A high fat diet with a high C18:0/C16:0 ratio induced worse metabolic and transcriptomic profiles in C57BL/6 mice

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Abstract
Background: Differential effects of individual saturated fatty acids (SFAs), particularly stearic acid (C18:0) relative to the shorter-chain SFAs have drawn an interest for more accurate nutritional guidelines. But specific biologic and pathologic functions that can be assigned to particular SFAs are very limited. The present study was designed to compare changes in metabolic and transcriptomic profiles in mice caused by high C18:0 diet and high palmitic acid (C16:0) diet. Methods: Male C57BL/6 mice were assigned to a normal fat diet (NFD), a high fat diet with high C18:0 / C16:0 ratio (HSF) or an isocaloric high fat diet with a low C18:0 / C16:0 ratio (LSF) for 10 weeks. Oral glucose tolerance test, 72h-energy expenditure measurement and CT scan of body fat were done before sacrifice. Fasting glucose and lipids were determined by an auto-biochemical analyzer. Blood insulin, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured by enzyme linked immunosorbent assay methods. Free fatty acids (FFAs) profiles in blood and liver were determined by using Gas Chromatography-Mass Spectrometry. Microarray analysis was applied to investigate changes in transcriptomic profiles in liver. Pathway analysis and Gene Ontology analysis were applied to describe the roles of differentially expressed mRNAs. Results: Compared with NFD group, body weight, body fat ratio, fasting blood glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), triglyceride, IL-6, serum and liver FFAs including total FFAs, C16:0 and C18:0 were increased in both high fat diet groups, which were much higher in HSF group than those in LSF group. There were much more differentially expressed IncRNAs and mRNAs in HSF mice than those in LSF mice, with distinguishable IncRNA, microRNA, and mRNA expression profiles between these two groups. And some biological functions and pathways, except for energy metabolism regulation, were identified that differentially expressed mRNAs between HSF group and LSF group probably involved in. Conclusion: The high fat diet with a high C18:0/C16:0 ratio induced much severe glucose and lipid metabolic disorders and inflammation, and affected more IncRNAs and mRNAs expression than an isocaloric low C18:0/C16:0 ratio diet in mice.

Introduction
Excessive intake of a high fat diet commonly leads to obesity, abnormal blood lipids profile, and even
insulin resistance [1, 2]. Increased plasma saturated fatty acids (SFAs), induced by a high fat diet, are mainly responsible for these pathologic progresses [3–5]. Therefore, control of dietary SFAs intake has been widely applied to prevent metabolic risks and cardiovascular diseases [6, 7].

Recently, differential effects of individual SFAs, particularly stearic acid (18:0) relative to the shorter-chain SFAs have drawn an interest for more accurate nutritional guidelines [8]. But specific biologic and pathologic functions that can be assigned to particular SFAs are very limited and basic researches need to be carried out [9]. Palmitic acid (C16:0) and C18:0 are the most common and abundant long chain SFAs in food and human body, and C16:0 can be conversed to C18:0 in the body [10]. A high fat diet with an increase in C18:0 has been found to induce a metabolic state favoring lower oxidative metabolism and severe hepatic insulin resistance in mice, compared with an isocaloric high fat diet [11]. In mice deficient for Elovl6, a gene encoding the elongase that catalyzes the conversion of C16:0 to C18:0, the level of C18:0 decreased while the level of C16:0 increased in serum and liver, and the mice became obese and developed hepatosteatosis, but were protected from insulin resistance when fed a high-fat diet [12]. These studies collectively suggest that dietary fatty acids composition rather than dietary fat content contribute more to insulin sensitivity.

The longer chain length of C18:0 probably contributes to the difference in its effect on insulin resistance as compared to C16:0. On one hand, C18:0 as compared to C16:0 and C14:0 is poorly incorporated into triacylglycerol in the liver [13]. On the other hand, the efficiency of SFAs oxidation has been demonstrated to be related to its chain length: the longer the chain length of the SFAs, the slower the rate of oxidation [14, 15]. Thus, when C18:0 increases in the body, intermediate lipids metabolites, such as diacyl-glycerols and ceramides are more likely to accumulate, which have been reported to interfere with insulin signaling pathway [16–19].

As the structure and metabolism between C16:0 and C18:0 are not exactly same, they may play many different roles in the body except for some similar functions [20]. In our study, the mice were fed isocaloric high fat diets with different C18:0/C16:0 ratios to induce obesity and insulin resistance. Metabolic parameters in serum and liver were determined and microarray analysis of the liver was done to investigate the changes in IncRNA, micorRNA and mRNA expressions. We aimed to
systematically compare the different effects between C16:0 and C18:0 at metabolic and even gene transcriptional level in mice, in order to provide more details in their pathophysiological activities.

Materials And Methods

Animals

Eight-week-old male C57BL/6 mice (18-22 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The mice were housed individually in pathogen-free metabolic cages in an environmentally controlled room at 21 ± 2 °C and 50 ± 5 % humidity with 12-h light/dark cycles; lights were on at 0700 h and off at 1900 h. The study was approved by the Institutional Animal Care Committee from Harbin Medical University and conducted in accordance with the University guidelines for the care and use of laboratory animals.

Treatments

The mice had ad libitum access to water and standard laboratory chow (Keaoxieli, Beijing, China). After acclimation for 1 week, the mice were randomly divided into 3 groups (n = 20 for each group). The mice in normal fat diet group (NFD) were fed a normal standard laboratory chow diet (15% of the energy in the form of fat). The mice in high C18:0/C16:0 group (HSF) were fed a high fat diet (36% of the energy in the form of fat) with a C18:0/C16:0 ratio of 1:2. The mice in low C18:0/C16:0 group (LSF) were fed a high fat diet (36% of the energy in the form of fat) with a C18:0/C16:0 ratio of 1:8. The fatty acids composition of high fat diets was determined by a Gas Chromatography-Mass Spectrometry. Food intake and body weight were measured daily and weekly, respectively. At the end of the 10th week, the blood was collected directly from the heart by cardiac puncture under deep anesthesia. Livers and pancreas were weighed and stored at -80°C for further use.

Energy expenditure measurement

Energy expenditure was assessed using the TSE PhenMaster (TSE Systems GmbH, Bad Homburg, Germany). At the end of the 9th week, six mice were randomly picked from each group and placed in the metabolic chambers and acclimated for 24 h. Energy expenditure analysis was performed for consecutive 72 hours. Oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were measured at 27-min intervals. VO₂ and VCO₂ values were in mL/h. The total energy expenditure (EE)
was calculated from the sum of fat and carbohydrate oxidation. Respiratory exchange rate (RER) was calculated as $VCO_2/VO_2$. Fat and carbohydrate oxidation rates were calculated according to the following equation [11]:

Carbohydrate oxidation rate (kcal/h) = $(4.585 \times VCO_2) - (3.226 \times VO_2) \times 4/1000$

Fat oxidation rate (kcal/h) = $(1.695 \times VO_2) - (1.701 \times VCO_2) \times 9/1000$

**Body fat distribution analysis**

At the end of the 10th week, six mice were randomly picked from each group and were scanned with a Latheta LCT-200 (Hitachi, Japan) in a prone position to image the fat distribution. Visceral fat, subcutaneous fat, and muscle mass were calculated based on the scanned CT value. Total fat ratio = total fat mass/(total fat mass + muscle mass) x 100%. Liver fat ratio = (muscle CT value - liver CT value)/(muscle CT value - fat CT value) x 100%.

**Oral glucose tolerance test (OGTT) and homeostasis model assessment of insulin resistance (HOMA-IR) index**

At the end of the 10th week, OGTT was done in each group before sacrifice. Following a 15-hour fasting, 25% glucose (w/v) was given by gavage at a dose of 0.2ml/10g·BW. Blood sample was collected from the tail vein at 0, 30, 60, 90 and 120 min. Insulin resistance was estimated by HOMA-IR based on fasting glucose and insulin levels as follows: HOMA-IR = fasting glucose (mmol/L) x fasting insulin (mU/L)/ 22.5.

**Measurement of blood biochemical parameters and inflammatory markers**

Fasting blood glucose, total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) were determined with an auto-biochemical analyzer (Hitachi 7100, Japan). Blood insulin (R&D Systems, Abnova, USA), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured by enzyme linked immunosorbent assay methods (Sigma-Aldrich, Beijing).

**Measurement of fatty acids profile in serum and liver**

The levels of fatty acids in serum and liver of mice were detected by a Gas Chromatography-Mass
Spectrometry (TRACE GC/PolarisQ MS, Thermo Finnigan, San Jose, USA) as previously described [21].

**Detection of cell ultra-structural changes in liver and pancreas**

Liver and pancreas tissues were fixed by 2.5% glutaraldehyde solution and 1% osmium acid solution. After dehydration by ethanol and acetone, tissues were embedded in acetone and embedding solvent. Ultra-thin sections were prepared by Reichert-Jung Ultracut E ultramicrotome and stained with uranyl acetate and lead citrate solution. Transmission electron microscopy was employed to observe and analyze the changes of mitochondria in liver and insulin granules in pancreas.

**Analysis of IncRNA/miRNA/mRNA expression in liver**

Gene expression in liver of mice (n = 3 per group) was determined by Kangcheng Bio-tech Inc (Shanghai, China). Total RNA from each sample was quantified using the NanoDrop ND-1000 and the RNA integrity was assessed using standard denaturing agarose gel electrophoresis. For microarray analysis, Agilent Array platform was employed. The sample preparation and microarray hybridization were performed based on the manufacturer’s standard protocols with minor modifications. Agilent Feature Extraction software (version 11.0.1.1) was used to analyze the acquired array images. Quantile normalization and subsequent data processing were performed using the GeneSpring GX v11.5.1 software package (Agilent Technologies). Differentially expressed genes with statistical significance were identified through Volcano Plot filtering. For IncRNAs, those with fold changes ≥2.0 and $P$-value < 0.05 were considered significantly different. For microRNAs and mRNAs, those with fold changes ≥1.5 and $P$-value < 0.05 were considered significantly different. Hierarchical Clustering was also performed to show the distinguishable IncRNAs, microRNAs and mRNAs expression pattern among samples. Pathway analysis and Gene Ontology analysis (GO analysis) were applied to describe the roles of these differentially expressed mRNAs played in these biological pathways or GO terms.

**Statistical analysis**

All data were expressed as the mean ± SEM. Comparisons between groups were performed using the t test or ANOVA with a Bonferroni post hoc test of pairwise comparisons where appropriate. $P < 0.05$ was considered to be statistically significant.

**Results**
The effect of dietary C18:0/C16:0 ratios on body weight and body fat content

At the end of the 6th week, the body weight of mice in HSF group became higher than that in LSF group (Fig. 1a). No significant changes in diet intake were observed among the three groups (data not shown). CT images of body fat distribution showed the richest visceral and subcutaneous fat were in the HSF group, followed by the LSF group, which were much higher than those in NFD group (Fig. 1b). The total body fat ratio and liver fat ratio were also higher in HSF group than those in LSF group (Fig. 1c, d).

The effect of dietary C18:0/C16:0 ratios on energy metabolism

There was no difference in energy expenditure among the 3 groups (Additional file 1: Fig. S1a). Carbohydrate oxidation in HSF and LSF groups was significantly lower, while fat oxidation was much higher, compared with those in NFD group (Fig. S1b, c). Moreover, fat oxidation in LSF group was significantly higher than that in HSF group, indicating a lower oxidation rate of C18:0 than C16:0. There was a trend of decrease in RER in HSF and LSF groups, but it did not reach a statistical significance, compared with that in NFD group (Fig. S1d).

The effect of dietary C18:0/C16:0 ratios on blood biochemical parameters, glucose tolerance and inflammatory markers

Mice in both high fat diet groups exhibited decreased glucose tolerance, but the glucose tolerance was impaired much severe in HSF group than that in LSF group (Additional file 2: Fig. S2). Fasting blood glucose, insulin, TC, TG, HDL-c, LDL-c, TNF-α, IL-6 and HOMA-IR in high fat diet groups were significantly higher than those in NFD group, and glucose, insulin, HOMA-IR, TG and IL-6 levels in HSF group were higher than those in LSF group (Additional file 3: Table S1).

The effect of dietary C18:0/C16:0 ratios on fatty acids profile in serum and liver

The levels of serum total fatty acids, saturated fatty acids, unsaturated fatty acids, C16:0, C18:0, C18:1, C18:3 were significantly higher in mice fed with high fat diets, and these fatty acids were much higher in HSF group than those in LSF group (Table 1). The levels of C20:2 and C20:4 were also increased in high fat diet groups, but there was no difference between HSF group and LSF group. The changes in liver fatty acids profile were similar with those in serum among groups (Additional file 4:...
### Table 1

| FAs (µg/ml) | NFD           | LSF        | HSF        |
|------------|---------------|------------|------------|
| C14:0      | 5.15 ± 0.97   | 5.55 ± 0.73| 8.3 ± 2.13 |
| C16:0      | 257.4 ± 78.21 | 330.05 ± 58.69 | 377.67 ± 44.33 *# |
| C16:1      | 37.07 ± 5.08  | 39.68 ± 8.51 | 52.18 ± 10.33 *# |
| C18:0      | 55.39 ± 42.35 | 80.86 ± 29.84 * | 120.08 ± 26.37 *# |
| C18:1      | 159.06 ± 15.99 | 236.41 ± 22.68 * | 272.41 ± 23.83 *# |
| C18:2      | 313.71 ± 54.58 | 256.11 ± 17.52 * | 286.88 ± 31.07 |
| γ-C18:3    | 13.04 ± 8.41  | 8.42 ± 4.16  | 12.06 ± 5.64 |
| C18:3      | 201.41 ± 43.73 | 82.31 ± 15.27 * | 114.14 ± 8.37 *# |
| C20:2      | 35.96 ± 40.21 | 150.16 ± 98.72 * | 98.11 ± 46.96 * |
| C20:4      | 81.72 ± 10.27 | 144.23 ± 37.63 * | 148.17 ± 49.17 * |
| C20:5      | 6.02 ± 1.78   | 2.6 ± 1.04   | 6.66 ± 2.36 |
| saturated fatty acid | 317.94 ± 89.10 | 406.24 ± 98.47 * | 506.05 ± 69.91 *# |
| unsaturated fatty acid | 847.98 ± 145.63 | 885.06 ± 165.88 | 990.63 ± 130.54 *# |
| total free fatty acids | 1128.85 ± 243.39 | 1296.7 ± 129.58 * | 1444.79 ± 184.27 *# |

All values are presented as mean ± SEM (n = 10). NFD, normal fat diet group; HSF, high stearic acid diet group (C18:0/C16:0 = 1:2); LSF, low stearic acid diet group (C18:0/C16:0 = 1:8). * Compared with the NFD group, p < 0.05. # Compared with LSF group, p < 0.05.

The effect of dietary C18:0/C16:0 ratios on cell ultrastructure in liver and pancreas

Liver mitochondria in NFD group appeared as typical mitochondria, elongated or round in shape, with tubular cristae and a few electron dense granules in the mitochondrial matrix (Additional file 5: Fig. S3a). Mitochondria in high fat diet groups were severely distended compared with NFD group, and mitochondrial distension in HSF group was more serious than that in LSF group (Fig. S3b, c). Some mitochondria in HSF group showed an exvaginated inner mitochondrial membrane. For pancreas, integritied β cell structure was observed in NFD group, with abundant insulin secretion granules in the cytoplasm (Fig. S3d). The amounts of insulin granules decreased significantly in both LSF and HSF groups, and there were obvious vacuoles induced by denatured insulin granules in HSF group (Fig. S3e, f).

Differentially expressed IncRNAs in liver among groups

A total of 34,523 IncRNAs was analyzed. As shown in Heat map and Volcano Plot, there were distinguishable gene expression profilings among groups (Fig. 2). Compared with NFD group, 258 IncRNAs were differentially expressed in LSF group including 152 up-regulated and 106 down-regulated (Fig. 2a, d), while 751 IncRNAs were differentially expressed in HSF group including 364 up-regulated and 387 down-regulated, which were much more than those in LSF group (Fig. 2b, e).
Among these differentially expressed lncRNAs in comparison with NFD group, there were 148 same lncRNAs in both LSF and HSF groups. There were 216 differentially expressed lncRNAs between LSF and HSF groups, including 116 up-regulated and 100 down-regulated (Fig. 2c, f).

**Differentially expressed microRNAs in liver among groups**

As shown in Heat map and Volcano Plot, 3544 microRNAs were analyzed and there were distinguishable microRNA expression profiles among groups (Fig. 3). Compared with NFD group, 44 microRNAs were differentially expressed in LSF group including 20 up-regulated and 24 down-regulated (Fig. 3a, d), while 42 microRNAs were differentially expressed in HSF group including 29 up-regulated and 13 down-regulated (Fig. 3b, e). Among these differentially expressed microRNAs in comparison with NFD group, there were 10 same microRNAs in both LSF and HSF groups. There were 32 differentially expressed microRNAs between LSF and HSF groups, including 24 up-regulated and 8 down-regulated (Fig. 3c, f).

**Differentially expressed mRNAs in liver among groups**

Among 23,047 mRNAs, a total of 302 differentially expressed mRNAs were identified between LSF group and NFD group, including 140 up-regulated and 162 down-regulated (Fig. 4a, c). While 808 differentially expressed mRNAs were identified between the HSF group and NFD group, including 433 up-regulated and 375 down-regulated (Fig. 4b, e). There were much more differentially expressed mRNAs in HSF group than those in LSF when compared with NFD group. And among these differentially expressed mRNAs, there were 150 same mRNAs in both LSF and HSF groups. There were 275 differentially expressed mRNAs between LSF and HSF groups, including 230 up-regulated and 45 down-regulated (Fig. 4c, f).

**Function analysis of differentially expressed mRNAs in liver among groups**

The GO analysis covered three domains: biological process (Fig. 5), cellular component (Additional file 6: Fig. S4), molecular function (Additional file 7: Figure S5), and we mainly focused on biological processes. Compared with NFD group, differentially up-regulated mRNAs were involved in biological processes including nitric oxide mediated signal transduction, and cellular response to glucose starvation (Fig. 5a, c), and differentially down-regulated mRNAs were involved in positive regulation
of fatty acid oxidation, arachidonic acid metabolic process, thioester metabolic process, acyl-CoA metabolic process, long term synaptic depression, and urate metabolic process in both LSF group and HSD group (Fig. 5b, d). Compared with LSF group, differentially up-regulated mRNAs were involved in inclusion body assembly, regulation of gene silencing, mammary gland involution (Fig. 5e), and differentially down-regulated mRNAs were involved in monocyte chemotaxis, antigen processing and presentation of exogenous peptide antigen via MHC class II, and lymphocyte chemotaxis in HSF group (Fig. 5f).

Regarding the up-regulated transcripts, the common pathways involved in both LSF group and HSD group included cytokine-cytokine receptor intervention, and prolactin signaling pathway, compared with NFD group (Fig. 6a, c). Regarding the down-regulated transcripts, the common pathways in LSF group and HSD group included retinol metabolism, fatty acid degradation, peroxisome, steroid hormone biosynthesis, PPAR signaling pathway, and arachidonic acid metabolism (Fig. 6b, d). When compared the difference between LSF group and HSD group, the top 3 score enrichment pathways were chemical carcinogenesis, retinol metabolism, steroid hormone biosynthesis for up-regated mRNAs (Fig. 6e), and steroid hormone biosynthesis, endocytosis and MAPK signaling pathway for down-regated mRNAs (Fig. 6f).

Discussion
In the present study, we compared the changes in metabolic and transcriptomic profiles between two high fat diet groups with different C18:0/C16:0 ratios in mice for the first time. We observed glucose and lipid metabolic disorders and inflammation were much severe in HSF mice than those in LSF mice. Compared with NFD group, there were much more differentially expressed IncRNAs and mRNAs in HSF mice than those in LSF mice, with distinguishable IncRNA, microRNA, and mRNA expression profiles between these two groups. And some biological functions and pathways, except for energy metabolism regulation, were identified that differentially expressed mRNAs between HSF group and LSF group probably involved in.

Many studies have shown that a high fat diet induces passive overfeeding, which leads to obesity and poor lipids profile [22–24]. But limited studies focused on the effect of diet saturated fatty acids
compisiton, especially for C18:0/C16:0 ratio on metabolim [25]. In our present study, compared with
NFD mice, mice in both high fat diet groups developed obesity. But the body weight and body fat ratio
of mice in HSF group were much higher than those in LSF. Also, there were more severe mitochondrial
injury in liver and pancreas in HSF group. This may in part be explained by the decrease in fat
oxidation in HSF group when compared with LSF group, as observed in energy metabolism monitor.
And previous studies support our finding that saturated fatty acids with longer chain length have
slower oxidation rate [14, 15]. These results suggest that obesity induced by high fat diets is
associated with the chain length of the saturated fatty acids in diets, a high C18:0 diet is more likely
to lead to obesity than an isocaloric high C16:0 diet.
Increased circulating FFAs, especialy SFAs are key factors that cause insulin resistance in obesity
[26]. C16:0 and C18:0 are the most common long chain saturated fatty acids in food and human
body, which are most closely related to insulin resistance and type 2 diabetes [19, 27]. Human
studies have revealed that circulating SFAs, especialy C16:0 and C18:0 were associated with higher
diabetes risk [28, 29]. But whether there is any difference between C16:0 and C18:0, especially from
diet, in their effect on insulin resistance and diabetes has not been reported in human. A study form
van den Berg SA demonstrated that a high fat diet rich in stearate induced a metabolic state favoring
low oxidative metabolism and whole body insulin resistance characterized by severe hepatic insulin
resistance [11]. But he did not detect the circulation and organism FFAs levels in his study. Our
previous study found that C18:0 exhibited a stronger lipotoxic role than C16:0 in both mouse islets
and rat insulinoma INS-1 cells [30]. In our present study, we also found that the high fat diet with high
C18:0 induced severe insulin resistance in mice than the isocaloric high C16:0 diet, in accordance
with previous studies. In addition, the levels of FFAs, including C16:0 and C18:0 in serum and liver
were much higher in HSF group, compared with LSF group. The high fat diet rich in C18:0 leads to the
higher level of C18:0 in the body in HSF group. This results in more intermediate lipids metabolites
due to relatively lower capacity of C18:0 in oxidation and incorporation into triacylglycerol. Moreover,
the higher level of C18:0 probably inhibits the conversion of C18:0 from C16:0 in the body, leading to
the accumulation of C16:0 in HSF group. These collectively contribute to the severe insulin resistance
in HSF group.

Fatty acids, not only an energy resource, but also important messenger molecules, are involved in multiple pathophysiological processes in the body [31]. Therefore, we did gene microarray analysis of IncRNAs, microRNA and mRNA profiles to further explore whether high fat diets with different C18:0/C16:0 ratio result in different changes in biological processes and which signaling pathways are affected. As expected, both HSF group mice and LSF group mice exhibited distinguishable gene expression profiles, compared with NFD mice. And more differentially expressed IncRNAs and mRNAs were observed in HSF group than those in LSF, indicating that high C18:0 probably leads to changes in more biological processes or signaling pathways. Results from GO analysis indicated that C16:0 and C18:0 were involved in some same biological processes, including nitric oxide mediated signal transduction, cellular response to glucose starvation, positive regulation of fatty acid oxidation, arachidonic acid metabolic process, thioester metabolic process, acyl-CoA metabolic process, long term synaptic depression, and urate metabolic process. Most of these biological processes have been widely explored in studied on fatty acids metabolism and function [32–34]. They also exihbied significantly different effects in many other biological processes, including inclusion body assembly, regulation of gene silencing, mammary gland involution, monocyte chemotaxis, antigen processing and presentation of exogenous peptide antigen via MHC class II, and lymphocyte chemotaxis. Studies about the effects of specific fatty acids on these biological processes are very limited so far, especially for C16:0 and C18:0. Further pathway analysis showed that the common pathways possibly regulated by C16:0 and C18:0 include cytokine-cytokine receptor intervention, prolactin signaling pathway, retinol metabolism, fatty acid degradation, peroxisome, steroid hormone biosynthesis, PPAR signaling pathway, and arachidonic acid metabolism. These pathways have been found mostly involved in glucose and lipid metabolism [35–38], in accordance with the common biological processes found between LSF group and HSD group. For the different pathways that the differentially expressed mRNAs regulated between the two groups, the score enrichment pathways included chemical carcinogenesis, retinol metabolism, steroid hormone biosynthesis, endocytosis and MAPK signaling. These signaling pathways have been found involved in the regulation of immunological
processes [39-42], which are consistent with the GO analysis results. Several studies have reported the high fat diet regulated immune function [43, 44]. But the pathways for immunological regulation by C18:0 and C16:0 in has seldom been reported. Most of the in our data have not been investigated. It is worthwhile to further investigate the differentially expressed IncRNAs and microRNAs between the two high fat diet groups in order to highlight the different biological functions and related mechanisms between C18:0 and C16:0.

Conclusions
A high fat diet with high C18:0/C16:0 ratio induces a worse lipids profile, severe insulin resistance and affects more IncRNAs and mRNAs expression than an isocaloric low C18:0/C16:0 ratio diet in mice. Our results provide new insights into the different biological functions and related mechanisms between C18:0 and C16:0.

Abbreviations
C16:0: Palmitic acid; C18:0: Stearic acid; EE: Energy expenditure; FFAs: Free fatty acids; GO analysis: Gene Ontology analysis; HDL-c: High density lipoprotein cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance; HSF: High fat diet with high C18:0 / C16:0 ratio; IL-6: Interleukin-6; LDL-c: Low density lipoprotein cholesterol; LSF: High fat diet with a low C18:0 / C16:0 ratio; NFD: Normal fat diet; OGTT: Oral glucose tolerance test; RER: Respiratory exchange rate; SFAs: Saturated fatty acids; TC: Total cholesterol; TG: Triglyceride; TNF-α: Tumor necrosis factor-α.

Declarations
Acknowledgements
Not applicable.

Authors’ contributions
LN designed and supervised the implementation of this study; LW, FX, SZ, and DH performed experiments; LW, FX, DH, JZ, and LC analyzed data; LW wrote the paper. All the authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Institutional Animal Care Committee from Harbin Medical University and conducted in accordance with the University guidelines for the care and use of laboratory animals (approval number:12-0003).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Supplementary Information
Additional file 1: Fig. S1. Indirect calorimetry of mice for consecutive 72 hours.

Additional file 2: Fig. S2. Oral glucose tolerance test of mice.

Additional file 3: Table S1. The levels of fasting serum indices in different groups of mice.

Additional file 4: Table S2. Liver fatty acids profile of mice.

Additional file 5: Fig. S3. Detection of cell ultra-structures in liver and pancreas by transmission electron microscopy.

Additional file 6: Fig. S4. GO analyses of cellular component for the differentially expressed mRNAs in mice.

Additional file 7: Fig. S5. GO analyses of molecular function for the differentially expressed mRNAs in mice.

Captions

**Fig. S1** Indirect calorimetry of mice for consecutive 72 hours. a, Energy expenditure. b, Carbohydrate oxidation. c, Fatty acid oxidation. d, Respiratory exchange rates. NFD, normal fat diet group; HSF, high stearic acid diet group (C18:0/C16:0=1:2); LSF, low stearic acid diet group (C18:0/C16:0=1:8). EE, energy expenditure. RER, respiratory exchange rate. N=6 for each group. *P < 0.05, compared with NFD group; # P < 0.05, compared with LSF group.

**Fig. S2** Oral glucose tolerance test of mice. NFD, normal fat diet group; HSF, high stearic acid diet group (C18:0/C16:0=1:2); LSF, low stearic acid diet group (C18:0/C16:0=1:8). N=10 for each group. *P < 0.05, compared with NFD group at the same time point; # P < 0.05, compared with LSF group at the same time point. NFD, normal fat diet group; HSF, high stearic acid diet group (C18:0/
C16:0=1:2); LSF, low stearic acid diet group (C18:0/ C16:0=1:8).

**Fig. S3** Detection of cell ultra-structures in liver and pancreas by transmission electron microscopy. a-c, Changes in mitochondria in liver; d-f, Changes in insulin granules in pancreas. a and d, normal fat diet group; b and e, low stearic acid diet group (C18:0/ C16:0=1:8); c and f, high stearic acid diet group (C18:0/ C16:0=1:2).

**Fig. S4** GO analyses of cellular component for the differentially expressed mRNAs in mice. a, c and e, Top ten fold enrichment terms of cellular components for mRNAs down-regulated. a, NFD vs LFD; c, NFD vs HFD; e, LFD vs HFD. b, d and f, Top ten fold enrichment terms of cellular components for mRNAs up-regulated. b, NFD vs LFD; d, NFD vs HFD; f, LFD vs HFD. The bar plot shows the top ten fold enrichment value of the significant enrichment terms.

**Fig. S5** GO analyses of molecular function for the differentially expressed mRNAs in mice. a, c and e, Top ten fold enrichment terms of molecular functions for mRNAs down-regulated. a, NFD vs LFD; c, NFD vs HFD; e, LFD vs HFD. b, d and f, Top ten fold enrichment terms of molecular functions for mRNAs up-regulated. b, NFD vs LFD; d, NFD vs HFD; f, LFD vs HFD. The bar plot shows the top ten fold enrichment value of the significant enrichment terms.

Figures
Figure 1

Body weight and body fat content of mice. a, The changes in the body weight of mice among groups (n=20 for each group). b, CT images of mice fat distribution in the 3 groups. c, Comparisons of mice body composition among groups (n=6 for each group). d, Comparisons of fat radios among groups (n=6 for each group). NFD, normal fat diet group; HSF, high stearic acid diet group (C18:0/ C16:0=1:2); LSF, low stearic acid diet group (C18:0/ C16:0=1:8). * P < 0.05, compared with NFD group; # P < 0.05, compared with LSF group. Yellow for subcutaneous fat and pink for visceral fat in Fig. 1b.
Differentially expressed IncRNAs in liver of mice. a-c, Differentially expressed IncRNAs in liver analyzed using hierarchical clustering; red indicates high relative expression, and green indicates low relative expression. a, NFD vs LFD; b, NFD vs HFD; c, LFD vs HFD. d-f, Differentially expressed IncRNAs in liver using Volcano plot. The red point in the plot represents the differentially expressed IncRNAs with statistical significance. d, NFD vs LFD; e, NFD vs HFD; f, LFD vs HFD. NFD, normal fat diet group; HSF, high stearic acid diet group (C18:0/ C16:0=1:2); LSF, low stearic acid diet group (C18:0/ C16:0=1:8).
Differentially expressed mRNAs in liver of mice. a-c, Differentially expressed mRNAs in liver analyzed using hierarchical clustering; red indicates high relative expression, and green indicates low relative expression. a, NFD vs LFD; b, NFD vs HFD; c, LFD vs HFD. d-f, Differentially expressed mRNAs in liver by Volcano plot. The red point in the plot represents the differentially expressed mRNAs with statistical significance. d, NFD vs LFD; e, NFD vs HFD; f, LFD vs HFD. NFD, normal fat diet group; HSF, high stearic acid diet group (C18:0/C16:0=1:2); LSF, low stearic acid diet group (C18:0/C16:0=1:8).
Differentially expressed microRNAs in liver tissues. a-c, Differentially expressed microRNAs in liver analyzed using hierarchical clustering; red indicates high relative expression, and green indicates low relative expression. a, NFD vs LFD; b, NFD vs HFD; c, LFD vs HFD. d-f, Differentially expressed microRNAs in liver by Volcano plot. The red point in the plot represents the differentially expressed microRNAs with statistical significance. d, NFD vs LFD; e, NFD vs HFD; f, LFD vs HFD. NFD, normal fat diet group; HSF, high stearic acid diet group (C18:0/C16:0=1:2); LSF, low stearic acid diet group (C18:0/C16:0=1:8).
Figure 5

GO analyses of biological process for the differentially expressed mRNAs. a, c and e, Top ten fold enrichment terms of biological processes for mRNAs down-regulated. a, NFD vs LFD; c, NFD vs HFD; e, LFD vs HFD. b, d and f, Top ten fold enrichment terms of biological processes for mRNAs up-regulated. b, NFD vs LFD; d, NFD vs HFD; f, LFD vs HFD. The bar plot shows the top ten fold enrichment value of the significant enrichment terms.
Figure 6

Pathway analyses of the differentially expressed mRNAs. a, c and e, Top ten score enrichment terms of pathways for mRNAs down-regulated. a, NFD vs LFD; c, NFD vs HFD; e, LFD vs HFD. b, d and f, Top ten score enrichment terms of pathways for mRNAs up-regulated. b, NFD vs LFD; d, NFD vs HFD; f, LFD vs HFD. The bar plot shows the top ten Enrichment score value of the significant enrichment pathways.

Supplementary Files
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