Tree shrew (*Tupaia belangeri chinensis*), a novel non-obese animal model of nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is becoming a severe public health problem that is affecting a large proportion of population in the world. Generally, NAFLD patients are usually accompanied with obesity, hyperglycemia, insulin resistance (IR) and type 2 diabetes (T2D), for which numerous animal models have been generated in order to explore the pathogenesis and therapies of NAFLD. On the contrary, quite a number of NAFLD subjects especially in Asian regions are non-obese and non-diabetic. However, few animal models are available for the research of non-obese NAFLD. Here, four approaches (approach 1 to 4) divided by the variable compositions of diets were used to treat tree shrews (Tupaia belangeri chinensis), which have closer evolutionary relationship to primates than rodents. Analysis of plasma biochemical parameters, hepatic histology, and the expression of hepatic lipid metabolic genes revealed that all approaches led to hepatic lipid accumulation, liver injury and hypercholesterolemia; but had no effect on body weight and adipose tissue generation, as well as glycemia. Hepatic gene expression in tree shrews treated by approach 4 might suggest a different or non-canonical pathway leading to hepatic steatosis. In conclusion, the tree shrew displays hepatic steatosis, dyslipidemia, but non-obese and non-diabetic under high energy diets, which suggests that the tree shrew may be useful as a novel animal model for the research of human non-obese NAFLD.

Key words: Nonalcoholic fatty liver disease (NAFLD); tree shrew (Tupaia belangeri chinensis); high energy diet; animal model; non-obese fatty liver
Introduction

Nonalcoholic fatty liver disease (NAFLD), is a clinicopathological liver disorder characterized by macrovesicular hepatic lipids accumulation and occurs in patients who consume little or even no alcohol (Angulo, 2002; Mulhall et al., 2002; Neuschwander-Tetri and Caldwell, 2003; Sanyal, 2002). Clinically, the risk factors and the output of hepatic histopathology and pathophysiology among human NAFLD are numerous and complex, encompassing a spectrum of liver damage varying from simple steatosis, in which more than 5% of hepatocytes present lipid accumulation in the form of lipid droplets (Szczepaniak et al., 2005), to nonalcoholic steatohepatitis (NASH), and eventually fibrosis and cirrhosis (Angulo and Lindor, 2002; Matteoni et al., 1999). Generally, NAFLD subjects usually display other associated metabolic symptoms including obesity, dyslipidemia, insulin resistance and type 2 diabetes (Adams and Lindor, 2007; Angulo and Lindor, 2002; Clark et al., 2002; Targher and Arcaro, 2007). However, NAFLD also occurs in patients who show non-obese and non-diabetic symptoms (Chow et al., 2007; Goh et al., 2015; Omagari et al., 2002), -as a high percentage (15–21%) of Asia-Pacific NAFLD subjects have been found to be non-obese.

Due to the difficulty of clinical studies, animal models are absolutely necessary to explore the pathogenesis and therapies of NAFLD. To date, numerous animal models especially rodents have been generated by different approaches for the research of human NAFLD, including spontaneously genetic mutation models such as ob/ob mouse (Mayer et al., 1951), db/db mouse (Hummel et al., 1966), Zucker rats (Yang et al., 1997), as well as dietary or pharmacological manipulated models. For example, high energy diets (high fat diet, high cholesterol diet, or high carbohydrate diet) induced NAFLD in rodents (Takahashi et al., 2012), rabbits (Fu et al., 2009;
Regardless of the types of animal models used, the common characteristics are hepatic steatosis accompanying with obesity, dyslipidemia, hyperglycemia, insulin resistance and hyperinsulinemia (Anstee and Goldin, 2006; Ibrahim et al., 2015; Kucera and Cervinkova, 2014; Sanches et al., 2015), which largely reflect the histopathology and pathophysiology of human NAFLD in obese and/or diabetic patients. Therefore, these animal models are extremely valuable to study the pathogenesis and therapies of human NAFLD in obese and/or diabetic patients. However, very few animal models have been reported to be useful for the research of non-obese and non-diabetic human NAFLD. Hence, to develop new animal models of non-obese and non-diabetic NAFLD appears to be great urgent and essential.

The tree shrew (*Tupaia belangeri chinensis*), is a close relative of primates in terms of evolution (Fan et al., 2013), and has been used in biological research especially for hepatitis B virus (HBV) and C virus (HCV) research for decades (Su, 1987; Walter et al., 1996; Yan et al., 1996). Previously, we reported that the relationships between body weight, fasting blood glucose concentration, sex and age in tree shrews are similar to that of human beings. Additionally, we recently showed that a high fat diet combined with cholesterol successfully induced liver steatosis to inflammation and fibrosis progressively within 10 weeks, but induced no change in body weight (Zhang et al., 2015b), which was distinct from the cases of mice and Ossabaw pigs. This raised the question of whether or not the tree shrew could be an alternative animal model specifical for the research of non-obese and non-diabetic human NAFLD. Therefore, we applied different types of high energy diets including high fat or high sucrose diets to treat tree shrews. Remarkably, all these diets successfully induced liver steatosis, but did not affect body weight,
suggesting that the tree shrew is a novel animal model of human non-obese NAFLD.

Results

1. High energy diets caused liver injury in tree shrew.

As markers of liver necroinflammation, plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels have been widely used to indicate the degree of liver injury. In approach 1, the plasma levels of AST and ALT were normal in the control (CON) group, but increased gradually and significantly in both the high sucrose (HS), and the high fat and high sucrose (HFHS) groups at 5 weeks and 10 weeks (Fig. 1A). In approach 2, the plasma levels of AST and ALT were mostly normal throughout the experimental duration (24 weeks) even though the level of AST was slightly higher in the HFHS group than the CON group at 24 weeks (Fig. 1B). Astoundingly, in approach 3, a great increase of plasma AST and ALT levels was seen in the high fat and high cholesterol (HFHC) group from 4 weeks to 8 weeks, which was about 3 times higher at 4 weeks and 9 times higher at 8 weeks than the CON group (Fig. 1C). In approach 4, the plasma levels of AST and ALT showed no difference among four groups at 4 weeks, but significantly increased in the HFHS group at 8 weeks (Fig. 1D). Altogether, high energy diets using in approaches 1, 3 and 4, but not in approach 2 caused liver injury in tree shrew.

2. Different diet approaches changed blood lipid profiles in tree shrew

NAFLD patients often display dyslipidemia. Consistently, high energy diets often caused dyslipidemia in animal models of NAFLD (Supplementary Table 2). In order to investigate whether or not high energy diets lead to dyslipidemia in tree shrews, we tested four common
indicators of dyslipidemia, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c). In approach 1, the plasma TC levels were elevated gradually yet significantly in both the HS and the HFHS group. More specifically, both the plasma TG level and the LDL-c level increased significantly in the HS group but not in the HFHS group, whereas the change in the HDL-c level was opposite (Fig. 2A). In approach 2, only in the HFHS (40%) diet group did the plasma levels of TC, TG and HDL-c in the tree shrew increase significantly (Fig. 2B). In approach 3, the HF diet significantly elevated the TC level at 8 weeks, and the HDL-c level at both 4 weeks and 8 weeks (Fig. 2C). Remarkably, the HFHC diet greatly increased the levels of TC, HDL-c and LDL-c, and reversely decreased the level of TG at both 4 weeks and 8 weeks (Fig. 2C), which was consistent with our previous report (Zhang et al., 2015b). Additionally, in approach 4, all four diets did not affect the TG level throughout the experimental duration, or the levels of TC, HDL-c and LDL-c at 4 weeks. Only the HFHS diet significantly increased the levels of TC, HDL-c and LDL-c (Fig. 2D). Taken all these lines of evidences together, different approaches with distinct diets can indeed cause dyslipidemia to various degrees within tree shrew.

3. High energy diets did not cause hyperglycemia

In all four approaches, the levels of FBG and HbA1c showed no significant change at any experimental time point when compared to the CON group no matter for the HF (high fat), HFHC, HS, or HFHS diet group (Fig. 3). Thus, the tree shrew displays no hyperglycemia under the induction of high energy diets (Supplementary Table 2).
4. **High energy diets led to hepatic steatosis**

Lipids accumulation in the liver is the most remarkable feature of NAFLD in numerous animal models as well as in human patients. As the golden standard to reflect hepatic steatosis, pathological section by haematoxylin and eosin (H&E) staining is often used to detect liver lipid accumulation in both clinical diagnosis (reviewed by Tannapfel *et al.* (Tannapfel et al., 2011)) and animal research (He et al., 2013; Huang et al., 2015; Lee et al., 2015). Therefore, liver tissues from the end time point of each approach were processed by histopathology (H&E staining) and the histological changes were visualized by microphotographs (Fig. 4). The liver sections from the control groups displayed normal hepatocytes, whereas the sections from all high energy diet groups exhibited numerous huge pathological vacuoles in hepatocytes (Fig. 4), suggesting that lipid droplets accumulated in liver, and that these animals developed hepatic steatosis (Supplementary Table 2).

5. **High energy diets had no effect on adipose tissues**

High energy diets generally enlarge adipose tissues leading to obesity in most experimental animals and humans. Interestingly, when compared with control groups, neither the HF diet, the HS diet, nor the HFHS diet in approach 1, 2 and 4 affected body weight during the experiment (Fig. 5A-D, Supplementary Table 2). Although the high fat diet (HF) did not affect body weight during the experiment in approach 3, the high fat and high cholesterol (HFHC) diet eventually led to a slightly decreased body weight at the end of the experiment (8 weeks) (Fig. 5C, Supplementary Table 2), which was consistent with our previous report (Zhang et al., 2015b). In addition, animal dissection showed that adipose tissues including subcutaneous adipose tissues,
visceral adipose tissues and epididymal adipose tissues were apparently all absent, except the
enlarged and pale livers in high energy diets induced animals (Fig. 5E), even though there were
no differences in food consumption among the groups within any approach (data not shown).
Altogether, these results indicate that high energy diets only lead fat to accumulate in liver
exclusively, without accompanying obesity in tree shrew.

6. High energy diets impaired the expression of lipid metabolic genes.

In order to investigate the underlying mechanisms of lipid accumulation in the liver of tree
shrews, the mRNA expression of some genes involved in lipid synthesis, degradation, uptake and
secretion were tested by QPCR in approach 4. Interestingly, the expression of all 17 lipid
metabolic genes was significantly down-regulated in the HF, HS, and HFHS groups compared to
the control group (Fig. 6A-D), suggesting that high energy diets do impair lipid metabolism in
the liver of tree shrews.

Discussion

Excessive intake of energy is regarded as one of the crucial inducements of NAFLD;
therefore, high energy diets are commonly applied to generate animal models of NAFLD. In this
study, four types of high energy diets were applied, namely high sucrose diet (HS), high fat diet
(HF), high fat and high sucrose diet (HFHS), as well as high fat and high cholesterol diet
(HFHC). We used the combination of different diets in approaches 1 to 4 to treat tree shrews, and
found that all high energy diets lead to hepatic steatosis.
Although the majority of human NAFLD patients are associated with metabolic risk factors such as obesity, insulin resistance and type 2 diabetes, which have been extensively studied, concerns regarding non-obese human NAFLD are raising because it is not uncommon especially in Asian subjects who often possess lower BMI cut-offs (Liu, 2012). Clinically, the waist circumference, total abdominal fat levels, and subcutaneous fat levels were significantly higher in obese NAFLD patients than in non-obese NAFLD patients who did not show obvious insulin resistance (Yasutake et al., 2009). The same can be found of the prevalence of type 2 diabetes, the plasma TG and HDL-c levels (Honda et al., 2016). The laboratory animals, mice and rats, have been extensively used for the research of human NAFLD since they generally display hepatic steatosis, obesity including increased body weight and white adipose tissue (WAT), hypertriglyceridemia, hypercholesterolemia and hyperglycemia when fed on high energy diets (Supplementary Table 2). Consistent to human and rodent NAFLD, tree shrew NAFLD presented hepatic lipid accumulation, and with hypercholesterolemia on all four diets, as well as hypertriglyceridemia on the HS diet (Supplementary Table 2). However, the tree shrews treated by these four approaches did not gain body weight (Fig. 5A-D), epididymal or intra-abdominal adipose tissues (Fig. 5E), as well as gain changes in the levels of FBG and HbA1c (Fig. 3). Therefore, those lines of evidences indicate that unlike rodent models, the tree shrew model of NAFLD is non-obese and non-diabetic, which fundamentally mimics the principal symptoms of non-obese human NAFLD subjects.

In rodent models of NAFLD, once hit by excessive energy, the livers displayed a disorder of lipid metabolism which consequently led to lipid accumulation. In general, genes involved into lipid synthesis (srebp1, acc, fasn, dgat2 and hmgcr) and uptake (ldlr and cd36) have
up-regulated, while genes involved into lipid degradation (ppara and ctp1) and secretion (abcg5/8 and mttp) have down-regulated at mRNA or protein level (Ann et al., 2015; Gou et al., 2016; Hou et al., 2016; Kang et al., 2016; Komiya et al., 2016; Li et al., 2016; Liu et al., 2016; Ren et al., 2014; Shindo et al., 2010; Van Rooyen et al., 2011; Zhang et al., 2015a). Consistent with rodent models, the mRNA expression of genes involved in lipid degradation and secretion was decreased in tree shrews treated by approach 4 (Fig. 6B, D). However, surprisingly, both the mRNA expression of lipid synthesis and uptake pathway genes were decreased (Fig. 6A, C).

Although the underlying mechanisms of lipid accumulation in tree shrew need to be further studied in the future, the discrepancy of lipid gene expression between tree shrew and rodents somehow suggests that a new or non-canonical pathway may exist in tree shrews as the result of hepatic steatosis under excessive energy induction, which hopefully will provide new clues to investigate the pathogenesis of human non-obese NAFLD.

Theoretically, in rodent models, high energy intake often results in fat distribution to both adipose tissues especially epididymal adipose tissue and visceral adipose tissue, and to the liver, thus leading to obesity and fatty liver. However, in tree shrews, fat distribution to adipose tissues is shut down by uncharacterized mechanisms; while the liver of tree shrews are quite sensitive to accumulating fat under excessive energy conditions (Fig. 7). The discrepancy between the rodent and tree shrew model of NAFLD may facilitate researchers to select an appropriate animal model for the research of human NAFLD. In brief, we have successfully established tree shrew models of human NAFLD by the induction of high energy diets, in which they display dyslipidemia, and hepatic steatosis, but not hyperglycemia and obesity. Thus, tree shrews can be used as a completely novel animal model to study human non-obese NAFLD.
Materials and methods

Animals and experimental design

All tested tree shrews were males of around one year of age raised in the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS). All animal experiments were carried out according to the guidelines approved by the Animal Ethics Committee of the Kunming Institute of Zoology, Chinese Academy of Science. Animals were housed one animal per cage at room temperature maintained at 21 ± 2 °C, humidity at 50-70%, with natural lighting and free access to food and water.

The diets and processes of the four approaches were listed in Supplementary Table 1. At the sampling time point, animals were fasted overnight (~14 h), and then euthanized by ethyl ether anesthesia. Blood and liver samples were harvested, livers of tree shrews were fixed in 10% formalin or snap frozen in liquid nitrogen and stored at -80°C for further analyses.

Plasma biochemical parameters

Femoral vein blood of each tree shrew (0.5 ml) was taken after fasting 14 h, collected into the EDTAK₂-containing glass tubes (Shandong Aosaite Medical Devices Co’ LTD, Heze, Shandong, China), and then centrifuged at 3,000 rpm for 5 min at room temperature. The plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-c), high density lipoprotein-cholesterol (HDL-c), fasting blood glucose (FBG) and glycated haemoglobin A1c (HbA1c) were assayed by an automatic blood biochemistry analyzer (Abbott CI16200, Chicago, IL USA) at the First People’s Hospital of Yunnan Province, Kunming, China.
**Hepatic histology**

From the live sample, 5 μm-thick sections of formalin-fixed and paraffin-embedded livers were processed for hematoxylin-eosin (H&E) staining. Liver sections were then visualized and photographed using a light microscope (Olympus BX53, Kanngawa, Japan).

**Analysis of hepatic gene expression by real-time quantitative PCR (QPCR)**

The extraction of total RNA from liver tissues and the performance of real time quantitative PCR (QPCR) followed as we described previously (Zhang et al., 2015b).

**Statistical analysis**

Data were presented as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by LSD multiple comparison tests by SPSS20.0 (IBM SPSS Statistics, Armonk, NY, USA). All figures were made using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA).
Competing interests

The authors declare no competing or financial interests.

Author contributions

B.L., L.Z. and X.W. conceived and designed the experiments, L.Z., X.W., S.L., Y.L., Z.Z., Q.C. and R.X. performed the experiments; B.L., L.Z., X.W., and S.L. analyzed the data; L.Z., X.W., S.L., Y.L., Z.Z., Q.C., R.X. and B.L. contributed reagents/materials/analysis tools; and B.L., L.Z. wrote the paper. All authors were involved in editing the manuscript and approving the submitted version.

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Figures

A

Approach 1

B

Approach 1

C

Approach 2

D

Approach 2

C

Approach 3

D

Approach 3

C

Approach 4

D

Approach 4
Fig. 1. The plasma levels of AST and ALT from approach 1 (A), 2 (B), 3 (C), and 4 (D). Data were presented as mean ± SEM. Biological repeats (n) are shown within the figure. Significant difference between the CON diet group and an indicated diet group at the same time point (*P<0.05, **P<0.01, ***P<0.001). Abbreviations: Control diet (CON), High sucrose diet (HS), high fat diet (HF), high fat and high sucrose diet (HFHS), high fat and high cholesterol diet (HFHC).
Fig. 2. Blood lipid profiles of approach 1 (A), 2 (B), 3 (C), and 4 (D). The HDL-c and LDL-c levels of HS and HFHS group in approach 1 at 5 weeks were not detected. Data were presented as mean ± SEM. Biological repeats (n) are shown within the figure. Significant difference between the CON diet group and an indicated diet group at the same time point (*P<0.05, **P<0.01, ***P<0.001). Abbreviations: Control diet (CON), High sucrose diet (HS), high fat diet (HF), high fat and high sucrose diet (HFHS), high fat and high cholesterol diet (HFHC).
Fig. 3. The levels of fasting blood glucose (FBG) and glycated haemoglobin A1c (HbA1c) from approach 1 (A), 2 (B), 3 (C), and 4 (D). Data were presented as mean ± SEM. Biological repeats (n) are shown within the figure. Abbreviations: Control diet (CON), High sucrose diet (HS), high fat diet (HF), high fat and high sucrose diet (HFHS), high fat and high cholesterol diet (HFHC).
Fig. 4. Haematoxylin and eosin (H&E) staining of liver sections from approach 1 (A), 2 (B), 3 (C), and 4 (D). Scale bars: 100 μm. Abbreviations: Control diet (CON), High sucrose diet (HS), high fat diet (HF), high fat and high sucrose diet (HFHS), high fat and high cholesterol diet (HFHC).
Fig. 5. The body weight from approach 1 (A), 2 (B), 3 (C), and 4 (D), as well as the dissections of animals fed on CON diet (Normal state) and HFHC diet (fatty liver) from the approach 3 as presented examples (E). Data were presented as mean ± SEM. Biological
repeats (n) are shown within the figure. Abbreviations: Control diet (CON), High sucrose diet (HS), high fat diet (HF), high fat and high sucrose diet (HFHS), high fat and high cholesterol diet (HFHC).
Fig. 6. The mRNA expression of lipid metabolic genes involved in lipids synthesis (A), degradation (B), uptake (C), and secretion (D). Data were presented as mean ± SEM. Biological repeats (n) are shown within the figure. Significant difference between the CON diet group and an indicated diet group at the same time point (*P<0.05, **P<0.01, ***P<0.001).

Abbreviations: Control diet (CON), High sucrose diet (HS), high fat diet (HF), high fat and high sucrose diet (HFHS).
Fig. 7. A model of energy flow and distribution between rodent models and tree shrew model of NAFLD.
**Supplementary Table 1. The main compositions of the diets**

<table>
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<tr>
<th></th>
<th>D10012G</th>
<th>D10001</th>
<th>D12451</th>
<th>D12450J</th>
<th>D12492</th>
<th>D12109C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>20.0</td>
<td>20.3</td>
<td>20.3</td>
<td>20.8</td>
<td>24.0</td>
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<td>Carbohydrate</td>
<td>64.0</td>
<td>63.9</td>
<td>66.0</td>
<td>67.7</td>
<td>41.0</td>
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<tr>
<td>Sucrose</td>
<td>10.0</td>
<td>10.0</td>
<td>50.0</td>
<td>51.3</td>
<td>20.1</td>
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<tr>
<td>Fat</td>
<td>7.0</td>
<td>15.8</td>
<td>5.0</td>
<td>11.5</td>
<td>24.0</td>
<td>45.0</td>
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<tr>
<td>Cholesterol</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

All diets were purchased from Research Diets Company.
### Supplementary Table 2. The diets and processes of four approaches.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Animal number</th>
<th>Feeding mode of different diets</th>
<th>Experimental time point</th>
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</thead>
<tbody>
<tr>
<td>Approach 1</td>
<td>D10012G (CON)=CON group</td>
<td>8</td>
<td>every day</td>
</tr>
<tr>
<td></td>
<td>D10001 (HS)=HS group</td>
<td>6</td>
<td>every day</td>
</tr>
<tr>
<td></td>
<td>D12451 (HFHS)=HFHS group</td>
<td>5</td>
<td>every day</td>
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<td></td>
<td>D10012G (CON)=CON group</td>
<td>5</td>
<td>every day</td>
</tr>
<tr>
<td></td>
<td>D12451 (HFHS)=C3H1 group</td>
<td>5</td>
<td>every day</td>
</tr>
<tr>
<td></td>
<td>30% D12451 (HFHS)+70% D10012G (CON)=HFHS 30% group</td>
<td>5</td>
<td>3 days CON diet and 1 day HFHS diet</td>
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<td></td>
<td>40% D12451 (HFHS)+60% D10012G (CON)=HFHS 40% group</td>
<td>5</td>
<td>every day</td>
</tr>
<tr>
<td></td>
<td>D12450J (CON)=CON group</td>
<td>5</td>
<td>every day</td>
</tr>
<tr>
<td>Approach 2</td>
<td>D12492 (HF)=HF group</td>
<td>6</td>
<td>every day</td>
</tr>
<tr>
<td></td>
<td>D12109C (HFHC)=HFHC group</td>
<td>7</td>
<td>every day</td>
</tr>
<tr>
<td></td>
<td>D10012G (CON)=CON group</td>
<td>7</td>
<td>every day</td>
</tr>
<tr>
<td></td>
<td>D10001 (HS)=HS group</td>
<td>6</td>
<td>1 day CON diet and 1 day HS diet</td>
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<td>1 day CON diet and 1 day HF diet</td>
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<tr>
<td></td>
<td>D12451 (HFHS)=HFHS group</td>
<td>5</td>
<td>1 day CON diet and 1 day HFHS diet</td>
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</table>

All diets were purchased from Research Diets. Abbreviation: CON, control diet; HF, high fat diet; HS, high sucrose diet; HFHS, high fat high sucrose diet; HFHC, high fat high cholesterol diet.
**Supplementary Table 3. Effects of high energy diets on different animal models**

<table>
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<th>Diets</th>
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<th>Rats</th>
<th>Tree shrews</th>
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</thead>
<tbody>
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<td>HF</td>
<td>Hepatic lipids accumulation</td>
<td>Y (Sato et al., 2010; Savard et al., 2013; Yimin et al., 2012)</td>
<td>Y (Auberval et al., 2014; Ganji et al., 2014; Lee et al., 2015)</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>Increased body weight</td>
<td>Y (Sato et al., 2010; Savard et al., 2013; Yimin et al., 2012)</td>
<td>Y (Auberval et al., 2014; Ganji et al., 2014; Lee et al., 2015)</td>
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</tr>
<tr>
<td></td>
<td>Increased WAT</td>
<td>Y (Sato et al., 2010)</td>
<td>Y (Sato et al., 2010)</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Hypertriglyceridemia</td>
<td>Y (Ha and Chae, 2010; Yimin et al., 2012)</td>
<td>Y (Auberval et al., 2014; Ganji et al., 2014)</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>Hypercholesterolemia</td>
<td>Y (Sato et al., 2010; Savard et al., 2013; Yimin et al., 2012)</td>
<td>Y (Yimin et al., 2012)</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia</td>
<td>Y (Sato et al., 2010; Yimin et al., 2012)</td>
<td>Y (Yimin et al., 2012)</td>
<td>Y</td>
</tr>
<tr>
<td>HS</td>
<td>Hepatic lipids accumulation</td>
<td>Y (Deaciuc et al., 2008; Pierce et al., 2016; Song et al., 2007)</td>
<td>Y (Chen et al., 2011; Lima et al., 2016)</td>
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HF, high fat diet; HS, high sucrose diet; HFHS, high fat high sucrose diet; HFHC, high fat high cholesterol diet.

“Y”, means “yes”; “N”, means “no”.

Hazarika et al., 2016; Panchal et al., 2013; Roberts et al., 2015; Matsuzawa et al., 2007; Park et al., 2011; Savard et al., 2013; Elshazly, 2015; Xu et al., 2010; Shin et al., 2016.
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