Regulation of gene expression during the fasting-feeding cycle of the liver displays mouse strain specificity

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Running title: Transcriptomes of two mouse strains during fasting-feeding

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ABSTRACT

The liver maintains metabolic homeostasis by integrating the regulation of nutrient status with both hormonal and neural signals. Many studies on hepatic signaling in response to nutrients have been conducted in mice. However, no in-depth study is currently available that has investigated genome-wide changes in gene expression during the normal physiological fasting-feeding cycle in nutrient-sensitive and -insensitive mice. Using two strains of mice, C57BL/6J and BALB/cJ, and deploying deep RNA-Seq complemented with quantitative-RT-PCR, we found that feeding causes substantial and transient changes in gene expression in both mouse strains. The majority of significantly changed transcripts fell within the areas of biological regulation and cellular and metabolic processes. Among the metabolisms of three major types of macronutrients, \textit{i.e.} carbohydrates, proteins, and lipids, feeding affected lipid metabolism the most. We also noted that the C57BL/6J and BALB/cJ mice significantly differed in gene expression and in changes in gene expression in response to feeding. In both fasted and fed states, both mouse strains shared common expression patterns for about 10,200 genes, and additional 400–600 genes were differentially regulated in one strain but not the other. Among the shared genes, more lipogenic genes were induced upon feeding in BABL/cJ than in C57BL/6J mice. In contrast, in the population of differentially enriched genes, C57BL/6J mice expressed more genes involved in lipid metabolism than BALB/cJ mice. In summary, these results reveal that the two mouse strains used here exhibit several differences in feeding-induced hepatic responses in gene expression, especially in lipogenic genes.

Introduction
The liver plays several essential roles to maintain metabolic homeostasis including the integrated regulation of dietary and nutrient status with hormonal and neural signals. In particular, the liver undergoes dramatic changes in gene expression during the fasted to fed transition with a marked down regulation of gluconeogenic gene expression and induction of lipogenic gene expression in the fed state (1-4). Although most studies on hepatic nutrient/hormonal signals have been focused on these and related lipid and carbohydrate metabolic pathways, the liver expresses approximately 11,000 protein-encoding genes with nearly 3,000 up and down-regulated by the fasting-feeding cycle, which need more in-depth studies.

To date most transcriptomic studies in the liver have used the C57BL/6J mice that are relatively sensitive to diet-induced obesity and compared steady-state differences between mice maintained on a low fat or high-fat diet (5-9). Although a few studies have examined other strains of mice that are relatively resistant to diet-induced obesity, for instance BALB/cJ mice, on low fat versus high-fat diets (6,10,11), there have not been any direct comparisons between strains or the systematic analyses of the temporal changes in gene expression during the normal physiologic fasting-feeding cycle. To examine the dynamic changes in hepatic protein-encoding transcriptome in the fasting and during the postprandial states, we performed deep RNA-seq analyses of C57BL/6J versus BALB/cJ mice. These data indicate marked qualitative and quantitative differences in the normal physiologic responses of the liver between these two strains of mice, with BALB/cJ mice displaying greater extent of responsiveness in lipid processing pathways as compared to C57BL/6J. In contrast, in the population of enriched genes, C57BL/6J mice express more genes involved in lipid metabolism than BALB/cJ mice.

Results

Data validation

As shown in Table 1, in this study, there were 8 groups with 5 mouse replicates for each group, resulting in a total of 40 livers samples from 40 individual mice. The RNA-seq data for all samples have been deposited in the GEO database for public access. The quality of mRNA-seq data from the 40 samples, is summarized in Table S1, with error rates for all samples lower than 0.03% and greater than 90% of reads with error rates of 1/1000 (Q30). The reproducibility and reliability of these datasets are demonstrated in Fig. 1A showing that gene expression reads as FPKM of all samples in group 1 were well correlated. R^2’s between all samples in group 1 and the other remaining 7 groups are listed in Table S2, all with R^2 greater than 0.92. The correlation coefficient matrix of all samples shown in Fig. 1B demonstrates clear patterns, confirming that all the data within each group were well correlated. These data verify that all the mRNA-seq results obtained were reproducible and reliable.

Conformation of mRNA-seq Data by qPCR

To confirm the changes in gene expression determined by RNA-seq over the time course of the fasting/fed transition, we performed 600 qRT-PCR assays for 15 representative genes in the glucoenogenic, lipogenic and fatty acid metabolism pathways from the livers of C57BL/6J and BALB/cJ mice (Fig. S1). The patterns of gene expression determined by qRT-PCR for these 15 transcripts were found to be in excellent agreement with the mRNA-seq data, validating that mRNA-seq is a reliable and quantitative method to determine global gene expression.

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Genome wide gene expression and clustering analysis

We then carried out hierarchical clustering analysis of gene expressions in all 8 groups. Average FPKM for each gene of each group was calculated and log10(FPKM+1) of all genes were plotted in a heatmap (Fig. 2A). This heatmap shows clear differences between two strains of mice. At the various time points, intra-strain groups closely clustered while inter-strain groups separated from each other. This is confirmed by the group distance shown in Fig. 2B, in which Euclidean distances among groups were calculated based on log10(FPKM+1) of all genes.

To quantify the differentially expressed genes, we used DESeq2 R package to compare the expression level of each gene in BALB/cJ mice versus C57BL/6J mice, and plotted -log10(padj) versus log2(fold change) at all four time points (Figs. 2C-2F). Genes with padj < 0.05 (-log10(padj) > 1.3) were viewed as significantly differentially expressed. Genes significantly highly expressed in C57BL/6J are in green and genes significantly highly expressed in BALB/cJ are in red. Genes with expression levels that were not significantly different between these two strains are in blue. Fig. 2G summarizes the percentage of total significantly differentially expressed genes between two strains. In the fasted state, there were about 12% of genes significantly differentially expressed. Following feeding, the percentage of differentially expressed genes increased from 3 to 6 hours, but decreased at 12 hour time point to the level close to that in the fasting state. The highest percentage occurred at the 6 hour time point.

Temporal changes of gene expressions from the fasted to fed state

To examine the impact of feeding on gene expressions, we first plotted -log10(padj) versus log2(fold change) of each gene from the fasted state to the various time points following feeding. Significantly downregulated genes are in green and significantly upregulated genes are in red. Genes with expression levels that were not significantly changed by feeding are in blue (Fig. 3A-3F). The numbers of significantly changed genes in the two strains are summarized in Fig. 3G. Overall the numbers of genes with expression levels significantly changed by feeding first increased and then decreased in a temporal manner. In C57BL/6J mice, the number of feeding changed genes gradually increased over time and reached peak levels approximately 6 hour following feeding and subsequently began to return to the fasted levels. In BALB/cJ mice, the number of feeding changed genes more rapidly reached peak levels approximately 3 hour following feeding. These levels maintained to 6 hour and returned to fasted level at 12 hour postprandial. The number of changed genes was greater in BALB/cJ mice than in C57BL/6J mice.

Classification of genes with the expression levels changed by feeding

Since the maximum changes in the number of gene expression occurred at 6 hour following feeding, we chose this time point as our focus for the rest of studies. At this time point as compared to fasted state, 1,189 genes were significantly downregulated and 882 genes significantly upregulated (padj ≤ 0.05) in C57BL/6J mice. Of those 1,189 significantly downregulated genes, 273 genes were reduced by greater than or equal to 2-fold (log2(fold change) ≤ -1) and were uploaded onto Panther Classification System for GO enrichment analyses. Fig. 4A shows that 22% of those 273 genes participated in metabolic process,
which is further classified as shown in Fig. 4B. This sub-classification shows that 25% involved in cellular metabolic process. Of the genes involved in cellular metabolic process, 20% have a role in lipid metabolism and 5% in amino acid metabolism (Fig. 4C). Similarly, the 882 genes that were significantly upregulated, 185 were increased by 2-fold or more (log2(fold change) $\geq 1$). Fig. 4D shows the classification of those genes in the biological process. While the highest percentage (38%) of genes involved in cellular process, 25% of genes participated in metabolic process, and were further classified into 19% having a role in lipid metabolism and 3% in amino acid metabolism (Fig. 4F).

In comparison, 6 hour postprandial BALB/cJ mice had 1,192 genes significantly downregulated and 1,180 genes significantly upregulated (p_adj $\leq 0.05$). Of those 1,192 significantly downregulated genes, 425 genes were reduced by 2-fold or more. GO enrichment analyses showed that 18% participated in metabolic process while 27% participated in cellular process (Fig. 5A). Sub-classification of genes in metabolic process showed that 25% involved in cellular metabolic process (Fig. 5B), similar to the classification in C57BL/6J mice (Fig. 4B). Further classification of genes in cellular metabolic process resulted in 17% having a role in lipid metabolism and 4% in amino acid metabolism (Fig. 5C). In the case of the 1,180 significantly upregulated genes in BALB/cJ mice, 444 genes were increased by 2-fold or more (log2(fold change) $\geq 1$) and 21% of these genes were classified in metabolic process (Fig. 5D), of which 34% involved in cellular metabolic process (Fig. 5E). Further classification showed that 25% was in lipid metabolism and 3% in amino acid metabolism (Fig. 5F).

Both downregulated and upregulated genes involved in metabolic processes in both strains are listed in Table 2. Although the number of downregulated genes in lipid metabolic processes of BALB/cJ mice is similar to that in C57BL/6J mice, the number of upregulated genes in BALB/cJ mice is 3-fold greater than in C57BL/6J mice. In comparison, the numbers of amino acid metabolic genes in BALB/cJ and C57BL/6J mice are similar.

### Networks formed by genes with the expression levels changed by feeding

To examine the causal relationships among the significantly changed genes and to predict the signal pathways involved, we performed IPA of genes listed in Table 2. Figs. 6A and 6C are networks in lipid metabolism processes for both strains. In both strains, PPARα (Pparα) is the center of both networks formed by direct relationships, whereas Leptin (Lep) is the center of both networks formed by indirect relationships. PPARα is a transcription factor and a major regulator of lipid metabolism in the liver. It is activated under conditions of energy deprivation. Upon feeding it was downregulated in both strains. In both strains, Pnpla2 (a lipase), Cyp4a11 (a monoxygenase), Socs2 (a suppressor of cytokine signaling), and Pck1 (a key gluconeogenic enzyme) were downregulated. The differences between two strains are that Acot1 (acyl-CoA thioesterase) was downregulated in C57BL/6J mice and that Lipin, which encodes a phosphatidate phosphatase, was downregulated in BALB/cJ mice.

The major differences between those two strains occur in the upregulated genes and networks. In addition to upregulated Pparδ, Fasn and Acyl in both strains, Acs3, Cyp2c18, Scd1, Elovl5 and Elovl6 were upregulated in BALB/cJ strain. All of these upregulated genes play important roles in fatty acid and lipid biosynthesis. The networks in amino acid metabolic processes are similar in two strains (Figs. 6B, 6D). In
both strains, \textit{Cpt1} was downregulated, while \textit{Ethe1} was upregulated.

\textbf{Differences in gene expression and classification in two strains of mice}

These data indicate that there are evident differences in liver gene expressions between C57BL/6J and BALB/cJ mice. To verify the differences, we directly compared gene expressions in those two strains. Here average FPKM of each gene in the same group was used. If average FPKM \( \geq 1 \), we viewed this gene as expressed in the group. Otherwise, we viewed it as unexpressed in the group. Gene populations of two strains are shown in the Venn diagrams (Fig. 7A).

In both fasted and fed states, the numbers of shared genes by both strains were in the range of 10,139 to 10,389. The numbers of genes uniquely expressed in C57BL/6J mice were in the range of 383 to 511, whereas the numbers of unique genes expressed in BALB/cJ mice were in the range of 403 to 602, slightly higher than that in C57BL/6J mice.

In the fasted state, 479 unique genes were expressed in C57BL/6J mice and 519 genes were expressed in BALB/cJ mice. We uploaded those two sets of genes onto Panther Classification System separately and performed GO enrichment analyses, respectively. Classifications of genes at all three levels (biological process, metabolic process and cellular metabolic process) are shown in Fig. S2. There were differences, although subtle, at the biological process level. Obvious differences could be observed at the metabolic process level, as 35\% genes in C57BL/6J mice involved in cellular metabolic process and only 26\% of genes in BALB/cJ mice involved in cellular metabolic process. Greater differences occurred at the level of cellular metabolic process, which are also shown in Figs. 7B and 7C. In C57BL/6J mice, the highest percentage of genes (32\%) were involved in lipid metabolism (Fig. 7B), whereas in BALB/cJ mice there were only 6\% of genes in this category. However, there was no gene in this subgroup in C57BL/6J mice involved in either carbohydrate or amino acid metabolism, while there were 6\% and 5\% of genes in BALB/cJ mice involved in carbohydrate metabolism and amino acid metabolism, respectively.

At the 6 hour postprandial time point, 396 genes were uniquely expressed in C57BL/6J mice and 602 genes were expressed in BALB/cJ mice. Classifications of genes at all three levels (biological process, metabolic process and cellular metabolic process) are shown in Fig. S3. Similar to the classifications in the fasted state, there were differences in gene classifications at both levels of biological and metabolic processes. Much greater differences were found at the cellular metabolic process. At this level, 36\% of genes in C57BL/6J mice, while only 12\% in BALB/cJ, participated in lipid metabolic process (Figs. 7D, 7E). Again, there was no gene in this subgroup in C57BL/6J mice involved in either carbohydrate or amino acid metabolism, whereas there were 2\% and 2\% of genes in BALB/cJ mice participated in carbohydrate metabolism and amino acid metabolism, respectively (Figs. 7D, 7E).

To quantify the gene expression differences between those two strains, we calculated gene expression fold changes in BALB/cJ mice versus that in C57BL/6J mice and \( p_{\text{adj}} \) values to determine the differences and the significance of differences, respectively. Volcano plots of -log10(\( p_{\text{adj}} \)) versus log2(fold change) are shown in Figs. 8A and 8D. The genes with expression levels significantly higher in C57BL/6J mice than in BALB/cJ mice are plotted in green, whereas genes with expression levels significantly higher in
BALB/cJ mice than in C57BL/6J mice are plotted in red.

In the fasted state, there were 736 genes with expression levels significantly higher in C57BL/6J mice than in BALB/cJ mice and 608 genes with expression levels significantly higher in BALB/cJ mice than in C57BL/6J mice. The number of genes in C57BL/6J and BALB/cJ mice livers with a log2(fold change) $\leq -1$ and a log2(fold change) $\geq 1$ was 551 and 440, respectively. At both biological and metabolic process levels, the classifications of genes in C57BL/6J and BALB/cJ mice were very similar (Fig. S4). However, there were significant differences in classifications at the cellular metabolic process between those two strains (Figs. 8B, 8C). In contrast, the percentage of genes participating in amino acid process in C57BL/6J mice was much higher than that in BALB/cJ mice (29% versus 18%). In contrast, the percentage of genes participating in amino acid process in C57BL/6J mice was lower than that in BALB/cJ mice (2% versus 3%). Uniquely, 2% of genes in this sub-group in BALB/cJ mice participated in carbohydrate metabolism, while no gene in this sub-group in C57BL/6J mice participated in carbohydrate metabolism.

At the 6 hour time point, there were 1,632 genes with expression levels significantly higher in C57BL/6J mice than in BALB/cJ mice, and 1,470 genes with expression levels significantly higher in BALB/cJ mice than in C57BL/6J mice. The number of genes in C57BL/6J and BALB/cJ mice livers with a log2(fold change) $\leq -1$ and a log2(fold change) $\geq 1$ was 564 and 452, respectively. As shown in Fig. S5, at both biological and metabolic process levels, the classifications of genes in C57BL/6J and BALB/cJ mice were very similar. Yet, there were differences in classifications at the cellular metabolic process between those two strains (Figs. 8E, 8F). The percentage of genes participating in lipid process in C57BL/6J mice was much higher than that in BALB/cJ mice (27% versus 16%). The percentage of genes participating in amino acid was also higher in C57BL/6J than in BALB/cJ mice (3% versus 2%), which is in contrary to that in the fasted state. As to the percentage of genes participating in carbohydrate metabolisms, the situation is the same as in the fasted state.

Enriched genes in either strain from the volcano plots (Figs. 8A, 8D) involved in metabolic processes are listed in Table 3. In lipid metabolism, the number of enriched genes in C57BL/6J mice is about 3-fold of that in BALB/cJ mice in both fasted and fed states. In amino acid metabolic processes, the numbers of enriched genes in C57BL/6J and BALB/cJ mice were similar. In carbohydrate metabolism, while Synj2 was enriched in BALB/cJ mice, there was no gene enrichment in C57BL/6J mice.

**Discussion**

Nutrients are essential for fueling energy production, providing intermediates for macromolecular synthesis and regulation of enzymatic activities. The uptake of macronutrients in the forms of food and drink are digested and metabolized by biological processes, which are dictated through allosteric regulated signaling pathways and via transcriptional signals to control gene expression. Liver is a major organ controlling metabolism of nutrients to maintain energy homeostasis by regulating the dynamics of lipogenesis, lipolysis, gluconeogenesis, glycogenolysis, and glycolysis (12). To examine the changes in liver gene expression regulated during the fasted to fed transitions, we carried out a non-hypothesis-driven study of C57BL/6J and BALB/cJ mice by deep mRNA-seq. Here we report unbiased and reliable information on how gene expression levels changed during the fed/fasting transition.
The importance of the data presented in this manuscript is: i) to provide a reliable normal physiologic liver gene expression dataset that can be used as a reference for the normal controls of other studies; and ii) to establish the biologic variabilities between mouse strains as critical factors in understanding the changes in physiologic and pathophysiologic states.

Overall feeding caused significant, substantial and transient changes in gene expressions in both C57BL/6J and BALB/cJ mice (Fig. 3). As expected and well-established, gluconeogenic gene expression was suppressed by feeding while lipogenic gene expression was increased. However, many genes outside of these established pathways were also dynamically regulated. In fact, of the 2,071 regulated gene transcripts in C57BL/6J mice, 42.6% (882) increased and 57.4% decreased (1,189) at 6 hour postprandial. In BALB/cJ mice, 2,372 genes were regulated, of which 49.7% (1,180) were increased and 50.3% (1,192) were decreased. Although these genes represent a variety of biological process, the majority of transcripts fall within the areas of biological regulation, cellular and metabolic processes (Figs. 4, 5). Among metabolisms of three major types of macronutrients (carbohydrate, protein and lipid), feeding exerted the greatest impact on lipid metabolism.

There are also significant differences in the responses to feeding between C57BL/6J and BALB/cJ mice. Within the categories of cellular metabolic processes, feeding in BALB/cJ mice resulted in the suppression of 31% and induction of 31% of genes involved in cellular macromolecular metabolic pathways. In contrast, in C57BL/6J mice feeding suppressed 9% and induced 32% of genes in cellular macromolecular metabolic pathways. In the case of lipid metabolic process, feeding suppressed 20% and induced 19% of genes in C57BL/6J mice (Fig. 4). In BALB/cJ mice these percentages were 17% and 25%, respectively (Fig. 5). In feeding induced genes there were more genes involved in lipid metabolism in BALB/cJ mice than in C57BL/6J mice (Table 2). However, in gene population enriched in either strain, there were more genes involved in lipid metabolism in C57BL/6J mice than in BALB/cJ mice (Table 3).

Detailed analyses revealed that among those 2071 genes significantly changed by feeding in C57BL/6J mice (Fig. 2G), there were only 35 genes enriched in C57BL/6J mice only (g11 in Fig. 7A), i.e. there was only 1.7% of total feeding changed genes was uniquely expressed in C57BL/6J mice. Similarly, among those 2,372 genes significantly changed by feeding in BALB/cJ mice, there were only 66 genes enriched in BALB/cJ mice only (g15 in Fig. 7A), i.e. only 2.8% of total feeding changed genes was uniquely expressed in BALB/cJ mice. These data indicate that the majority of genes with expression levels significantly changed by feeding are shared genes by these two strains and in this shared gene population, there were more genes participating in lipid metabolism in BALB/cJ mice than in C57BL/6J mice.

Among 396 genes enriched in C57BL/6J at 6 hour postprandial, there were only 35 genes with their expression levels significantly changed by feeding, i.e. there were only 8.8% of genes changed by feeding. Among 602 genes enriched in BALB/c mice, there were only 66 genes with their expression levels significantly changed by feeding, i.e. there were only 10.9% of genes changed by feeding. In the enriched gene population in both strains, there were about 90% of genes with expression levels not significantly changed by feeding. In these respective enriched populations, there were more genes...
participating in lipid metabolism in C57BL/6J mice than in BALB/cJ mice. Several members of major urinary protein (MUP) family are uniquely enriched in C57BL/6J mice (Table 3). MUPs are members of a large family of low-molecular weight proteins known as lipocalins (13), which transport small hydrophobic molecules including steroids and lipids. It has been shown that MUPs are associated with the regulation of energy expenditure (14) and that they restrict glucose production by directly inhibiting lipogenic genes in liver (15). In the genes uniquely enriched in BALB/cJ mice, some of them (6%) (Fig. 7C) participate in carbohydrate metabolism.

Moreover, among the gene shared by both strains, in the fasted state there was about 7% of total number of genes highly expressed in C57BL/6J and 6% of total number of genes highly expressed in BALB/cJ mice. The percentage of sum of differentially expressed genes is 11.5% (Fig. 2G). Feeding exacerbated these differences. At 6 hour following feeding, the percentage of total differentially expressed genes tripled (Fig. 2G). The livers of C57BL/6J and BALB/cJ also responded to feeding with distinct patterns. Upon feeding, there were a larger number of genes that more rapidly responded in BALB/cJ mice compared to C57BL/6J mice, and the changes dissolved faster in BALB/cJ mice than in C57BL/6J mice (Fig. 3). These data suggest that BALB/cJ mice are more sensitive to feeding and have greater capacity to resolve feeding induced changes in gene expressions more completely, i.e. the changes were not sustained. This is perhaps one of the reasons that ultimately BALB/cJ mice are more resistant to diet induced metabolic/physiologic changes, for instance, high fat diet induced obesity (6,11), even though their genes respond to diet at a faster speed and to a greater extent.

In summary, this study provides unbiased, reproducible, reliable and comprehensive information on the liver transcriptomes of two strains (C57BL/6J and BALB/cJ) of mice in fasted and fed states that is now made available for public access. These data demonstrate that both strains of mice respond in an overall similar pattern of changes in gene expression but with unique and distinct differences in the rate, extent and combination of various gene regulator subsets controlling cellular metabolic processes. Given these differential aspects of gene expression, these likely account for the differential sensitivity of these two mouse strains to the effects of diet induced obesity and insulin resistance. Moreover, these data also demonstrate the presence of unique subsets of genes 4% - 6% that are exclusively expressed in one strain, but not the other (Fig. 7A). Further studies will now be needed to explore the molecular basis and functional significance of these differentially expressed genes.

**Experimental procedures**

**Mice**

All following experimental procedures done with mice were approved by the Institutional Care and Use Committee at the Albert Einstein College of Medicine in accordance with the “Guide for the Care and Use of Laboratory Animals” published by the National Institute of Health. Wild type C57BL/6J and BALB/cJ mice at age of 8 weeks were purchased from the Jackson Laboratory. One day after arrival, they were trained for 1 week for regulated fasting (from 10 pm to 7 pm) and feeding of low-fat chow diet (PicoLab Mouse Diet 5053, 10 % of calories from fat and 64.5% of calories from carbohydrates) (from 7 pm to 10 pm). After one week of training, mice were fasted from 10 pm the day before to 7 pm the day of the experiment. The mice were allowed to eat *ad libitum* until 10 pm at which point...
food was removed. Mice were sacrificed at 7 pm as fasted controls and at 10 pm (3 h postprandial), 1 am (6 h postprandial) and 7 am (12 h postprandial) following the initiation of feeding, respectively. Livers were harvested and snap frozen in liquid nitrogen. This protocol resulted in the generation of 8 groups of mice with 5 replicate mice per group (Table 1).

**Total RNA extraction**

Approximately 5mg of frozen liver powder was completely dissolved in 500 µL of Trizol reagent at room temperature. One hundred µL of chloroform was added to the Trizol-liver mixture. The mixture was kept at room temperature for 2 min and subsequently centrifuged at 12,000 rpm for 15 min at 4°C. The top layer (aqueous) was isolated and mixed with 100% ethanol at 1:1 ratio. Up to 700 µL of the mixture was transferred onto RNeasy mini column from Qiagen kit (catalogue No. 74106). Thereafter the rest of procedure of the same protocol part 1 from step 3 to the end was followed to finish total RNA extraction. All total RNA samples were sent to Novogene Corporation Inc in Sacramento, CA 95826 for genome wide mRNA sequencing. All samples passed through the following three steps before library construction: 1) Nanodrop for RNA purity check, OD260/OD280 in a range of 1.95 – 2.05; 2) agarose gel electrophoresis for RNA integrity and potential contamination; and 3) Agilent 2100 for confirming RNA integrity.

**Quantitative RT-PCR**

SuperScript VILO cDNA synthesis kit (Invitrogen) and PowerUp SYBR Green Master Mix (Applied Biosystems) were used for cDNA strand synthesis from purified total RNA and qPCR StepOnePlus Real-time PCR system (Applied Biosystems) was used for quantification. Samples were normalized to Ppib gene to determine relative mRNA levels. The primer sequences used in this study are listed in Table S3.

**Library construction and sequencing**

Library construction and sequencing were conducted by Novogene. Briefly, mRNA was purified from total RNA by using poly-T oligo attached magnetic beads and fragmented. cDNA was synthesized. cDNA fragments with 150 bp, paired and two-ended reads were generated and selected. Libraries (30,000,000 fragments / library) were constructed and fed into Illumina machines.

**Data Processing**

STAR v2.5 was used to align clean data to mm10 reference genome with parameter mismatch = 2 (16). HTSeq v 0.6.1 was used to count the read numbers mapped of each gene (17). Differential expression analysis between two conditions/groups was performed using the DESeq2 R package (v 2_1.6.3), which generated log2 fold change and adjusted p value (padj) (18). Fragments per kilobase of transcript per million mapped reads (FPKMs) were calculated to normalize read counts. Based on FPKM of each gene of each sample, square of Pearson coefficients (R²’s) were calculated to show correlations between samples and reproducibility. FPKM of each gene was averaged in each group, and log10(FPKM+1) were used to generate a heatmap, in which groups and genes were clustered, respectively. Euclidean distance between groups were also calculated based on log10(FPKM+1) of all genes. Differentially expressed genes were annotated and classified with Gene Ontology (GO) terms using Panther Classification system [15], and networks were generated using Ingenuity Pathway Analysis (IPA) [16].
Conflicts of interest: The authors declare that they have no conflicts of interest with the contents of this article.
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Footnotes
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Abbreviations
FPKMs, Fragments per kilobase of transcript per million mapped reads; GO, Gene Ontology; IPA, Ingenuity Pathway Analysis; LEP, leptin.
Figure Legends.
Figure 1. Correlations of liver gene expressions of replicate samples. (A) Correlations of gene expressions between each sample with the other 4 samples in the control group (fasted C57BL/6J). (B) Correlation matrix of all 40 individual samples.

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Figure 3. Feeding induced differentially expressed genes during the time course of 12 hours in two strains. (A-F) Volcano plots of -log10(padj) versus log2(fold change) at various time points following feeding. padj and log2(fold change) of all genes in C57BL/6J mice (A-C) or BALB/cJ mice (D-F) at 3 (A, D), 6 (B, E), or 12 (C, F) -hour time point following feeding versus corresponding genes in fasted state were calculated using R package DESeq2. Feeding significantly suppressed genes are in green. Feeding significantly induced genes are in red. Genes that were not significantly changed by feeding are in blue. (G) Bar graph of number of feeding significantly changed genes.

Figure 4. Classification of liver genes with expression levels significantly suppressed with log2(fold change) ≤ -1 (A-C) or induced with log2(fold change) ≥ 1 (D-F) by feeding in C57BL/6J mice according to biological processes with Gene Ontology (GO) terms using Panther Classification system.

Figure 5. Classification of liver genes with expression levels significantly suppressed with log2(fold change) ≤ -1 (A-C) or induced with log2(fold change) ≥ 1 (D-F) by feeding in BALB/cJ mice according to biological processes with Gene Ontology (GO) terms using Panther Classification system.

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classifications of genes expressed uniquely in C57BL/6J (g11) (D) or uniquely in BALB/cJ (g15) (E) mice at 6 hour following feeding.

Figure 8. Differentially expressed liver genes in two strains of mice in fasted and fed states and sub-classifications of genes with expression levels significantly different between two strains of mice. (A, D) Volcano plots of -log10(p_{adj}) versus log2(fold change) in fasted state (A) and at 6 hour postprandial (D). p_{adj} and log2(fold change) were calculated using R package DESeq2. Significantly highly expressed genes in C57BL/6J mice are in green. Significantly highly expressed genes in BALB/cJ mice are in red. Genes with expression levels not significantly different between two strains are in blue. (B-C, E-F) Sub-classifications of genes significantly highly expressed in C57BL/6J (B, E) or in BALB/cJ (C, F) mice in fasted state (B, C) or at 6 hour postprandial (E, F).

Supporting Information.
Figure S1. Gene expression changes determined by qRT-PCR for selected transcripts identified by mRNA-seq analyses.

Figure S2. Classifications of liver genes uniquely expressed in C57BL/6J (A-C) or BALB/cJ (D-F) mice in fasted state according to biological processes with Gene Ontology (GO) terms using Panther Classification system.

Figure S3. Classifications of liver genes uniquely expressed in C57BL/6J (A-C) or BALB/cJ (D-F) mice at 6 hour postprandial according to biological processes with Gene Ontology (GO) terms using Panther Classification system.

Figure S4. Classifications of liver genes significantly highly expressed in C57BL/6J (A-C) or BALB/cJ (D-F) mice with log2(fold change) ≤ -1 or log2(fold change) ≥ 1 in fasted state according to biological processes with Gene Ontology (GO) terms using Panther Classification system.

Figure S5. Classifications of liver genes significantly highly expressed in C57BL/6J (A-C) or BALB/cJ (D-F) mice with log2(fold change) ≤ -1 or log2(fold change) ≥ 1 at 6 hour postprandial according to biological processes with Gene Ontology (GO) terms using Panther Classification system.
Table 1. Information on groups of samples.

| Group | Strain   | Treatment | Time following feeding (hour) | n  |
|-------|----------|-----------|-------------------------------|----|
| g1    | C57BL6/J | Fasted    | 0                            | 5  |
| g3    | C57BL6/J | Fed       | 3                            | 5  |
| g11   | C57BL6/J | Fed       | 6                            | 5  |
| g19   | C57BL6/J | Fed       | 12                           | 5  |
| g2    | BALB/c   | Fasted    | 0                            | 5  |
| g7    | BALB/c   | Fed       | 3                            | 5  |
| g15   | BALB/c   | Fed       | 6                            | 5  |
| g23   | BALB/c   | Fed       | 12                           | 5  |
Table 2 Classification of genes significantly changed by feeding in two strains of mice into different metabolic processes.

| Metabolic Process | Feeding Induced Changes in Gene Expressions |         |         |
|-------------------|-------------------------------------------|---------|---------|
|                   | C57BL6/J                                  | BALB/c  |         |
|                   | Gene Name | Log2(Fold Change) | p_adj | Gene Name | Log2(Fold Change) | p_adj |
| Lipid             | Gpcpd1    | -2.7728           | 9.20E-19 | Soc2      | -2.79           | 6.3E-08 |
|                   | Cish      | -2.3285           | 6.97E-11 | Cyp4a32   | -2.6369         | 6.82E-25 |
|                   | Soc2      | -2.2685           | 6.91E-12 | Pnpla2    | -1.8954         | 1.72E-08 |
|                   | Acot3     | -1.579            | 9.25E-07 | Pck1      | -1.8644         | 2.97E-05 |
|                   | Ppara     | -1.373            | 1.89E-05 | Apoa5     | -1.6325         | 7.75E-19 |
|                   | Cyp4a32   | -1.3553           | 1.11E-07 | Ppara     | -1.613          | 2.28E-06 |
|                   | Pnpla2    | -1.2806           | 8.30E-08 | Brc1      | -1.5072         | 0.000476 |
|                   | Pck1      | -1.1051           | 0.030689 | Lpin2     | -1.4706         | 1.24E-07 |
|                   | Cers6     | -1.0876           | 0.000106 | Acsl5     | 1.0773          | 0.001533 |
|                   | Itga5     | 0.62872           | 0.008832 | Scd1      | 1.2232          | 0.008949 |
|                   | Acnat2    | 1.1885            | 0.001069 | Acsl3     | 1.4191          | 1.65E-10 |
|                   | Ptgds     | 1.2158            | 0.003509 | Neu2      | 1.4268          | 2.8E-06  |
|                   | Acly      | 1.2355            | 6.77E-05 | Elov15    | 1.4309          | 1.29E-05 |
|                   | Ppard     | 1.7707            | 1.17E-13 | Lcn12     | 1.4539          | 0.014005 |
|                   | Elov13    | 2.0721            | 2.04E-07 | Aacs      | 1.4545          | 2.1E-05  |
|                   |           |                   |         | Acnat2    | 1.5257          | 2.8E-06  |
|                   |           |                   |         | Scd3      | 1.6095          | 0.002007 |
|                   |           |                   |         | Ptgds     | 1.7043          | 0.001283 |
|                   |           |                   |         | Elov13    | 1.7081          | 0.003178 |
|                   |           |                   |         | Ptgds     | 1.8201          | 3.55E-05 |
|                   |           |                   |         | Cyp2c55   | 1.8654          | 7.05E-07 |
|                   |           |                   |         | Acss2     | 1.8823          | 2.99E-19 |
| Amino Acid        |           |                   |         | Ppard     | 2.0301          | 8.38E-22 |
|                   | Cpt1a     | -1.7772           | 9.64E-11 | Chac1     | -3.0505         | 2.39E-13 |
|                   | Chac1     | -1.304            | 0.007439 | Cpt1a     | -1.8878         | 3.24E-23 |
|                   | Eth1      | 1.6048            | 5.57E-10 | Ethel1    | 1.2171          | 1.06E-09 |
|                   |           |                   |         | Ptgds     | 1.7043          | 0.001283 |
Table 3 Classification of genes enriched in C57BL6/J or BALB/c mice into different metabolic processes.

| Metabolic process | C57BL6/J  | BALB/c  | Fed-6h | C57BL6/J  | BALB/c  |
|-------------------|-----------|---------|--------|-----------|---------|
| Lipid             | Mup18     | Cyp4a32 | Mup18  | Cyp4a32   |         |
|                   | Mup1      | Cyp2a22 | Mup1   | Cyp2a22   |         |
|                   | Cyp2c69   | Cyp2b13 | Cyp2c69| Cyp2b13   |         |
|                   | Cyp2c55   | Synj2   | Cyp2c23| Synj2     |         |
|                   | Cyp2c23   | Apoa4   | Cyp2d11| Bco1      |         |
|                   | Cyp2d11   | Bco1    | Cyp4f3 | Scd3      |         |
|                   | Mup17     | Cyp2c67 | Mup17  | Cyp2c29   |         |
|                   | Mup10     | Cyp2a4  | Mup10  | Cyp2a4    |         |
|                   | Mup6      | Dgki    | Mup6   | Elov15    |         |
|                   | Acss2     | Cyp2c39 | Cyp4a31| Cyp2c39   |         |
|                   | Scd1      |         | Mup14  |           |         |
|                   | Mup14     |         | Mup7   |           |         |
|                   | Mup7      |         | Mup20  |           |         |
|                   | Mup20     |         | Mup19  |           |         |
|                   | Mup19     |         | Cyp4a12b|         |         |
|                   | Cyp4a12b  |         | Cish;ortholog|       |         |
| Carbohydrate      | Mup8      |         | Mup8   |           |         |
|                   | Acsm2     |         | Lpgat1 |           |         |
|                   | Lpgat1    |         | Enpp2  |           |         |
|                   | Enpp2     |         | Lcn2   |           |         |
|                   | Elov16    |         | Mup11  |           |         |
|                   | Mup11     |         | Obp2a  |           |         |
|                   | Fasn      |         | Cyp2c68|           |         |
|                   | Cyp2c68   |         | Cyp2c50|           |         |
|                   | Acsl3     |         | Cyp2c37|           |         |
|                   | Cyp2c50   |         | Mup6   |           |         |
|                   | Cyp2c37   |         | Mup15  |           |         |
|                   | Mup6      |         | Socs2  |           |         |
|                   | Aacs      |         |        |           |         |
|                   | Dgkh      |         |        |           |         |
|                   | Mup15     |         |        |           |         |
| Amino acid        | Vnn3      | Ahcy    | Vnn3   | Ahcy      |         |
|                   | Vnn1      | Dio3    | Ahcy   | Vnn1      |         |
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