Metabolic adaptation to nutritional state requires alterations in gene expression in key tissues. Here, we investigated chromatin interaction dynamics, as well as alterations in cis-regulatory loci and transcriptional network in a mouse model system. Chronic consumption of a diet high in saturated fat, when compared to a diet high in carbohydrate, led to dramatic reprogramming of the liver transcriptional network. Long-range interaction of promoters with distal regulatory loci, monitored by promoter capture Hi-C, was regulated by metabolic status in distinct fashion depending on diet. Adaptation to a lipid-rich diet, mediated largely by nuclear receptors including Hnf4α, relied on activation of preformed enhancer/promoter loops. Adaptation to carbohydrate-rich diet led to activation of preformed loops and to de novo formation of new promoter/enhancer interactions. These results suggest that adaptation to nutritional changes and metabolic stress occurs through both de novo and pre-existing chromatin interactions which respond differently to metabolic signals.

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Animals have evolved to cope with fluctuations in food availability and nutritional content of food that can have major consequences on health. The responses to nutrient quality and availability range on a continuum from complete lack of nutrients to overabundance. These physiologic states have differing homeostatic requirements ranging from the mobilization of storage depots that provide energy and critical biomolecules to the biosynthesis of molecules mediating deposition of energy and metabolic precursors, with accompanying alterations in gene expression programs to facilitate biological responses. Such metabolic adaptation can lead, in the extreme, to the development and progression of obesity and related diseases including non-alcoholic fatty liver disease (NAFLD)\(^1\)\(^2\). With the spread of the Western lifestyle, including consumption of an energy rich diet high in fat, obesity and NAFLD are an emerging health concern\(^3\).

Under conditions of calorie excess, the liver adapts to overnutrition through dynamic reprogramming of the transcriptome, one of many adaptations necessary to maintain metabolic homeostasis. Gene regulatory mechanisms mediating liver metabolic adaptation to excessive calorie intake are incompletely understood. Detailed analysis of the role(s) of promoter–enhancer interaction dynamics may provide mechanistic insights into the progression from nutrient-induced metabolic adaptation to disease.

Within nuclei, chromosomes are organized at multiple levels, from nucleosomal building blocks to more complex structures. Current models suggest that domain organization can reflect genome accessibility as well as regulatory contacts with promoters that modulate gene expression\(^4\). These epigenomic events are cell-type specific and relative stable, but still have the flexibility to be fine-tuned in response to environmental cues\(^5\)\(^6\). Numerous studies indicate that diet can reprogram the epigenome and gene expression by affecting enhancers\(^8\)\(^9\)\(^10\). However, enhancers are usually located long distances away from their target genes, which makes it difficult to accurately assign specific enhancers to their target genes and delineate the details of gene regulation. Hi-C was recently used to identify genome-wide chromatin interactions and provide unique insights into higher-order chromatin organization\(^11\). Although unbiased, Hi-C requires very deep sequencing to provide unique insights into higher-order chromatin organization\(^12\). Promoter capture Hi-C (PCHi-C) was developed to facilitate identification of promoter–anchored chromatin interactions at high resolution\(^12\)\(^13\), enabling characterization of both stable and stimulus-responsive promoter–enhancer loops\(^14\). To date, limited data are available describing the dynamics of promoter–enhancer interactions during metabolic adaptation to changes in diet and progression to overt physiologic changes leading to disease and how these processes impact gene expression.

Given this dearth of information, a deeper understanding of the how the liver epigenome responds to diet, obesity, and the dysfunctional metabolic and physiologic state associated with obesity should provide insights into adaptation and disease. Here, we employ a long-standing model of diet-induced obesity in C57BL/6 mice. Male animals are placed on a long-standing model of diet-induced obesity in C57BL/6J mice. Male animals are placed on de novo lipogenesis (DNL) and upregulation of genes involved in fatty acid oxidation (FAO). We examined expression of genes in these two critical metabolic pathways in our data, observing that lipid-rich diet and associated chronic obesity and adaptation to fatty liver led to a marked suppression of de novo lipogenesis as compared with the carbohydrate-rich diet (Fig. 2d). Hallmark genes\(^22\) activated by sterol regulatory element binding protein 1c (SREBP-1c) including elongation of long-chain fatty acids family member 6 (Elovl6), fatty acid synthase (Fasn), and stearoyl-CoA desaturase (Scd1) were downregulated in animals on lipid-rich as compared with animals on carbohydrate-rich diet. We also observed upregulation of hallmark genes integral to other aspects
**Fig. 1 Effects of diet on metabolic parameters.**

a. Schematic overview of the study.

b. Body weight (mean and SEM) of study animals (n = 5 per group) after 20 weeks of carbohydrate-rich diet (CD) or lipid-rich diet (LD).

c. d. IPGTT and IPITT tests in mice fed the LD or CD (n = 5 per group).

e, f. Fasting plasma insulin and leptin level in mice fed the LD or CD (n = 4 per group).

g–l. Metabolic cage analysis during light and dark phase of energy expenditure (EE), oxygen consumption rate (VO2), carbon dioxide production (VCO2) (n = 3–4 per group).

m, n. Fasting plasma lipids and enzymes in mice fed the LD or CD.

o. Liver, brown adipose tissue (BAT), subcutaneous adipose tissue (sWAT), and epididymal adipose tissue (eWAT) mass in mice fed the LD or CD.

p–s. Hematoxylin and eosin (H&E) staining of adipose tissue and liver (×40 magnification; scale bar indicates 200 μm). Significance is indicated (two-tailed t test). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
of fatty acid metabolism in obese animals (Fig. 2e). The type one acyl-CoA thioesterases, Acot2, Acot3, Acot4, Acot5, and Acot6, which are clustered on mouse chromosome 12 and known to be regulatory targets of peroxisome proliferator-activated receptor alpha (PPAR-α), were upregulated23. Likewise, the type two acyl-CoA thioesterases Acot7, Acot8, Acot9, and Acot13 are upregulated in obese animals. These enzymes catalyze the hydrolysis of the thioester linkage between CoA and fatty acids, leading to the generation of free fatty acid and CoA in mitochondria, peroxisomes, and the cytosol24. Their upregulation in dietary conditions where intracellular fatty acid concentration is elevated reflects participation in fatty acid oxidation in mitochondria and peroxisomes as well as potential roles in signaling pathways dependent on unconjugated fatty acids25. Also upregulated in obese animals are genes classically associated with beta oxidation of fatty acids for energy production such as acyl-CoA dehydrogenases (Acadm, Acads, and Acadvl), enoyl-CoA hydratase (Ehhadh), and hydroxyacyl-CoA dehydrogenase (Hadh) as well as the mitochondrial carnitine-dependent lipid transporter, Cpt1. These differences in the transcriptome are reflective of the vastly different metabolic states and regulatory networks active in livers of animals on lipid-rich versus carbohydrate-rich diets. We selected for validation a subset of DEGs, those involved in the DNL and FAO pathways (Supplementary Fig. 1a).

Chronic obesity rewrites the enhancer network in liver. In order to understand how adaptation to chronic obesity affects enhancer activity, we profiled genomic H3K27ac (3 animals per group) by chromatin immunoprecipitation sequencing (ChIP-seq) in liver. H3K27ac peaks were called by SICER and showed a significant overlap (∼73–89%) across biological replicates, regardless of group (Supplementary Data 2a). Analysis with Diffbind revealed about 5000 loci (from a total of 50,205 peak loci) with significantly differential enrichment (p < 0.05, fold change > 1.5) across biological replicates, regardless of group (Supplementary Data 2b). A depiction of differentially enriched regions is presented in Fig. 2f. The top eight differentially enriched regions were validated by ChIP-qPCR (Supplementary Fig. 2a). As expected, these differentially enriched loci were mostly located far from TSS, and they were classified as enhancers (Supplementary Fig. 2b, c). GO and MSigDB analysis indicated that lipid-rich diet-induced...
regions were enriched with genes related to immune function, including immune system process, leukocyte activation and regulation of immune response (Supplementary Fig. 2d, i), which is also consistent with the pathological process of NAFLD. Carbohydrate-rich diet-induced regions were enriched with genes related to metabolic process, including small molecule metabolic process and carboxylic acid metabolic process (Supplementary Fig. 2e, g).

Enhancer regions usually harbor transcription factors, which bind cognate cis-acting DNA sequences and enable selective gene expression and regulation. To this end, we used HOMER to determine which transcription factor binding motifs were present in differentially enriched loci. In the lipid-rich diet enriched H3K27ac regions, the top enriched motifs corresponded to the known consensus binding sequences for ETS (ETS1, EHF), bZIP (FOSL2, JUN-AP1, and ATF3), and C/EBP (C/EBPA, C/EBPB, and C/EBPE) family transcription factors (Fig. 2h). ETS/AP1 binding sequences are prototypical RAS-responsive elements, which regulate cell proliferation and differentiation in response to a variety of growth factors and cytokines. Lipid-rich diet and obesity is known to cause lipid accumulation in hepatic cells, leading to induction of stress signals and activation of ATF3, an adaptive-response gene. The transcription factors in the C/EBP family have been shown to regulate lipogenesis and motif analysis of lipid-rich diet enriched H3K27ac regions suggested that obesity and NAFLD-induced enhancer activity and gene transcription is likely regulated by transcription factors such as ETS or C/EBP family transcription factors. At carbohydrate-rich diet enriched loci, we also found that motifs for the bZIP family transcription factors and nuclear receptor (HNF1, HNF6) families were highly enriched (Fig. 2i). The HNF family transcription factors are involved in regulating the complex gene networks of lipid and carbohydrate metabolism. For this family, the ratio of carbohydrate/fat was reported to change their DNA binding as well as local histone acetylation.

**Topologically associated domains are not altered by obesity.** The biological interplay between promoters and distal regulatory loci is complicated, with enhancers frequently located 10’s to 100’s of kilobases from transcription start sites. Unambiguous determination of functional relationships between putative enhancers and promoters is facilitated by analysis of chromatin conformation. To obtain detailed insight into the chromatin interactions in our system, we performed in situ Hi-C in livers from animals on carbohydrate-rich and lipid-rich diets. In our system, we performed in situ Hi-C in livers from animals on carbohydrate-rich and lipid-rich diets (Supplementary Fig. 3c). Chromatin interactions linking enhancers to gene promoters are considered to have regulatory roles. Among our identified interactions, 28,682 and 30,502 chromatin interactions overlapped with gene promoters (TSS +/- 2 kb). To obtain a high-resolution view of promoter-anchored chromatin interactions in liver and their changes upon adaptation to obesity, we performed promoter capture Hi-C (PChi-C) (2 animals per group). Hi-C libraries were hybridized with 39,021 biotinylated probes targeting 25,747 promoters and each library was sequenced to an average depth of ~1 billion reads. After QC by HICUP, we obtained an average 400 million unique and valid di-tags per replicate in each group, respectively (Supplementary Data 3a). High quality PChi-C data in our study (Supplementary Fig. 4) reveals significant enrichment of promoter interactions compared with in situ Hi-C (Fig. 4a, b). The scores of chromatin loops called by CHiCAGO indicated the robustness and confidence of interactions. Using the CHiCAGO interaction score (cutoff = 5), we identified around 170,000 interactions in each diet sample, which is six times more than that identified in Hi-C. After filtering out trans-interactions and interactions that span more than 1 Mb, 149,798 and 151,203 cis interactions were retained (Fig. 4c). A large portion (54%) of interactions was shared across physiologic condition, suggesting many promoter interactions are stable even at high resolution (Fig. 4d). Almost 80% of the interactions span <500 kb with a mean size of 284 kb (Fig. 4e). Approximately 13% of the interactions were between two promoters (Fig. 4f), indicating a role for promoters to act as regulatory elements for distal genes.

To understand the potential regulatory roles of the promoter interactions on gene expression, we correlated promoter interactions with gene expression. Generally, an increased number of promoter interactions was positively correlated with gene expression, supporting the hypothesis of a regulatory role for these promoter interactions. We also observed that promoters for unexpressed genes were also enriched for interactions, which suggested that certain promoter interactions are preformed rather than induced by enhancer activation. Furthermore, these interactions significantly overlapped with cis-regulatory regions (DNase I hypersensitive sites, gene promoters, enhancers and CpG islands) defined using mouse ENCODE data (Fig. 4g). The presence of H3K27ac in promoters and enhancers is associated with gene transcription, thus, we also tested for the intersection of H3K27ac enrichment with promoter-interacting regions (PIRs). Notably, those PIRs with H3K27ac enrichment were significantly associated with increased gene expression, supporting models stipulating that promoter contacts with active enhancers upregulate gene expression (Fig. 4i). These data established an atlas of promoter interactions in liver under differing dietary regimens including adaptation to obesity and metabolic stress.

**Promoter chromatin interactions in metabolic adaptation.** In our Hi-C data, we found 34,982 significant chromatin interactions in liver from animals on lipid-rich diet and 37,185 from animals on carbohydrate-rich diet (Supplementary Fig. 3c). Chromatin interactions linking enhancers to gene promoters are considered to have regulatory roles. Among our identified interactions, 28,682 and 30,502 chromatin interactions overlapped with gene promoters (TSS +/- 2 kb). To obtain a high-resolution view of promoter-anchored chromatin interactions in liver and their changes upon adaptation to obesity, we performed promoter capture Hi-C (PChi-C) (2 animals per group). Hi-C libraries were hybridized with 39,021 biotinylated probes targeting 25,747 promoters and each library was sequenced to an average depth of ~1 billion reads. After QC by HICUP, we obtained an average 400 million unique and valid di-tags per replicate in each group, respectively (Supplementary Data 3a). High quality PChi-C data in our study (Supplementary Fig. 4) reveals significant enrichment of promoter interactions compared with in situ Hi-C (Fig. 4a, b). The scores of chromatin loops called by CHiCAGO indicated the robustness and confidence of interactions. Using the CHiCAGO interaction score (cutoff = 5), we identified around 170,000 interactions in each diet sample, which is six times more than that identified in Hi-C. After filtering out trans-interactions and interactions that span more than 1 Mb, 149,798 and 151,203 cis interactions were retained (Fig. 4c). A large portion (54%) of interactions was shared across physiologic condition, suggesting many promoter interactions are stable even at high resolution (Fig. 4d). Almost 80% of the interactions span <500 kb with a mean size of 284 kb (Fig. 4e). Approximately 13% of the interactions were between two promoters (Fig. 4f), indicating a role for promoters to act as regulatory elements for distal genes.

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Altered promoter contacts at differentially expressed genes. To understand whether metabolic adaptation to diet altered promoter looping events in liver, we used edgeR\textsuperscript{38} to identify differences in chromatin interaction patterns. We identified 1962 rewired promoter interactions ($N = 195691$, $p < 0.001$); 705 were increased in frequency in lipid-rich diet and 1257 were more frequent in animals on carbohydrate-rich diet (Fig. 5a, b, Supplementary Data 3c). The interactions that were altered...
overlapped with 109 and 228 DEGs, respectively. Gene Ontology (GO) analysis indicated DEGs with carbohydrate-rich diet-induced promoter interactions were enriched in metabolism-related pathways, including small molecular metabolic process, lipid metabolism, and carboxylic acid metabolic process (Fig. 5c). DEGs with lipid-rich diet-induced promoter interactions were enriched in cell motility, regulation of transmembrane transport, and positive regulation of cell communication (Fig. 5d). This set of pathways indicates the potential role of promoter interactions in a broad spectrum of cellular metabolic events.

We next asked whether rewiring promoter interactions contributes to altered gene expression. Compared with the DEGs associated with static loops, we found that only the promoter/enhancer interactions responsive to carbohydrate-rich diet
significantly associated with increases in gene expression (Mann–Whitney U test, p < 0.0001) (Fig. 5e). Taken together, our findings demonstrate that rewiring of promoter interactions contributes to transcriptional regulation of metabolic genes and may be diet dependent.

Preformed promoter–enhancer interactions. An enhancer usually regulates a distal gene through physically connecting to the gene’s promoter. To understand whether the activity status (using H3K27 acetylation as a proxy) of the enhancers identified as interacting with a given promoter in our capture Hi-C data reflected gene expression, we examined the overlap of the distal end of promoter interactions with H3K27ac differentially enriched loci, regardless of whether the number of contacts changed with condition. We discovered 4449 promoter–enhancer interactions in which H3K27ac was differentially enriched in lipid-rich diet and 2003 cases where this mark was enriched in carbohydrate-rich diet (Fig. 5f, g, Supplementary Data 3c). The promoter–enhancer interactions with H3K27ac enrichment that were altered overlapped with 616 and 317 DEGs, respectively. Next, we considered how gene expression changes as a function of the altered activity of enhancers. As shown in Fig. 5h, DEGs where the promoter interacts with an acetylated enhancer were upregulated, regardless of diet (Mann–Whitney U test, p < 0.0001). We note that the number of promoter–enhancer loops in which activation status of the enhancer changes (6452) in a preformed loop greatly exceeds the number of new loops formed following a change in conditions (1962). These findings suggest that activation of enhancers already interacting with a promoter represents an important mode of distal gene regulation during metabolic stress (Fig. 5f).

Transcriptome analysis links Hnf4α to metabolism. To evaluate which transcription factor binding events were involved in gene expression changes, we performed motif analysis in the gene promoters. Using HOMER, we observed enrichment of several TF motifs at the DEG promoters (Fig. 6a), including Hnf4α. Hnf4α, which is a signal responsive transcription factor, has an important role in regulating lipid metabolism and is activated by lipid ligands. We previously found that Hnf4α serves as a modulator to regulate the epigenome and gene expression during diet-induced obesity in the colon. Since Hnf4α itself was differentially expressed in our system, we first validated its expression by qPCR and immunoblotting (Fig. 6b). We observed downregulation of Hnf4α at the protein and mRNA levels in obese animals. As the lipid-rich diet used in this study contains ~3% by weight C18 polyunsaturated fatty acids including linoleic acid (USDA, National Nutrient Database for Standard Reference [https://ndb.nal.usda.gov/ndb/foods/]) a known ligand for HNF4α, downregulation of HNF4α protein is potentially consistent with the increased turnover noted with other nuclear receptors upon ligand binding. We do not at this time understand downregulation of HNF4α mRNA. Next, we performed ChIP-seq to understand how Hnf4α localization responds to obesity (2 animals per group). Peak calling was done by HOMER and resulted in 45,830 peaks in carbohydrate-rich diet and lipid-rich diet. Most of the peaks were located within 500 kb near a TSS. We compared the differentially enriched Hnf4α binding regions across condition (N = 4384, FDR < 0.05, fold change > 1.5) and identified 3222 peaks more enriched in lipid-rich versus carbohydrate-rich diet and 1161 peaks with the opposite enrichment (Fig. 6c, Supplementary Data 4a). These findings demonstrate that rewiring of promoter interactions accompanied by dynamic promoter interaction events. We observed that interactions at the distal end of promoter interactions overlapped with Hnf4α binding (Chi square test, p < 0.0001) (Fig. 6g), which suggests that Hnf4α binds to sites of promoter interactions. We next considered Hnf4α binding at genes whose promoters had interactions with changes in occupancy (Fig. 6h). In agreement with previous findings, the DEGs that showed gains in Hnf4α peaks at the distal end of sites of promoter interactions also showed upregulated gene expression (Mann–Whitney U test, p < 0.0001, Fig. 6i). To extend our analysis and understand the relationship between differential Hnf4α binding and gene expression, we also assigned the condition-enriched Hnf4α binding sites to the nearest DEGs (TSS+1–50 kb). We found that DEGs with condition-induced Hnf4α binding had significantly higher expression than DEGs with unchanged Hnf4α binding (Mann–Whitney U test, p < 0.0001, Fig. 6j).

Hnf4α binding with additional transcription factors. Although we identified around 4000 Hnf4α binding sites that were changed, this number only represents about 10% of the genome-wide binding sites for Hnf4α. Of all Hnf4α binding sites, 79% are co-localized with loci enriched for H3K27ac, which is marker of active promoters and enhancers (Fig. 7a). These data suggest that most of the identified Hnf4α-bound regions in liver are active elements or open chromatin that exist in both conditions and are not readily changed. Since such a large percentage of Hnf4α binding sites are stable, we hypothesized that Hnf4α may interact functionally with other transcription factors during metabolic stress. To test our hypothesis, we used HOMER to determine which transcription factor binding motifs were present in these Hnf4α bound activated regions. As expected, among 36,125
Fig. 5 Promoter interactions rewired by diet. **a** A schematic of diet-induced interactions. **b** Example of interactions with increased in frequency under carbohydrate-rich diet (CD). Interactions with CHICAGO score > 5 were in gray color and red color indicated significantly increased interaction identified by EdgeR. **c, d** Gene ontology analysis of rewired interactions in CD and lipid-rich diet (LD). **e** Box and whisker plots showing changed gene expression as a function of diet-induced promoter interactions. Box denotes 25th to 75th percentile, horizontal bar is median, whiskers extend to 1.5*IQR beyond the box. **f** A schematic of diet-induced activation at promoter-enhancer interactions. **g** Genome browser view of transcript abundance (lower panels), H3K27ac (middle panels), and promoter interactions at the CD36/Gnat3 locus. Interactions with CHICAGO score > 5 were in red color. **h** Box and whisker plots showing altered gene expression as a function of diet at loci with activated enhancers. Box denotes 25th to 75th percentile, horizontal bar is median, whiskers extend to 1.5*IQR beyond the box. Significance is indicated (Mann–Whitney U test). **∗∗∗∗p < 0.0001.**
Hnf4α-bound activated regions, the most significantly enriched consensus motif is that for HNF4α. Interestingly, the second most common motif is that for the basic leucine zipper domain (bZIP) family of transcription factors, which includes the C/EBP family (Fig. 7b). C/EBPs are a family of six CCAAT/enhancer binding proteins, among which C/EBPα and C/EBPβ are most widely expressed and studied\(^4^7\). Compared with C/EBPβ, C/EBPα is more abundant and its expression changes in our RNA-seq data (Supplementary Data 1). Thus, we focused on investigating further the location and interactions of C/EBPα.

To validate the motif results, we performed C/EBPα ChIP-seq (2 animals per group) and found 55% of C/EBPα peaks colocalized with Hnf4a, rank1 $p = 1e^{-16}$.
Fig. 6 Transcriptome analysis links Hnf4α to regulation of metabolism genes in NAFLD. a Motifs enriched in the promoters of differentially expressed genes. b Abundance of Hnf4α at the transcript (left panel) and protein levels (right panel) in carbohydrate-rich diet (CD) or lipid-rich diet (LD) group. The column graph depicts mean and SEM (n = 5 animals). Significance is indicated (two-tailed t test). c Heatmap of Hnf4α differentially bound regions. Acetylation status at the same loci is indicated in the right-hand panel. d Metagene plot of Hnf4α and H3K27ac signal at differentially bound regions. e, f Example of differential binding of Hnf4α and acetylation at H3K27 at the Lcn2 and Adglyf loci. Regions with differential Hnf4α binding are indicated with blue shading in the figure. g Overlay of Hnf4α with the distal end of promoter/enhancer interactions compared with random samples. Significance is indicated in the figure (Chi square test). h Schematic overview of diet-induced Hnf4α binding at interactions. I Box and whisker plots showing changed gene expression as a function of diet-induced Hnf4α binding at promoter interactions. Box denotes 25th to 75th percentile, horizontal bar is median, whiskers extend to 1.5*IQR beyond the box. j Box and whisker plots showing changed gene expression as a function of diet-induced Hnf4α binding near a TSS (±/– 50 kb). Box denotes 25th to 75th percentile, horizontal bar is median, whiskers extend to 1.5*IQR beyond the box. Significance (Mann-Whitney U test) is indicated in the figure. ***p < 0.001, ****p < 0.0001.

with Hnf4α in liver (Monte-Carlo simulation, N = 10000, p < 0.0001, Fig. 7c, d). Analysis of overlapped regions by GREAT revealed that co-bound sites were enriched in genes involved in a variety of metabolic pathways, including carboxylic acid metabolism, fatty acid metabolism, steroid metabolism, and lipid catabolism (Fig. 7e). Next, we sought to understand how C/EBPα binding changes with diet and to identify the differentially bound sites (N = 7327, FDR < 0.05, fold change > 1.5). Using the same cutoff criteria as for Hnf4α, we observed 7327 differentially bound sites including 5102 more enriched in lipid-rich diet and 2225 sites more enriched in carbohydrate-rich diet (Fig. 7f, Supplementary Data 4b). GO analysis suggested that C/EBPα differentially bound sites were also enriched in metabolism-related pathways regardless of condition (Fig. 7g, h). Motif analysis of C/EBPα differentially bound sites also indicated an increased presence of the Hnf4α motif in both conditions (Fig. 7i, j). To investigate whether Hnf4α interacts with C/EBPα, we also examined the Hnf4α binding signal at C/EBPα differentially bound sites (Fig. 7k). Metagene plots indicated that Hnf4α signal was higher at differentially C/EBPα enriched regions, suggesting that, at a minimum, Hnf4α is always present at these sites (Fig. 7l). One example for our model is presented in Fig. 7m where the fatty acid translocase CD36 exhibits diet dependent alterations in C/EBPα binding and histone acetylation. To test our hypothesis, we divided the diet-induced C/EBPα binding sites into two categories: sites with Hnf4α or sites without Hnf4α. We observed that co-binding of C/EBPα with Hnf4α significantly changes the binding magnitude of C/EBPα in a condition-dependent manner (Fig. 7n). We also found promoter-interacting regions where co-binding with Hnf4α significantly increased the C/EBPα binding signals at diet-induced C/EBPα binding sites in both conditions (Fig. 7o). These data suggest that Hnf4α is frequently found at promoter-interacting regions where it frequently co-binds with C/EBPα to regulate gene expression during metabolic adaptation to diet (Fig. 8).

Discussion

Nonalcoholic fatty liver disease (NAFLD) is one of several obesity-related diseases that is part of an emerging epidemic of metabolism-based pathologies. NAFLD is characterized by fat accumulation in hepatocytes and comprises a spectrum of histopathologic abnormalities ranging from simple steatosis through steatohepatitis to fibrosis and cirrhosis. In a sense, NAFLD and associated pathologies can be considered as an extreme case of the spectrum of metabolic adaptation to diet which is an evolutionary necessity for animals. Here, we demonstrate that animals placed on extreme diets (an energy rich diet high in lipid, a less caloric diet rich in carbohydrate) for chronic periods exhibit alterations at the physiologic, cellular, and molecular levels. Animals consuming excess calories from a diet rich in lipid exhibit extreme weight gain at both the whole-animal level as well as in specific tissues including liver and adipose. Physiologic measures such as fasting serum hormone levels, serum lipid levels, glucose, or insulin tolerance, and measures of energy expenditure and respiration, are likewise aberrant. In addition, chronic consumption of a lipid-rich diet leads to visible accumulation of lipid droplets in liver, to increased size of adipocytes and to accumulation of liver enzymes in serum, indicative of hepatocyte damage. These multi-system changes reflect adaptation of metabolism and physiology of the animal to an extreme diet. Not surprisingly, the chronic consumption of extreme diet and the adaptive responses described above are accompanied by molecular alterations in hepatocytes underlying dramatic remodeling of the transcriptome. Our results define physiologic responses leading to dynamic activation of pre-existing promoter–enhancer interactions and to formation of new enhancer/promoter loops (Fig. 8a, b). In the case of physiologic and metabolic adaptation to lipid-rich diet, activation of enhancers that participate in preexisting interactions with promoter chromatin predominates; the responses to a carbohydrate-rich diet are more balanced.

That the metabolic response to different diets at the level of chromatin interactions differs is potentially reflective of the underlying biology. The liver response to carbohydrates is complex, but includes insulin signaling pathways that converge on the transcriptional responses mediated by the sterol regulatory element binding protein 1c (SREBP-1c). This transcription factor is sequestered in an inactive state outside the nucleus until signaling events mediate its nuclear accumulation at target genes, including a gene set differentially expressed in this study. We anticipate that such a mode of transcriptional regulation may be facilitated by the de novo creation of enhancer/promoter loops downstream of binding by SREBP-1c. In contrast, the hepatocyte transcriptome, in response to the many alterations in physiology initiated by chronic consumption of a lipid-rich diet involved differential expression of genes regulated by a pair of nuclear receptors, peroxisome proliferator-activated receptor alpha (PPARα) and hepatocyte nuclear factor 4 alpha (HNF4α). Both PPARα and HNF4α are constitutively localized to the nucleus and are activated by ligand binding. Preferential utilization of preformed promoter/enhancer loops by ligand-activated transcription factors may be reflective of a general feature of this class of transcription factors. Ligand availability for PPARα and HNF4α is expected to be modulated by diet, and potentially by the action of genes differentially expressed in this system such as the acyl-CoA thioesterases. Thus, the transcriptional outcomes of adaptation to chronic consumption of lipid-rich diet likely feed forward to impact activation of master regulatory transcription factors governing the response.

In contrast to differential promoter/enhancer loops, we observed that higher-order chromatin organization, including A/B compartments and TAD boundaries, in liver is largely unchanged, even when comparing animals with such vastly different physiologic and metabolic profiles. Long-range chromatin architecture is closely associated with transcriptional programs in a cell-type-specific manner. Although our mouse model has
Fig. 7 *Hnf4a* frequently co-binds with *C/EBPa*. a Overlap between *Hnf4a* and *H3K27ac* sites, regardless of diet conditions. b Motifs enriched in overlapping regions of *Hnf4a* and *H3K27ac*. c Overlap between *Hnf4a* and *C/EBPa* binding sites, regardless of diet. d The genome browser shot provides an example of colocalization of *Hnf4a* and *C/EBPa* binding at the *Elov6* locus. e GO analysis of *Hnf4a* and *C/EBPa* overlapping regions. f Heatmap of *C/EBPa* signal at diet-induced differentially bound regions. g, h GO analysis of *C/EBPa* differentially bound regions. i, j Motifs enriched at *C/EBPa* differentially bound regions. k Heatmap of *Hnf4a* and *H3K27ac* signal at *C/EBPa* differentially bound regions. l Metagene plot of *Hnf4a* signal at *C/EBPa* differentially bound regions. m Example of *Hnf4a* binding at a promoter interaction site as a bookmark for *C/EBPa*. n Heatmap of *C/EBPa* at differentially bound regions with or without premarked *Hnf4a*. o Metagene plot of *C/EBPa* at differentially bound regions with or without premarked *Hnf4a* at promoter interactions. Significance (Mann–Whitney U test) is indicated in the figure.
dramatic physiologic and metabolic alterations, the observed liver phenotypes are reversible on removal of the lipid-rich diet. In line with this notion, chromatin structure, as revealed by topologically associated domains, appears relatively stable in NAFLD despite the dramatic impact on the animal.

In hepatocytes, the balance between de novo lipogenesis (DNL) and fatty acid oxidation (FAO) maintains homeostasis. In NAFLD, cellular concentrations of fatty acids are higher than normal; only the oxidative conversion of fatty acids and a decrease in lipogenesis minimizes lipotoxicity in liver. Our gene expression data indicate that genes involved in FAO are upregulated and genes involved in DNLI are downregulated (Fig. 2d, e). This finding suggested that NAFLD-induced steatosis was likely an early stage in a two-hit process of the development of NAFLD, in which FAO and DNLI still cooperate. Gene expression change during NAFLD development is likely mediated through effects on transcription factors. Using HOMER, we found that the Hnf4α binding motif was enriched in the promoter regions of DEGs, a potential to both activate and repress transcription. In our study, we observed that nearly 80% of Hnf4α binding sites overlapped with H3K27ac enriched loci, which suggests that Hnf4α prefers to bind at regions of open chromatin. HNF4α is an orphan nuclear receptor involved in metabolic regulation with the potential to both activate and repress transcription. In our study, we observed that nearly 80% of Hnf4α binding sites overlapped with H3K27ac enriched loci, which suggests that Hnf4α prefers to bind at regions of open chromatin. HNF4α is primarily located in the nucleus and the protein level of Hnf4α decreased with NAFLD, suggesting that Hnf4α might be activated by its ligands and relocated in the genome to modify gene expression in NAFLD.

Combining our PChIC data and Hnf4α ChIP-seq, we found that Hnf4α binding was enriched at the promoter-interacting regions (Fig. 6g) involved in regulation of gene expression (Fig. 6i, j). A previous study also reported that HNF4α could recruit additional cofactors to regulate gene expression. In our study, around 90% of HNF4α binding sites were stable between carbohydrate-rich diet and lipid-rich diet group, which led us to hypothesize that Hnf4α co-binding with other transcription factors may play an important role in response to external stimuli and physiologic change. Motif analysis suggested that Hnf4α binding sites were also enriched for the binding motif of C/EBPα, another important transcription factor in metabolism. C/EBPα ChIP-seq data showed that more than half the binding sites overlapped with Hnf4α binding sites, suggesting they might work together to regulate gene expression. Importantly, we observed that more than 20% of C/EBPα binding sites were changed by different diets, which was more than the number of HNF4α sites that changed (10%). CD36 is target gene of both HNF4α and C/EBPα, and the knockout of CD36 protects against NAFLD during exposure to a lipid-rich diet. We found that CD36 expression was elevated under lipid-rich diet and showed an activated promoter-enhancer interaction. In the distal end of the interaction, HNF4α may function to act as a bookmark for C/EBPα, looping to the promoter of CD36 to upregulate its expression. Collectively, these data are consistent with a model in which HNF4α premarks specific positions for C/EBPα to regulate metabolism genes (Fig. 8c).

In summary, our study presents an atlas of promoter interactions in liver during adaptation to the physiologic and metabolic outcomes of chronic exposure to carbohydrate-rich or lipid-rich diet and offers understanding about how chromatin interactions and gene expression respond to metabolic and physiologic stress. We showed that genes are regulated through promoter interaction dynamics in two different ways: activation of preformed loops and de novo generation of new loops. Furthermore, we identified significant co-binding of Hnf4α with C/EBP alpha and propose that Hnf4α may function to mark open chromatin for C/EBP. Overall, our findings provide insights into chromatin organization and gene regulation during the development of metabolism-related pathologies.

**Methods**

**Mice study.** Five-week-old C57BL/6 mice were purchased from Jackson Laboratory, were acclimated to the NIEHS animal facility (on normal chow, NIH-31) for

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**Fig. 8 Promoter/enhancer interactions during metabolic adaptation to diet.** a, b Diet-induced differential gene expressions involves two types of promoter-enhancer interactions: de novo formation of promoter-enhancer interactions (a); activation of preformed promoter-enhancer interactions (b). c Enhancer activation can occur through multiple mechanisms. Nuclear receptors, including Hnf4α, can be activated by ligand binding, leading to local increases in histone acetylation and enhancer function (left panel). Alternatively, lineage-specifying transcription factors, including HNF4α, can bind constitutively at signal responsive enhancers and co-bind with other transcription factors, such as C/EBPα to regulate metabolism-related genes (right panel).
Glucose tolerance and insulin tolerance tests. Intraperitoneal glucose tolerance tests (IPGTTs) were performed at 19 weeks of LD and CD. Mice were fasted and glucose was injected intraperitoneally at a concentration of 2 g/kg body weight. Blood glucose was measured with a glucometer at 0, 20, 40, 60, and 120 min. Intraperitoneal insulin tolerance tests (IPTTs) were performed 1 week later. Mice were fasted and insulin was injected intraperitoneally at a dose of 0.75 IU/kg body weight. Blood glucose was measured with a glucometer at 0, 15, 30, 45, 60, and 120 min. 

Plasma parameters. Mice were fasted overnight and blood samples were collected after 20 weeks of LD and CD. Plasma was collected and subjected to insulin measurements using the Mouse Insulin ELISA kit (ALPCO, NH, USA) and leptin measurements using the Mouse/Rat Leptin ELISA (ALPCO, NH, USA) following the manufacturer’s instructions. Samples were measured in triplicate. Plasma cholesterol, HDL, LDL, ALT, and AST were measured by Clinical Pathology Laboratory in NIEHS.

Hi-C technology. In situ Hi-C was carried out as previously described with some modifications38,39. Livers tissue was collected, homogenized and filtered with a 70-μm nylon cell strainer. Around 10 million cells were fixed with 1% formaldehyde in PBS for 25 min at room temperature, then washed twice and cross-linked by phenol at 27 min. Energy expenditure was calculated according to the TSE default setting using the following equation: total energy expenditure (kcal/h) = 3.941 × VO₂ (l/h) + 1.106 × VCO₂ (l/h). Data were collected for 3 days and normalized by weight. The fasted day was considered the acclimation period and was excluded from data analysis. Mice had unlimited access to food and water for the entire duration in CalCages.

ChIP-seq and data analysis. Livers tissue was collected, homogenized, and fixed with 1% formaldehyde in PBS for 10 min at room temperature. Cross-linking was terminated by addition of glycine at 0.125 M for 5 min. Cells were lysed with 10 mM Tris-HCl pH 8.0, 10 mM NaCl, 0.2% NP-40 and protease inhibitors for 30 min on ice. Nuclei were resuspended in 1 × NEBuffer2 with 1% SDS and incubated at 62 °C for 10 min. After incubation, nuclei were collected and resuspended in 1 × NEBuffer2 with 1% Triton X-100. HindIII (NEB, R3104, 400 u) enzyme was added to the solution and the nuclei were digested at 37 °C overnight with agitation (750 rpm). Around 400 unit HindIII was added to the solution at 37 °C for another 1 h the next day. After digestion, 37.5 μl of 0.4 mM biontin-14-dATP (ThermoFisher, 19524016), 1.5 μl of 100 mM dCTP, 1.5 μl of 10 mM dGTP, 1.5 μl of 10 mM dTTP, and 8 μl of 5 U/μl Klenow (NEB, M0210) were added and rotated at 37 °C for 1 h. After dATP filled in, the solution was incubated at 65 °C for 10 min to inactive the enzyme. Ligation was performed by adding 667 μl of water, 120 μl of 10X NEB T4 DNA ligase buffer, 100 μl of 10% Triton X-100, 12 ml of 10 mg/ml BSA, and 0.5 μl of 400 U/μl T4 DNA Ligase (NEB, M0202) and incubating at 16 °C for 4 h. Reverse cross-linking was done by adding 10 μl of proteinase K (ThermoFisher, AM2545), 100 μl of 10% SDS, and 100 μl of 5 M NaCl, and incubated at 65 °C overnight. DNA was purified by phenol–chloroform extraction and 20 μg of DNA was used to ligate with T4 DNA polymerase (NEB, M0203) for 4 h at room temperature to remove the biotin from non-ligated ends. Then, DNA was sheared by Covaris and double size selected using AMPure XP beads as described in ref. 39. MyOne Streptavidin T1 DynaBeads (ThermoFisher, 656001, 150 μl) were washed, resuspended in 300 μl of wash buffer (5 mM Tris-HCl, 0.5 mM EDTA, 1 M NaCl, 0.05% Tween 20) and added in 300 μl of 2× binding buffer (10 mM Tris-HCl, 1 mM EDTA, 2 M NaCl) with sheared DNA for 15 min at room temperature. The pulled down fragments were end-repaired, adenine-tailed, and ligated to adaptors (Bioo Scientific, NOVA-5144-61) on beads as described in ref. 39. The immobilized Hi-C libraries were amplified using primers mix from NEXTFlex Pre and Post Capture Kit (Bioo Scientific, NOVA-5144-61) with 12 PCR amplification cycles. The resulting libraries were sequenced on Illumina Novaseq as 50 bp paired-end.

ChIP-Seq analysis. PCHi-C reads were also processed by HiCUP pipeline38. The percentage of mapping efficiency, unique and validated di-tags and capture efficiency were listed in Supplementary Data 3a. We used CHICAGO40 to call the significant interactions with score cutoff 5. All the trans-interactions and those interactions span more than 1 Mb were discarded. EdgeR was used to identify the significantly increased and decreased promoter interactions with cutoff 0.0003 as described in ref. 38. When calculating the correlation between gene expression and interaction number, TPM was divided in four categories: TPM = 0, TPM = 0–1, TPM = 1–10, and TPM > 10. To test whether H3K27ac at the distal end of the interaction, we categorized interactions according to whether at least one distal end overlap with H3K27ac peak, avoiding the dramatic decreased promoter interactions with cutoff 0.0003 as described in ref. 38. All interactions were merged together and the overlapping regions were sorted by their overlap score. The overlap score was calculated as the number of total associated loops, resulting no interaction, 1 interaction with/without H3K27ac, 2–5 interaction with/without H3K27ac, and > 5 interaction with/without H3K27ac. Overlap of interactions with functional genomic elements was performed using Epithinker41. Epithinker automatically calculates control sets for user-uploaded region sets, which is done by reshuffling the genomic positions while retaining the overall number of regions and the distribution of region sizes. The selected overlap criterion is at least 10% overlap. We divided the diet-induced promoter interaction in two categories: (1) rewired interactions that were significantly different between diet groups, which were defined by Epithinker; (2) interactions that were not significantly different but with differential H3K27ac enrichment at the distal end.

ChIP-seq and data analysis. ChIP-seq experiments were performed on HeLa cells using a Covaris to generate 200–250 bp fragments for immunoprecipitation as previously described42. The 20 μg chromatin in IP buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl (pH 8.1)) was subjected to immunoprecipitation with 1 μg of H3K27ac (Abcam, ab74297), 5 μg H3K9ac (Abcam, ab41850) or 5 μg C/EBPα (Abcam, ab40764) antibody and incubated overnight. The samples were washed with Dynabeads for another 3 times with saline-washed (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), high salt (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), LiCl (0.25 M LiCl, 1% NP-40, 1% deoxycholate, 1 mM EDTA, 15 mM Tris-HCl, pH 8.1) and then twice with TE buffer as previously described42. After elution from the beads, the protein-DNA complexes were reverse crosslinked and DNA was purified using AMPure XP beads (Beckman). ChIPed DNA (1–2 ng) was prepared using NEXTFlex Rapid Illumina DNA-Seq Library Prep Kit according to the manufacturer’s instructions (Bioo Scientific, NOVA-5144-02). The resulting libraries were sequenced on Illumina NextSeq500 as 35 bp pair-end.

Raw reads were filtered by quality score and aligned to the mouse genome (mm9) (Supplemental Data 6). Unique aligned and deduplicated reads were used for peak calling. SICER (v1.1) was used for H3K27ac with size 200, gap size 200, fragment size 200, and FDR cutoff 0.001 (Supplemental Fig. 5). For H3K9ac and C/EBPα, significant peaks were identified by the Homer tool with ‘–style-factor’ option. Differentially enriched loci were identified using the DiffBind package and results were filtered with FDR < 0.05 and fold change > 1.5. Top eight differentially expressed regions were identified using the DiffBind package and results were filtered with FDR < 0.05 and fold change > 1.5.
enriched loci were validated by ChiP-qPCR and primers are listed in Supplementary Data 5b. The HOMER package was used to extract motifs from the differentially bound regions (~size given) against a large set of randomly selected genomic fragments of the same size. The statistical significance of the overlap between HNF4α and C/EBPα peaks was determined by Monte-Carlo simulation, where BEDtools v2.24.0 ‘shuffle’ was used to select size-matched random peaks within enhancer regions (defined by H3K27ac peaks) followed by overlap assessment via BEDtools v2.24.0 ‘intersect’ for 10,000 iterations.

RNA-seq and data analysis. RNA-sequencing was done by the NJEHS epigenomics core. Libraries were sequenced as PE-76mers on an Illumina NextSeq500. Raw read pairs were filtered to require a minimum average base quality score of 20. Filtered read pairs were mapped against the mm9 reference genome by STAR with the following parameters: --outSAMattrIHstart 0 --outFilterType BySJout --alignSlorefWeightMin 8 -- outMultimapperOrder Random --limitRMAreadKRT 550000000000 (other parameters at default settings). Mapped read counts per gene were reported by Subread featureCounts (version 1.5.0-p1) with parameters -s -2 -Sfr -p2. Differentially expressed genes (DEGs) were identified by DESeq2 (v1.10.1) using filtering thresholds of FDR < 0.05 and fold change > 1.25. Normalized read counts are reported as transcripts per million (TPM). The gene models used for the RNA-seq analysis are taken from mm9 RefSeq annotations, as downloaded from the UCSC Genome Browser on September 9, 2016. Promoter motif analysis was done by HOMER with default setting. DEGs in DNL and FAO pathways were validated by qPCR or western blot and primers are listed in Supplementary Data 5a.

Histological analysis. Livers and adipose tissues were removed immediately after cervical dislocation and fixed in 10% buffered formalin. Paraffin sections (5 μm) were stained with hematoxylin and eosin (H&E) and analyzed using a microscope.

Statistical analysis. All statistical analyses were performed by two-tailed Student’s t test, Mann–Whitney U test or Chi-squared test using GraphPad Prism7 (San Diego, CA) and RStudio. The level of significance was set at p < 0.05 and indicated as follows: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. All data were expressed as means ± SEM.

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**Author contributions**

The study was designed by Y.Q. and P.A.W. Experiments were performed by Y.Q., J.D.R., and K.C. S.A.G. and Y.Q. performed data analysis. The paper was written by Y.Q., S.A.G., J.D.R., and P.A.W. All authors read and approved the final paper.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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