-specific traits, we performed RNA sequencing (RNA-seq) on control and Mettl14 L-KO male and female liver samples. PCA of genome-wide mRNA abundance showed clear segregation between groups, with m6A unexpectedly contributing to more variability in global RNA levels than sex (Fig. 6h). Remarkably, loss of Mettl14 abolished m6A-based differences in variance and significantly minimized sex-driven PCA variance, suggesting a strong interaction between RNA modifications and sex-biased gene regulation.

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**Fig. 5 | m6A machinery undergoes transcriptional regulation by sex-biased transcription factors.** a, qPCR analysis of Mettl14 expression in livers of male and female Apoe<sup>−/−</sup> mice (n = 3) with core four genotypes. b, UCSC browser screenshot of ATAC–seq data for the Mettl14 promoter region from male mouse liver under different diets and transcription factor motifs identified at this site. Screenshots are representative of five independent biological replicates. c, qPCR analysis of Bcl6 expression in male mouse livers under different diets (n = 4 WD, n = 5 chow, n = 5 HFD). The experiment was repeated three times with similar results. d, qPCR analysis of Mettl14 expression in livers of male and female WD-fed WT and Bcl6 L-KO mice (n = 5 WT mice, n = 6 Bcl6 KO mice). The experiment was repeated three times with similar results. NS, not significant. e, Western blot comparing Mettl14 protein levels in livers of male and female WD-fed Bcl6 KO mice compared to WD-fed WT mice. Equal amounts of protein were pooled from five animals per group and run in triplicate. The experiment was repeated two times with similar results. All mice were fed the indicated diet for 4 weeks and were fasted for 4 h before euthanization. Values are the mean ± s.e.m. of three (a), four (c), five (c and d) or six (d) independent biological replicates. P values were calculated using one-way ANOVA followed with multigroup comparison (Fisher’s) in c or two-way ANOVA followed by multigroup comparison (Tukey) in a and d. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. The precise n values, P values and details of the statistical testing are provided as source data.
Fig. 6 | Loss of m^6A significantly diminishes sex-specific differences in hepatic lipid composition. a, CIRCOS plot of hepatic lipid content under different diets (n = 5 per group). Width of connection indicates higher TAG content. b, Heat map of lipogenic gene expression in livers of male and female mice under different diets (n = 5 per group) based on RNA-seq. c, qPCR analysis of lipogenic gene expression in livers of WD-fed male and female mice (n = 4 per group). The experiment was repeated three times with similar results. d, Western blots comparing lipogenic protein levels in the livers of WD-fed WT male and female mice. Equal amounts of protein were pooled from eight animals per group and run in triplicate. The experiment was repeated three times with similar results. e, m^6A-IP–qPCR validation of m^6A enrichment on lipogenic genes in the livers of male and female mice (n = 4 per group). The experiment was repeated twice with similar results. f, Western blots comparing lipogenic protein levels in the livers of WD-fed WT male and female mice. Equal amounts of protein were pooled from eight animals per group and run in triplicate. The experiment was repeated three times with similar results. g, Quantification of hepatic triglyceride content by lipidomics in WT and Mettl4 L-KO male and female mice (n = 6 KO chow-fed females, n = 7 WT and KO WD-fed males and females, n = 8 WT and KO chow-fed males and KO chow-fed females). h, PCA of gene expression for male and female mouse livers in control and Mettl4 L-KO samples (n = 7 WT males, n = 8 all other groups). Clustering was obtained with data from all detected genes without additional filters. i, Bar plot of functional enrichment adjusted P values (hypergeometric P values after Benjamini–Hochberg correction) for genes significantly associated with sex-specific responses to Mettl4 L-KO (interaction genes). Ontology terms were grouped by gene member similarity. All mice were fed the indicated diet for 4 weeks and fasted for 4 h before euthanization. Values are the mean ± s.e.m. of four (c and e) or between six and eight (g) independent biological replicates. P values were calculated using an unpaired two-tailed t-test (c, e and g). **P < 0.01; ***P < 0.001; ****P < 0.0001. The precise n values, P values and details of statistical testing are provided as source data.

(Fig. 6h). Notably, fitting the RNA-seq data to various multivariate additive and interaction models showed that the interaction between sex and Mettl4 was significant for most sex-biased genes. This interaction resulted in a major reduction in sex-biased gene expression in Mettl4 L-KO mice (Extended Data Fig. 6a–c), and hierarchical clustering revealed that Mettl4 L-KO male mice consistently clustered with female samples (Extended Data Fig. 6c). Notably, we did not observe differences in key regulators of sex-biased gene expression such as Xist and Sry (Extended Data Fig. 6d), and the majority of the differentially regulated genes are...
not known to be direct targets of Xist. We used model-based clustering of all sex-biased and Mettl14-dependent genes to more rigorously evaluate the influence of sex on loss of m6A. Consistent with our global analysis, a number of clusters showed dramatic abrogation of sex-biased gene expression in the setting of loss of Mettl14 (Extended Data Fig. 6e). Many of the genes in these clusters have been reported to define differences between male and female livers34. In addition, unsupervised interrogation of genes in these clusters revealed that multiple metabolic processes are the most enriched pathways under the interaction between m6A and sex (Fig. 6i). In summary, our findings suggest that hepatic m6A levels are important contributors to sex-biased gene expression and sex-specific lipid traits.

**Discussion**

In this work, we find that lipogenic mRNAs undergo chemical modifications that powerfully impact transcript turnover, adding another layer of dynamic gene regulation in hepatic lipid metabolism. The transcription factor SREBP1C, which is reduced during fasting and is activated by insulin and the lipid-responsive nuclear receptor liver-X-receptor (LXR), plays key roles in the induction of lipogenic genes through direct binding to lipogenic promoters35–37. Our model fits well with classical transcriptional mechanisms controlling lipogenesis and suggests that m6A fine-tunes transcriptional outputs by enhancing mRNA degradation under conditions where hepatic fatty acid biogenesis may be in low demand.

A number of studies including our own have reported that female mice have higher basal fasting triglyceride levels than males, but the molecular basis for these observations are unknown38–40. We observed significant differences in the effects of m6A between male and female mice and a strong influence of m6A on the interaction between sex and diet composition. In male mice, loss of m6A robustly impacted fatty acid biosynthesis and increased triglyceride stores under fasting chow conditions, but its influence was diminished during western diet feeding, consistent with the observed decrease in m6A. An opposite pattern was observed in female mice. Our findings here fill important gaps in the mysterious sex-specific differences in lipid composition but also suggest that there must be other factors beyond m6A control that govern sex differences in lipid composition. Although sex differences in lipogenesis and triglyceride stores were dramatically diminished with loss of m6A, it is important to highlight that they were not entirely abrogated. Additionally, control of lipogenesis is one of many factors that influences hepatic lipid stores and it is likely that sex differences related to other pathways such as mitochondrial bioenergetics, nuclear receptor outputs or lipid uptake may be important. Finally, our study examined gene function during short-term dietary perturbations to more directly underpin mechanistic effects of m6A. Although the influence of hepatic RNA modifications during prolonged feeding mimicking fatty liver disease is not well defined, we predict, based on our findings, that the female sex might confer a fitness advantage in chronic liver disease models. Despite starting and ending knockout models to validate our main findings. However, we work focused on the role of m6A in lipid synthesis, it is important to point out that m6A may have other contributions in metabolism. Future studies should more thoroughly investigate these questions.

**Methods**

**Reagents, plasmids and cell transfection.** shRNAs targeting Mettl14 were obtained from Horizon Discovery and cloned into pLKO.1 vector (human: TRCN0000015935; mouse: TRCN0000084995). The resulting plasmids were transiently transfected using FuGene HD transfection reagent (Promega) or electroporated using the Neon transfection system (Invitrogen) and then tested for their ability to knock down Mettl14 at 48h. AAV-TBG-Pl-Cre-rRG (Addgene) was used for acute deletion of Mettl14 and Bcl6 in mouse liver, and AAV-TBG-Pl-eGFP-WPRE-BGH (Addgene) or AAV-TBG-Pl-null-BGH (Addgene) was used as the control. For overexpression of m6A writers, the following viruses were generated by VectorBuilder: AAV-TBG-mMettl3-WPRE, AAV-TBG-mMettl14-WPRE and AAV-TBG-mWtap-WPRE, and AAV-TBG-Pl-Null-BGH (Addgene) was used as the control. For targeted m6A modification of cellular mRNAs, plasmids encoding nuclease-dead Cas13b and truncated Mettl3 fused to a nuclear localization sequence (pCMV-dCas13-M3nls) and gRNA expression plasmid (pL6-PspCasi13b-gRNA) were obtained from Addgene (155366 and 155368). A complete list of antibodies and primers is available in Supplementary Tables 1 and 2.

**Animals and diets.** All animals used in this study were on the C57BL/6 background unless otherwise noted. Our study used both male and female mice. Mice were housed in a temperature-controlled room under a 12-h light/12-h dark cycle and pathogen-free conditions. Mice were fed a standard chow diet, WD (40% Kcal with 0.135% cholesterol and moderate sucrose) or HFD (60% Kcal with moderate sucrose and no cholesterol; Research Diets). The duration of the diet was 4 weeks for most experiments, but for male and female comparison, the duration of WD RNA-seq experiments was shorter (10d). For experiments regarding the
overexpression of m^A writers, mice were fed a FPC-NASH diet (Envigo) and cultured in William's E medium with 5% BSA. AML and Cell culture.

Advisory Committee.

using a homemade reverse transcriptase, as previously described^48. cDNA was Gene expression and immunoblot analysis.

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of male and female mice fed chow diet, WD or HFD for 2 or 4 weeks (for example, WD versus chow diet in males). In addition, count data were fitted to different types of multivariate models. For the diet dataset, we fitted the data to additive models (− sex + diet) and interaction models (− sex + diet + sex:diet) and performed contrast tests with reduced models to evaluate the global effect of the multivariable or interaction between variables while controlling for baseline differences in the remaining variables. The same strategy was used for the Mettl1 L-KO dataset with sex and Mettl1 genotype as covariates. For each test, genes were classified as differential if the adjusted P value < 0.01 (Wald test for pairwise contrasts, likelihood-ratio test for multivariate models), with additional thresholds for size effect (log fold change > 1) and baseline gene expression (FPKM > 1). The pool of most variable genes in each dataset, selected by PCA loadings or differential expression tests, was then subjected to model-based clustering using MBCluster.Seq^52 to classify them based on their overall abundance profile across samples. Functional enrichment for genes selected in the tests and clusters above was performed with Metascape^53. All figures were generated in MATLAB (v2017a, MathWorks; RRID: SCR_001622) using the ‘pdist’ and ‘seglinkage’ functions. Differential expression analyses were performed with DESeq2 (Bioconductor, v3.7; RRID: SCR_005687). All other differential tests were performed with pairwise contrasts for comparisons between levels of the same variable in a given group (for example, WD versus chow diet in males). In addition, count data were fitted to different types of multivariate models. For the diet dataset, we fitted the data to additive models (− sex + diet) and interaction models (− sex + diet + sex:diet) and performed contrast tests with reduced models to evaluate the global effect of the multivariable or interaction between variables while controlling for baseline differences in the remaining variables. The same strategy was used for the Mettl1 L-KO dataset with sex and Mettl1 genotype as covariates. For each test, genes were classified as differential if the adjusted P value < 0.01 (Wald test for pairwise contrasts, likelihood-ratio test for multivariate models), with additional thresholds for size effect (log fold change > 1) and baseline gene expression (FPKM > 1). The pool of most variable genes in each dataset, selected by PCA loadings or differential expression tests, was then subjected to model-based clustering using MBCluster.Seq^52 to classify them based on their overall abundance profile across samples. Functional enrichment for genes selected in the tests and clusters above was performed with Metascape^53. All figures were generated in MATLAB (v2017a, MathWorks; RRID: SCR_001622).

m^A-seq. We followed the protocol outlined previously^49 with minor modifications. Approximately 500 μg of total RNA was extracted from frozen liver tissue (n = 5 per group) using TRIzol and purified using Oligo(dT) Dynabeads (Invitrogen). Because of the large amount of input RNA required, we pooled approximately 1 μg of purified mRNA from each animal per treatment group (n = 5 per group). Approximately 5μg of pooled mRNA was then fragmented in fragment analysis (10 mM Tris-HCl (pH 7.0), 10 mM ZnCl2) at 94°C for 2 min and 15 s. NA samples were run on a 100 μg of mRNA (100 ng per group) for 200 cycles at 25°C as the input control for RNA-seq. About 5μg of fragmented mRNA was incubated with 12 μg of anti-m^A antibody (Synaptic Systems) in 1x IP buffer (10 mM Tris-HCl (pH 7.4), 150 mM NaCl and 0.1% IGEPA) at 4°C for 2 h at 4°C on a rotating wheel. While the fragmented RNA was incubated, mRNA for A/G magnetic beads (Pierce) were washed twice and blocked by incubating in 1x IP buffer supplemented with 0.5 μg/ml BSA for 2h at 4°C on a rotating wheel. The m^A-IP mixture was then added to the beads and incubated for an additional 2 h at 4°C on a rotating wheel. After three washes with IP buffer, bound mRNA was eluted by incubating with 100 μl elution buffer (6.7 mM N^6-methyladenosine-5′-monophosphate in 1x IP buffer) for 1 h at 4°C on a rotating wheel. Eluted mRNA fragments were collected, and the beads were incubated with an additional 100 μl elution buffer for 1 h at 4°C on a rotating wheel. The two 100 μl eluates were combined and precipitated overnight at −20 °C by adding 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100% ethanol. On average, 5μg of purified mRNA yielded tens of nanograms of immunoprecipitated mRNA from each group. Input and immunoprecipitated mRNA fragments were used as the input for first-strand cDNA synthesis and library preparation using a TruSeq Stranded mRNA Library Prep Kit (Illumina) and sequenced on an Illumina HiSeq 3000 with single-end 50-bp read length. Raw sequence files were aligned to the GRCh38 assembly of the mouse genome using the RNA-seq pipeline described above. Peak detection was performed using MACS2 (v2.1.1) on uniquely aligned reads using default parameters set for the −nomodel flag (−e = 200). Both enriched and depleted peaks (using the IP libraries as reference) were identified, pooled and consolidated into a single peak library. Both enriched and depleted peaks (using the IP libraries as the reference) were identified using a q value < 0.05, pooled and consolidated into a single peak library. Downstream peak visualization and analysis were performed using the MAGeCK tool. Downstream validation of peak annotations and computed enrichment scores using the Magnitude (m) score, which estimates the significance of m^A peaks using variance-stabilized data. Peak annotation was performed with PeakAnalyzer^54 and motif enrichment with the MEME suite^55. Peak counts per
sample were summarized with STAR's built-in gene counter by re-mapping the raw sequencing files to the peak database. Downstream differential peak analyses and normalization followed the same rules as for the RNA-seq data.

m6A-IP–qPCR. The relative abundance of select lipogenic mRNAs in m6A-Antibody IP samples was assessed by RT–qPCR in mouse liver. Total RNA was isolated from frozen mouse liver with TRIzol (Invitrogen). After DNase treatment, RNA was fragmented in fragmentation buffer (50 mM Tris-HCl (pH 8.0), 50 mM MgCl2) at 94 °C for 3 min. After portions of fragmented RNA was saved as the input control, and the remaining fragmented RNA (approximately 10 µg of total RNA) was used to perform m6A-IP–qPCR. m6A immunoprecipitation was performed as described in the m6A-seq procedure. Approximately 300 µg of total RNA was used to perform m6A-IP–qPCR. A 300-ng aliquot was saved and used as the input control. The remaining RNA was used to perform m6A immunoprecipitation as described in the library preparation procedure. After thorough wash and elution solution, immunoprecipitated RNAs, resulting pellets were suspended in 15 µl RNase-free water and analysed by qPCR. The abundance of lipogenic mRNAs in m6A antibody samples was determined by normalizing to input control for each gene and expressed as the IP:input ratio or the percentage input.

mRNA half-life studies. Primary hepatocytes were treated with actinomycin D (5 µg ml−1; Sigma) for 0 h, 2 h, and 4 h before trypsinization and collection. Total RNA was isolated by TRIzol, reverse transcribed and analysed by qPCR. The degradation rate of RNA was estimated as previously described using equations (1) and (2) below:−4

\[ \log_2 \left( \frac{A_t}{A_0} \right) = -kt \]

where \( t \) is transcription inhibition time (h), \( A_t \) and \( A_0 \) represent mRNA quantity at time \( t \) and time 0. Two \( k \) values were calculated as follows: time 2 h versus time 0 h, and time 4 h versus time 0 h. The final lifetime was calculated by using the average of \( k_{2h} \) and \( k_{4h} \).

\[ t_{1/2} = \frac{2}{k} \left( k_{2h} + k_{4h} \right) \]

Polysome profiling. Total cellular protein was isolated from approximately 300 µg of frozen liver tissue in 500 µl of lysis buffer (10 mM Tris (pH 7.4), 150 mM KCl, 5 mM MgCl2, 100 µg ml−1 CHX, 0.5% Triton X-100, 1.1% protease inhibition (Roche) and 40 µM SUPERasein). Extracts were left on ice for 15 min and then centrifuged at 15,000 g for 15 min at 4 °C. Supernatant was collected (250 µl), and a small aliquot (25 µl) was saved and used as the input control for each experiment. Each lysate was then split by ratio of 1:4 and centrifuged at 15,000 g for 15 min at 4 °C. Supernatant was collected (250 µl). Portion II (250 µl) was then layered onto the top of gradient and absorbance was measured at 260 nm on a nanospectrometer (Beckman, Rotor SW28). Aliquots of 250 µl were then collected starting from the top of gradient by dispensing slowly down the side of tube, making sure not to disturb gradient. Gradients were then centrifuged at 4 °C for 4 h at 27,500 r.p.m. (Beckman, Rotor SW28). Aliquots of 250 µl were then collected starting from the top of gradient and absorbance was measured at 260 nm on a nanospectrometer. Aliquots were then categorized into three main subtypes using the curve generated in the previous step (non-ribosome portion, 40–80S and polysome). Each aliquot was then combined with an equal volume of TRIzol to purify RNA and used for qPCR analysis. Abundance of lipogenic mRNAs in each fraction was determined by normalizing to the input control value for each gene. We also performed the analysis by normalizing to housekeeper within the same fraction, which did not alter the results.

Mitochondrial isolation and bioenergetics. Briefly, liver was minced, washed with PBS and homogenized with a glass Dounce homogenizer. Mitochondria were isolated by dual centrifugation (800g and 8,000g) and respiration was obtained with a Seahorse bioanalyzer XF24 (Agilent)5. Fatty acid oxidation was measured in the presence of 60 µM palmitoyl-carnitine, 250 µM malate and 4 µM FCCP.

ATAC–seq. ATAC–seq was optimized in the liver after several modifications from the original protocol6. A total of 100 µg of frozen liver was ground to fine powder with a cell crusher, and 1 ml of ice-cold nuclei isolation buffer was added (20 mM Tris-HCl, 50 mM EDTA, 5 mM spermidine, 0.15 M spermine, 0.1% mercaptoethanol, 40% glycerol (pH 7.5), mM EGTA and 60 mM MgCl2). After 5 min of cooling on ice, the cell suspension was filtered through Miracloth (Calbiochem) and (2) below. After 5 min at 4 °C and resuspension in PBS. A 300-ng aliquot was saved and used as the input control for each experiment. Each lysate was then split by ratio of 1:4 and centrifuged at 15,000 g for 15 min at 4 °C. Supernatant was collected (250 µl). Portion II (250 µl) was then layered onto the top of gradient and absorbance was measured at 260 nm on a nanospectrometer. Aliquots were then categorized into three main subtypes using the curve generated in the previous step (non-ribosome portion, 40–80S and polysome). Each aliquot was then combined with an equal volume of TRIzol to purify RNA and used for qPCR analysis. Abundance of lipogenic mRNAs in each fraction was determined by normalizing to the input control value for each gene. We also performed the analysis by normalizing to housekeeper within the same fraction, which did not alter the results.

Targeted m6A modification of cellular mRNAs. TRM editor plasmids encoding nucleic-acid-dead Cas3b and truncated Mettl3 fused to a nuclear localization sequence (pCMV-4-dCas3-Mtn3s) and gRNA expression plasmid (pU6-PspCas13b-gRNA) were manufactured by IDT and cloned using the Gibson method (NEB) into the gRNA expression plasmid. Targeted m6A modification of cellular mRNAs with TRM editors was then performed as previously described. AML cells were plated on six-well plates (for RNA) or 10-cm dishes (for protein) and at approximately 80% confluency were transfected using Fugene HD (Promega) with 2.3 µg of TRM editor plasmid and 1.1 µg of gRNA expression plasmid (for six-well plates) or 13.5 µg of TRM editor plasmid and 5.7 µg of gRNA expression plasmid (for 10-cm dishes). At 48 h after transfection, protein and total RNA were collected as described in the gene expression and immunoblot analysis section. The relative abundance of mRNAs at different sites on Scd1 mRNA in cells transfected with gRNA targeting these sites or nontargeting control gRNAs was assessed by m6A-IP–qPCR. To account for variability in RNA amounts, m6A abundance at each site on Scd1 was normalized to input or a nontargeted region on the TRM-edited transcript.

Statistical analysis. A non-paired Student’s t-test was used to determine statistical significance, defined at P < 0.05. For multiple-group experiments analysis of variance was used followed by multigroup analysis. Unless otherwise noted, error bars represent the s.e.m. Most experiments were independently performed at least twice. Sample size is based on statistical analysis of variance and prior experience with similar in vivo studies.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability RNA-seq, m6A-seq and ATAC–seq data can be accessed at the NIH Sequence Read Archive repository under accessions PRJNA65718 and GSE157907. Source data are provided with this paper.

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Author contributions
T.S. conceived and supervised the study. T.S. and D.A.S. designed the study and guided the interpretation of the results and preparation of the manuscript. D.A.S., X.W., J.K. and D.W. performed the majority of experiments and data analysis. Z.Z. performed
the ATAC–seq experiments and data analysis. L.V. and K.R. performed the fatty acid oxidation studies and studies determining the influence of chromosomal versus gonadal factors in Mettl14 regulation and guided the interpretation of results. K.J.W. performed lipidomics studies and analysis. A.H.M. and S.R.J. performed m^6A measurement via mass spectrometry. A.H.-V. analysed the data for MOBES. J.C. assisted with m^6A loss-of-function in vivo studies and cellular knockdown. D.C. performed m^6A and RNA-seq analysis. T.S. wrote the manuscript with input from all authors. All authors discussed the results and approved the final version of the manuscript.

Competing interests
J.C. is a scientific founder of Genovel Biotech and holds equities with the company, and is also a Scientific Advisor for Race Oncology. A patent related to this work is issued to T.S. The other authors declare no competing interests.

Additional information
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Extended Data Fig. 1 | Effect of sex and diet composition on lipid metabolism. **a**, Fat mass measured by MRI for male and female mice in 2-week cohort (n=5 per group). **b**, Body weight of male and female mice in 2-week cohort (n=5 per group). **c**, Quantification of serum cholesterol levels of male and female mice in 2-week cohort (n=5 per group). **d**, Quantification of total serum triglycerides of male and female mice in 2-week cohort (n=5 per group). **e**, Fat mass measured by MRI for male and female mice in 4-week cohort (n=5 per group). **f**, Body weight of male and female mice in 4-week cohort (n=5 per group). **g**, Quantification of serum cholesterol levels of male and female mice in 4-week cohort (n=5 per group). **h**, Quantification of total serum triglycerides of male and female mice in 4-week cohort (n=5 per group). **i**, Quantification of total serum NEFA from male and female mice in 4-week cohort (n=5 per group). Mice were fed indicated diet for 4 weeks and fasted for 4-hrs. prior to sacrifice. Values are mean ± s.e.m. of 5 independent biological replicates (a-i). P values were calculated using one-way analysis of variance (ANOVA) with multi-group comparison (Fisher’s) in a-i. *P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. The precise n, P values, and details of the statistical testing are provided in the source data file.
Extended Data Fig. 2 | Lipogenic genes are highly enriched for m6A under chow diet. a, Lipidomics PCA plot for 4-week cohort (n = 5 per group). b, Quantification of major lipid species identified in lipidomics analysis of male and female mouse liver for 2-week cohort (n = 5 per group). c, Lipidomics heatmap for 4-week cohort (n = 5 per group). d, Quantification of major lipid species identified in lipidomics analysis of mouse liver for 4-week cohort (n = 5 per group). e, Comparison of number of differentially expressed genes (>2-fold) in WD or HF versus chow-fed male livers determined by RNA-seq and m6A-seq. f, Nucleotide sequences containing the m6A motifs on lipogenic transcripts and relative position of each motif on full-length mRNA. g, Rank order table of genes with greatest fold-change in m6A in male livers (Chow vs. Western diet). h, UCSC browser screenshot showing changes in m6A enrichment for DGAT2 in chow and HF diet-fed male livers. Mice were fed indicated diet for 4 weeks and fasted for 4-hrs prior to sacrifice. Values are mean ± s.e.m. of 5 independent biological replicates (b,d). P values were calculated using one-way analysis of variance (ANOVA) with multi-group comparison (Fisher’s) in b and d. *P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. The precise n, P values, and details of the statistical testing are provided in the source data file.
Extended Data Fig. 3 | Loss of the m^6A methylase METTL14 increases lipogenesis and hepatic triglyceride accumulation. a, Western blot of Mettl14 in other tissues from WT and Mettl14 L-KO chow-fed males. Equal amounts of protein were pooled from four animals per group and run in triplicate.
b, Lipidomics heatmap comparing hepatic lipidome of WT versus Mettl14 L-KO chow-fed males. (n = 8 per group). c, Quantification of various lipid species from lipidomics analysis of chow-fed male livers (n = 8). Statistical analysis was performed using unpaired two-tailed t-test. Values are mean ± SEM.
d, Quantification of total food intake for chow-fed male WT and Mettl14 L-KO mice (n = 8 per group). e, Body weight and percent fat measured by MRI for chow-fed male WT and Mettl14 L-KO mice (n = 8 WT mice, n = 7 L-KO mice). f, Quantification of cellular respiratory rate in livers of chow-fed WT and Mettl14 L-KO mice using NADH as the acceptor (n = 5 per group). g, qPCR analysis of fatty acid oxidation gene expression from liver of WT and Mettl14 L-KO mice (n = 4 per group). The experiment was repeated two times with similar results. h, Quantification of serum triglyceride levels from chow-fed male WT and Mettl14 L-KO mice (n = 8 per group).
i, qPCR analysis of gene expression from liver of WT and Mettl14 L-KO mice (n = 4 per group). The experiment was repeated two times with similar results. j, Western blot of lipogenic protein levels in a second independent cohort of chow-fed male mice. Equal amounts of protein were pooled from eight animals per group and run in triplicate. The experiment was repeated three times with similar results. k, Western blot comparing levels of lipogenic proteins in the setting of chronic Mettl14 deficiency (albumin-cre transgenics or control). Equal amounts of protein were pooled from five animals per group and run in triplicate. The experiment was repeated two times with similar results. l, Quantification of hepatic triglyceride concentrations from livers of albumin-cre transgenic or control mice (n = 4 Cre-N mice, n = 5 Cre-P mice). m, Quantification of lipogenic transcript m^6A abundance in WT and Mettl14 L-KO chow-fed livers measured by m^6A-IP-qPCR analysis (n = 4). The relative enrichment of m^6A in each sample was determined by normalizing to ten-fold input. The experiment was repeated two times with similar results. n, Quantification of lipogenic transcript m^6A abundance in WT and Mettl14 L-KO chow-fed livers measured by m^6A-IP-qPCR analysis (n = 4). The relative enrichment of m^6A in each sample was determined by normalizing to GAPDH. The experiment was repeated two times with similar results. Mice were fed chow diet for 4 weeks and fasted for 4-hrs. prior to sacrifice. Values are mean ± s.e.m. of 4 (l,m), 5 (f,g), 6 (d), or 8 (c,e,h) independent biological replicates. P values were calculated using unpaired two-tailed t-test. (c,i,l-n) *P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. The precise n, P values, and details of the statistical testing are provided in the source data file.
Extended Data Fig. 4 | m^6^A regulates the stability and cytoplasmic distribution of lipogenic transcripts. a, qPCR analysis of lipogenic gene expression in livers of WT and Mettl4 L-KO chow-fed male mice (n = 6 per group). The experiment was repeated three times with similar results. b, qPCR analysis of lipogenic pre-mRNA expression using primers that amplify intronic regions from livers of WT and Mettl4 L-KO chow-fed male mice (n = 4 per group). The experiment was repeated three times with similar results. c, Western blot of SREBP-1c from nuclear fraction (mature SREBP-1 [mSREBP-1c]). mSREBP-1c levels in livers of re-fed (4 hr.) mice are shown as reference. Equal amounts of protein were pooled from five animals per group and run in triplicate. The experiment was repeated two times with similar results. d, qPCR analysis of lipogenic transcript levels in WT or Mettl4 L-KO livers at 72 hrs. after Mettl4 deletion (n = 4). e, Polysome profiling curve depicting three major cytoplasmic mRNA pools. f, qPCR analysis quantifying amount of lipogenic mRNAs in ribosome-bound fractions in livers of Mettl4 L-KO chow-fed males compared to WT (n = 3 per group). g, mRNA stability assay for SCD1 mRNA in primary hepatocytes harvested from WT and Mettl4 L-KO chow-fed male mice (n = 3 per group). h, qPCR analysis of Mettl4 expression in AML cells used for single-molecule RNA FISH. (n = 5 per group). i, Quantification of hepatic triglyceride content in WD-fed male mice injected with AAV_m^6^A writers (Mettl4 + Mettl3 + WTAP) or AAV_null (normalized to liver weight) (n = 6 per group). Mice were fed indicated diet for 4 weeks and fasted for 4-hrs. prior to sacrifice except for mice in (i). Mice in (i) were fed NASH diet for 8-weeks and fasted 4-hrs. prior to sacrifice. Values are mean ± s.e.m. of 3 (f,g), 4 (b,d), 5 (h), or 6 (a,i) independent biological replicates. P values were calculated using unpaired two-tailed t-test. (a,b,d,h,i). *P < 0.05; **P < 0.01; *** P < 0.001; **** P < 0.0001. The precise n, P values, and details of the statistical testing are provided in the source data file.
Extended Data Fig. 5 | Regulation of Mettl14 in response to dietary conditions. a, Quantification of major lipid species identified in lipidomics analysis of male WD-fed WT and Mettl14 L-KO livers (n = 7 WT mice, n = 8 KO mice). b, UCSC browser screenshots showing decreased H3K27ac at Mettl14 promoter under HF diet feeding compared to chow. Mice were fed either standard chow diet (Prolab Isopro RMH 3000, Purina) for 24 weeks or 8 weeks of standard chow diet followed by 16 weeks of HFD (Soltis et al. 2017. Cell Reports). c, UCSC browser screenshots showing regulation of Mettl14 promoter by BCL6 and STAT5a in human (Steube et. al 2017. Nat Comm; Gertz et. al 2013. Mol. Cell; respectively). d, UCSC browser screenshots showing ATAC-seq data for Mettl14 promoter region from male mouse liver under different diets and regulation of Mettl14 promoter by BCL6 and STAT5a in male and female mice fed WD (Zhang et. al 2011. Mol Cel Biol). e, qPCR analysis showing fast/re-feed regulation of Bcl6 expression in livers of chow-fed males (n = 5 per group). The experiment was repeated twice with similar results. f, Quantification of m6A abundance on lipogenic transcripts in fasted (4 hr) or re-fed (fasted overnight and then re-fed 4 hr) male livers as measured by m6A-IP-qPCR (n = 4). The experiment was repeated twice with similar results. g, Expression of Mettl3, WTAP in re-fed mice (fasted overnight and then re-fed 4 hr) compared to fasted (4 hr) mice as measured by qPCR analysis (n = 5). h, qPCR analysis of Mettl3 expression in male and female liver under various diets (n = 4 chow-fed females, n = 5 all other groups). Mice were fed the indicated diet for 4 weeks and fasted (4 hr) or re-fed (fasted overnight and then re-fed for 4 hrs. prior to sacrifice). Values are mean ± s.e.m. of 4 (f), 5 (e,g,h) or 7 (a) independent biological replicates. P values were calculated using unpaired two-tailed t-test (a,e-g) or one-way analysis of variance (ANOVA) followed by multi-group comparison (Fisher’s) in h. *P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. The precise n, P values, and details of the statistical testing are provided in the source data file.
Extended Data Fig. 6 | Loss of Mettl14 is associated with sex-biased gene expression. **a**, Comparison of gene expression fold changes between control and Mettl14 L-KO mice obtained from male (x axis) or female (y axis) samples. **b**, Comparison of gene expression fold changes between male and female mice obtained from control (x axis) or Mettl14 L-KO (y axis) samples. The linear fit of all fold changes (yellow) has a smaller slope than the Control=KO line (black), highlighting that male/female differences are higher in controls for most genes. **c**, Hierarchical clustering of samples harvested from liver based on pair-wise distances. Shown is the tree based on the Euclidean distance between all samples (n = 7 WT male mice, n = 8 mice all other groups) based on genome-wide mRNA abundance distributions. **d**, Expression of XIST and SRY in livers of WT and Mettl14 L-KO mice as measured by qPCR (n = 4). **e**, Heatmaps of selected differential genes with distinct responses to Mettl14 L-KO. Three specific clusters of genes with sex-specific response to Mettl14 are shown. Highlighted are the name of genes known to be involved in metabolic pathways. Gene names are ordered as in the heatmap. All mice were fed WD for 4 weeks and fasted for 4 hrs. prior to sacrifice. P values were calculated using unpaired two-tailed t-test (d). The precise n, P values, and details of the statistical testing are provided in the source data file.
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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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|     | ☑ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
|     | ☑ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
|     | ☑ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated |

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
No special computer codes or algorithms

Data analysis
We used readily available software packages including Excel, PRISM, R, Fiji 2.2.1, and Sciex Lipidizer Platform for targeted quantitative measurement of 1100 lipid species across 13 lipid sub-classes; tuned with SelExcel tuning kit [Sciex 5040141].

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All high-throughput data related to this manuscript can be accessed at NIH’s SRA repository PRJNA663718; GRCh38/mm10
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Life sciences study design

All studies must disclose these points even when the disclosure is negative.

Sample size
Sample size is based on statistical analysis of variance and on prior experience with similar in vivo studies over the past 10 years. For recent examples see Zhang et al. Nature Communications. 2020; Sallam et al. Nature. 2016; Sallam et al. Nature Medicine. 2018; Zhang et al. elife. 2017

Data exclusions
Based on pre-established methodology in our lab. If done usually due to samples that are deemed to be outliers by formal testing or technical reasons such as inability to obtain enough serum/tissue or animals reported to be in distress by DLAM staff. Except for figure 4 no exclusions were done (2 data points were excluded in Fig. 4h based on Grubbs outlier test, alpha=0.05 and non physiologic values).

Replication
A strength of our studies is showing reproducibility with repeat experiments and different approaches to show consistent results. Experiments were replicated at least twice.

Randomization
Randomization was done manually.

Blinding
Blinded to group allocation for in vivo studies and as much as possible. Blinding is not possible for every experiment since same individual allocating treatment may be involved in downstream analysis. We note that most of the work used unbiased approaches such as shotgun lipidomics and NGS.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a                             | Involved in the study |
| ☐ ☒ Antibodies                  | ☐ ChIP-seq |
| ☐ ☒ Eukaryotic cell lines       | ☐ Flow cytometry |
| ☒ ☒ Palaeontology and archaeology | ☒ MRI-based neuroimaging |
| ☒ ☐ Animals and other organisms |         |
| ☒ ☒ Human research participants |         |
| ☒ ☐ Clinical data               |         |
| ☐ ☐ Dual use research of concern|         |

Antibodies

Antibodies used
All antibodies with details are provided in supplementary data table1.

Validation

The antibodies used are established in the field and have been used by a number of groups. anti-m6A:
Reactivity: Reacts with human, rat, mouse, eukaryotes, prokaryotes. Other species not tested yet.
Specificity: Specific for N6-methyladenosine (m6A) with some cross-reactivity to m5Am.

anti-Methyl14 validated by the Human Protein Atlas (HPA) project (www.proteinatlas.org). Each antibody is tested by immunohistochemistry against hundreds of normal and disease tissues. These images can be viewed on the Human Protein Atlas (HPA) site by clicking on the Image Gallery link.

anti-PDI
over 130 citations
Huppa, J.B. and Ploegh, H.L. (1998) Cell 92, 145-148.
Ellgaard, L. and Ruddock, L.W. (2005) EMBO Rep. 6, 28-32.
Tu, B.P. and Weissman, J.S. (2004) J. Cell Biol. 164, 341-346.

anti-SCD1
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) ATCC, SCAP/-293T cells kindly provided by Dr. Peter Espenshade (Johns Hopkins University School of Medicine, Baltimore, MD, USA); AML-12 cell line (CRG-2254)

Authentication Validated by ATCC and were obtained directly from ATCC. The maintenance of cell identity is not very relevant here but authentication is validated in our gene expression patterns that is provided in the manuscript from these cell lines

Mycoplasma contamination We routinely test cells for mycoplasma. All cells used in the study tested negative.

Commonly misidentified lines (See lisaC register) No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals Melti14floxB and 8c16 floxB/; male and female mice, 8-12 weeks of age at start of experiment
| Category                  | Description                                                                                                                                 |
|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Wild animals             | C57Bl/6J WT are purchased directly from Jax or littermate controls are used when possible. No wild animals used in the study.                  |
| Field-collected samples  | Mice were housed in a temperature-controlled room under a 12-h light/12-hr dark cycle and pathogen-free conditions. No field animals were used. |
| Ethics oversight         | All animal studies were approved by the UCLA ARC.                                                                                                                                                      |

Note that full information on the approval of the study protocol must also be provided in the manuscript.