



ORIGINAL ARTICLE

## High fat diet for induced dyslipidemia and cardiac pathological alterations in Wistar rats compared to Sprague Dawley rats

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### KEYWORDS

Dyslipidemia;  
Cardiac pathology;  
High fat diet;  
Wistar rats;  
Sprague Dawley rats

### Abstract

**Introduction:** Wistar and Sprague Dawley (SD) rats are generally used as models for the cholesterol metabolism experiments. They are acceptable to high fat diet-induced disorders with individual variations, including dyslipidemia and abnormal cardiac pathology.

**Objective:** To compare the effects of high fat diet in inducing dyslipidemia and cardiac pathological alterations between Wistar and SD rats.

**Methods:** We compared the differences in plasma cholesterol levels and cardiac pathological alterations between Wistar and SD rats of standard diet (3.90 kcal/g) and high fat diet (5.40 kcal/g) after 4 weeks.

**Results:** SD rats fed with high fat diet showed significantly enhanced LDL concentration and the decreased HDL concentration when compared to Wistar rats. Additionally, SD rats showed cardiac pathological alterations such as infiltration of mononuclear cells referring to inflammatory response and high amounts of perivascular fat playing a key role in the impairment of vascular functions.

**Conclusions:** Our results indicate that SD rats may be the more suitable model for dyslipidemia and alteration of cardiac pathology induced by high fat diet.

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### PALABRAS CLAVE

Dislipidemia;  
Patología cardíaca;  
Dieta alta en grasas;  
Ratas Wistar;  
Ratas Sprague Dawley

**Dieta alta en grasas para la dislipidemia inducida y cardíaca. Alteraciones patológicas en ratas Wistar en comparación con ratas Sprague Dawley**

### Resumen

**Introducción:** Las ratas Wistar y Sprague Dawley (SD) se usan generalmente como modelos para los experimentos del metabolismo del colesterol. Son aceptables para los trastornos inducidos

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por la dieta alta en grasas con variaciones individuales, incluida la dislipidemia y la patología cardíaca anormal.

**Objetivo:** Comparar los efectos de la dieta alta en grasas en la inducción de dislipidemia y alteraciones patológicas cardíacas entre ratas Wistar y SD.

**Métodos:** Comparamos las diferencias en los niveles de colesterol en plasma y las alteraciones patológicas cardíacas entre ratas Wistar y SD de dieta estándar (3,90 kcal/g) y dieta alta en grasas (5,40 kcal/g) después de 4 semanas.

**Resultados:** Las ratas SD alimentadas con una dieta alta en grasas mostraron una concentración significativamente mejorada de LDL y una concentración disminuida de HDL en comparación con las ratas Wistar. Además, las ratas SD mostraron alteraciones patológicas cardíacas, como la infiltración de células mononucleares que se refieren a la respuesta inflamatoria y las altas cantidades de grasa perivascular que juegan un papel clave en el deterioro de las funciones vasculares.

**Conclusiones:** Nuestros resultados indican que las ratas SD pueden ser el modelo más adecuado para la dislipidemia y la alteración de la patología cardíaca inducida por una dieta alta en grasas.

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## Introduction

Research animals are considered as valuable tools for understanding pathophysiology and in developing therapeutic interventions in diseases for many decades. Rat is one of the most widely used research animals for studying physiology, pharmacology, and metabolism in basic medical research.<sup>1,2</sup> The benefits of using rat models in biomedical researches are the following: rats are easy to manage in terms of dietary feeding and controlling environmental factors including temperature, humidity, and lighting. As the result, nearly all environmental variations do not differ much from human studies. In addition, rats generally have shorter life span than human. The changes and adaptation can be observed throughout their life cycle or even across several generations. The blood and organs can be also collected for profound experiment and bio-molecular investigations. Moreover, anatomical basis and physiological functions in rats, especially cardiac pathology, are similar to humans as well.<sup>3</sup>

Dyslipidemia can be present in rats with abnormal cholesterol metabolism. Inducing dyslipidemia in rats is often through a high fat diet using variety of fats such as lard, coconut, soybean, or palm oil, etc. It may be because prolonged ingestion of saturated fats is directly related to dyslipidemia and atherosclerosis.<sup>4</sup>

In experiment associated with cholesterol metabolism, Wistar and Sprague Dawley (SD) rats are vulnerable to diet-induced obesity and dyslipidemia with other variations including the alterations of cardiac pathology.<sup>5</sup> Previous studies reported that feeding diet containing as little as 42% fat in both Wistar and SD rats can change peripheral cholesterol levels in various circumstances.<sup>6–9</sup> Nevertheless, there are influencing variations such as age, feeding duration, and fat composition in diet used that may also be affect the experimental outcomes.<sup>6</sup> Therefore, the present

study aimed to compare the effects of high fat diet in inducing dyslipidemia and cardiac pathological alterations between Wistar and SD rats.

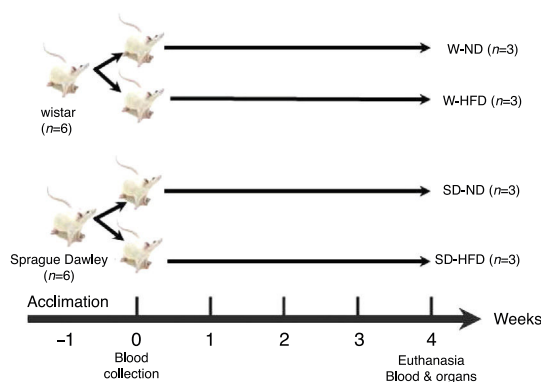
## Materials and methods

### Chemicals and reagents

Commercial kits for lipid profiles (BioSystems S.A, Barcelona, Spain), formalin buffer solution, neutral buffered, 10% (Sigma–Aldrich, St Louis, MO, United States), paraffin–polyisobutylene mixture (Leica Biosystems, Teban Gardens Crescen, Singapore), hematoxylin – eosin solution (Sigma–Aldrich, St Louis, MO, United States).

### Animals

Five-week-old male Wistar rat and Sprague Dawley rats weighing 180–190g were purchased from the National Laboratory Animal Center at Salaya Campus, Mahidol University. Two rats were housed in a plastic cage, opening on the top and covered with a rod lid in a room at 55–65% ambient humidity maintained and temperature controlled at 20–24°C under the 12-h light-dark cycle. The rats were raised with free access to drinking water and standard diet for 1 week before being assigned to the variable dietary groups. All procedures were conducted and approved by the Animal Care Ethical Committee of Laboratory Animal Science Center, Faculty of Tropical Medicine, Mahidol University (Approval No. FTM-ACUC 010/2018). FTM-ACUC memberships include doctor of veterinary medicine, researcher experienced in research involving animals and nonscientific background public member. The ethical standards and procedures were according to the Guide for the Care and Use of Laboratory Animals.<sup>10</sup>



**Figure 1** The experimental design of twelve rats. Both Wistar and Sprague Dawley (SD) rats (6 per each) were randomly divided into 4 groups and fed in selective diets for 4 weeks. W-ND; Wistar rats fed standard diet, W-HFD; Wistar rats fed a high fat diet, SD-ND; Sprague Dawley rats fed standard diet, SD-HFD; Sprague Dawley rats fed a high fat diet.

## Experimental design

Our experimental design consisted of 3 rats per experimental group. The strategic rationale for sample size calculation of experimental groups come from equation of  $n_1 = [(Z_{1-\alpha/2} + Z_{1-\beta})^2 (\sigma_1^2 + [\sigma_2^2/r])]/\delta^2$  which  $n_1$  = sample size,  $\sigma$  = standard deviation,  $r = n_2/n_1$ ,  $\delta$  = difference between two group of means. The sample size was calculated according to total cholesterol<sup>5</sup> using power  $(1 - \beta) = 0.9$  and type I error ( $\alpha$ ) = 0.01. After acclimation for 1 week, the 12 rats, 6 Wistar rats and 6 Sprague Dawley rats (SD) were divided into 4 groups ( $n=3$ ) for 2 different diets; standard diet (ND) or high fat diet (HFD) for 4 weeks according to the experimental design (Fig. 1). The standard diet and HFD were prepared according to the American Institute of Nutrition (AIN) 76A formula and D12492 formula with some modifications. The standard diet contained 11.5% fat, 20.8% protein, 67.7% carbohydrate and 3.90 kcal/g, and HFD consisted of 58.3% fat, 20.2% protein and 21.5% carbohydrate and 5.40 kcal/g. The composition of diets is shown in Table 1. At the end of the experiment, each rat was overnight fasted and euthanized with carbon dioxide. The heart and the adipose tissue were collected, weighed and kept for further analysis.

**Table 1** The composition of rat diets.

Ingredients	ND (%)	HFD (%)
Casein	20.0	20.0
Cornstarch	15.0	15.0
Sucrose	50.0	33.0
Cellulose	5.0	5.0
Methionine	0.3	0.3
Mineral mixture	3.5	3.5
Vitamin mixture	1.0	1.0
Choline bitartrate	0.2	0.2
Corn oil	5.0	5.0
Lard oil	–	17.0
Total	100.0	100.0

ND: standard diet, HFD: a high fat diet.

## Laboratory measurements

Body weight, food, and water consumption were measured and recorded. The amount of food consumed was calculated for calorie intake. Weight gain and weight of the visceral fat were determined. Serum total cholesterol (TC), high density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglyceride (TG) from lateral tail vein were measured by commercial kits for lipid profiles.

## Histopathological analysis

After euthanasia, the heart was harvested, trimmed of fat, and weighed. The middle cross-section of the heart was fixed on a filter paper in a 10% formalin buffer solution, and the fixed tissues were dehydrated and subsequently embedded in a paraffin–polyisobutylene mixture. Then, they were sectioned (4  $\mu$ m) on slides and stained with hematoxylin and eosin (H&E).<sup>11</sup> To facilitate a histopathological evaluation of ventricular cardiac tissue, a light microscope (Zeiss Axio Observer A1, Germany) with an attached camera (Canon, USA) and the ZoomBrowser EX computer-based image analysis software (Canon, USA) were used. The morphological changes were classified based on vascular morphological changes which were assessed by two independent researchers.

## Statistical analysis

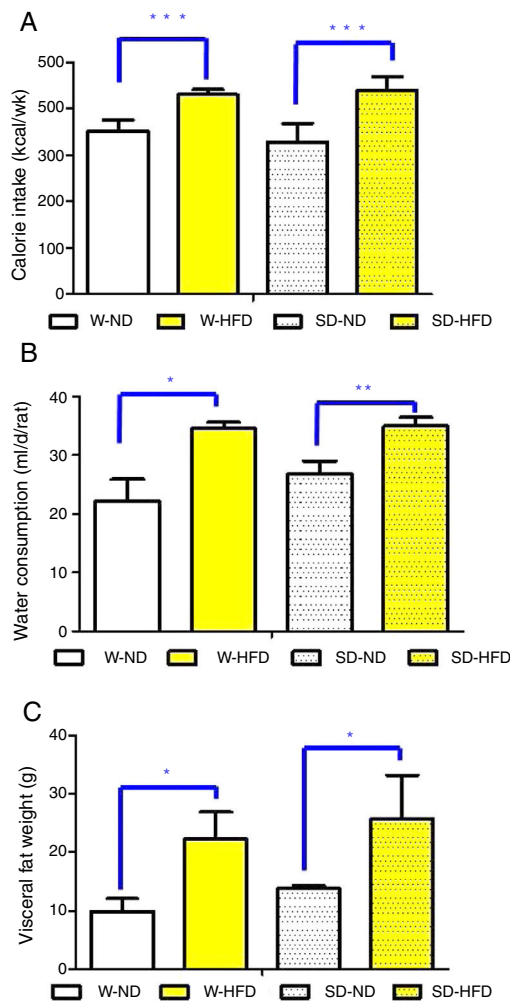
Numerical data were analyzed using SPSS version 18.0 statistics software (Chicago, IL, USA). Data are represented as means  $\pm$  standard deviations. Group differences were analyzed using the analysis of variance (ANOVA), followed by a post hoc Tukey's test as appropriate. The *p* value of less than 0.05 was acceptable for a statistically significant difference. Graphs were performed using GraphPad Prism 5 statistical software (GraphPad Software Inc., La Jolla, CA, USA).

## Results

### Food and water consumption, calorie intake, body weight and visceral fat weight

All animals survived and had no abnormal symptoms or behavioral changes throughout the study period. After feeding different types of diets for 4 weeks, food and water consumption in Wistar and Sprague Dawley (SD) rats were thoroughly observed. Although significant difference in food consumption was not found, there was significantly higher energy intake and water consumption in rats fed with HFD compared to those fed with ND (Fig. 2A and B).

At baseline, Wistar and SD rats in the 4 divided groups had similar average initial body weight. High energy intake led to remarkably increased body weight, especially in SD-HFD group. The significant difference in body weight was found in SD-HFD group when compared with SD-ND group (Fig. 3A). Moreover, the significant differences of visceral fat weight were found in both Wistar and SD rats fed with standard diet when compared to those fed with high fat diet (Fig. 2C). No significant difference of visceral fat weight was

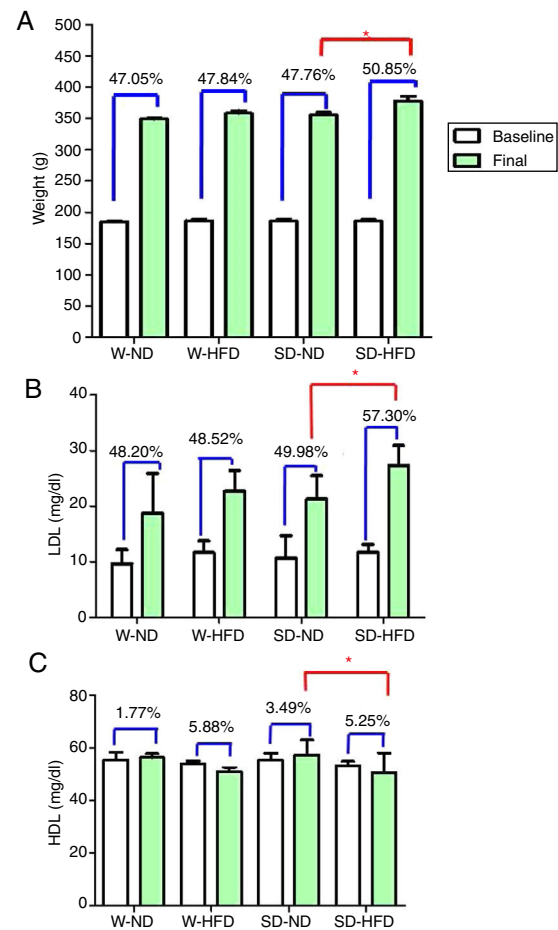


**Figure 2** The comparison of calorie intake (A), water consumption (B) and visceral fat weight (C) in Wistar and Sprague Dawley rats fed either standard diet (ND) or high fat diet (HFD). Data are expressed as mean  $\pm$  standard deviations. W-ND; Wistar rats fed standard diet, W-HFD; Wistar rats fed a high fat diet, SD-ND; Sprague Dawley rats fed standard diet, SD-HFD; Sprague Dawley rats fed a high fat diet. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

found between Wistar and SD rats fed with a high fat diet. Additionally, the significant difference was not observed in weight of heart among different groups.

### Lipid profiles

After feeding different kinds of diet in both strains of rats for 4 weeks, LDL levels increased from baseline. The significant increase of LDL was found in SD rats fed with HFD in comparison to rats in SD-ND groups (Fig. 3B). HDL levels changed from baseline (Fig. 3C). The significant decrease in HDL levels was found in SD rats fed with HFD in comparison to rats in SD-ND groups. Nevertheless, no significant changes in total cholesterol and triglyceride were observed (Table 2).



**Figure 3** The body weight (A), LDL (B) and HDL (C) at baseline and at the end of the experiment. Data are expressed as mean  $\pm$  standard deviations. W-ND; Wistar rats fed standard diet, W-HFD; Wistar rats fed a high fat diet, SD-ND; Sprague Dawley rats fed standard diet, SD-HFD; Sprague Dawley rats fed a high fat diet. \* $p < 0.05$ .

### Histopathological analysis

According to histopathology, microscopic images in Fig. 4A and B showed that the myocardial fibers of the rats fed with standard diet in both strains were found to be arranged in regular pattern with clear striations. There was no evidence of degeneration or necrosis. The nuclei appeared round or oval. In contrast, after feeding a high-fat diet for 4 weeks, the cardiac muscle structure of SD-HFD rats showed higher amounts of the mononuclear cell infiltration associated inflammation, dilated congestion of vascular related to myocyte damage, and enhancement of perivascular fat surrounding the blood vessel when compared with W-HFD rats (Fig. 4C and D).

### Discussion

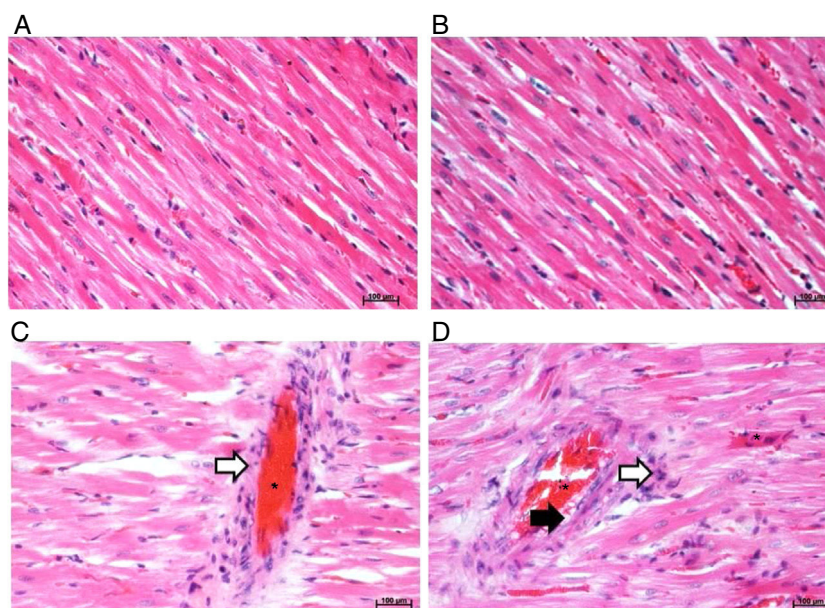
Obesity and overweight induced by high fat diet have been shown to be associated with cardiac pathology alterations.<sup>12,13</sup> High fat diet can also have influences on increasing body weight, visceral fat, and lipoprotein



**Table 2** Changes of total cholesterol and triglyceride between at baseline and at the end of the experiment.

Groups	W-ND		W-HFD		SD-ND		SD-HFD	
	Baseline	Final	Baseline	Final	Baseline	Final	Baseline	Final
Total cholesterol (mg/dL)	98.33 ± 4.51	105.67 ± 17.39	96.67 ± 4.93	123.67 ± 10.67	96.67 ± 6.11	106.00 ± 17.35	97.00 ± 8.00	124.33 ± 4.62
% change		6.95		21.83		8.80		21.98
Triglyceride (mg/dL)	58.33 ± 5.03	87.33 ± 13.07	64.00 ± 1.19	86.33 ± 24.85	62.33 ± 7.53	78.67 ± 5.86	55.67 ± 5.13	79.33 ± 17.21
% change		33.20		25.87		20.77		29.82

Data are expressed as mean ± SD,  $n = 3$ . W-ND; Wistar rats fed standard diet, W-HFD; Wistar rats fed a high fat diet, SD-ND; Sprague Dawley rats fed standard diet, SD-HFD; Sprague Dawley rats fed a high fat diet.  $p < 0.05$  represent significant differences compared with rats fed standard diet in the same species. % change compared between final and baseline.



**Figure 4** Histopathology of H&E stained ventricular cardiac tissues (200x magnification). A: Wistar rat fed standard diet, B: SD rat fed standard diet, C: Wistar rat fed high fat diet and D: SD rat fed high fat diet. Asterisks represent dilated congestion of blood vessel. White arrows represent the accumulation of mononuclear cells associated with inflammation. Black arrow represents perivascular fat surrounding the blood vessel.

metabolism. In the present study, the higher body and adipocyte weights were found in all groups of rats fed with high fat diet when compared to those fed with standard diet. Rats which received diet with excess energy may also synthesize and accumulate more adipose tissue as triacylglycerol<sup>14</sup> than those which get fed with standard diet, resulting in higher visceral fat weight and the dyslipidemic effects associated with obesity and overweight.<sup>15</sup>

Our data also indicated that Sprague-Dawley (SD) rats showed more complicated effects from high fat diet consumption when compared to Wistar rat. Similarly to our study, other studies in SD rats showed that rats which were fed with 61% fat diet for 8 weeks,<sup>16</sup> 40–45% fat diet for 3–4 weeks<sup>17</sup> and 60–70% fat diet for 2 weeks<sup>18</sup> could develop obesity and increase visceral fat mass. However, it was reported that Wistar rats could increase weight, body fat mass more pronouncedly than SD rats when fed with high fat diet for 17

weeks.<sup>5</sup> This may be the reason that Wistar rats consumed higher amounts of food and energy than SD rats at that time.

Lard oil, a major fat of diet in this study, contains high levels of saturated fatty acid (SFA) and cholesterol which are the greatest adverse factors affecting LDL and HDL concentrations which are associated with cardiovascular diseases (CVD), such as arteriosclerosis, stroke, and myocardial infarction.<sup>7</sup> Fatty acids regulate transcription factors, such as the peroxisome proliferator activated receptors (PPARs) and sterol regulatory element binding proteins (SREBPs) which are related to the synthesis and the reverse uptake of cholesterol, fatty acids, triglycerides, and phospholipids.<sup>19</sup> Consequently, lard oil in this study contributed to obesity and dyslipidaemia in rats, especially in SD rats. Similar to the study of Jia and colleagues,<sup>7</sup> they studied in 3-week-male SD rats fed with high fat diet (4.39 kcal/g, 47% calories from fat). After 4-week feeding diet, serum LDL concentrations of

rats increased while HDL concentrations of rats significantly decreased. AbdulKadir and his team<sup>20</sup> also showed that in 8-week-old male SD rats fed with high fat diet (4.14 kcal/g and 40% fat) for 10 weeks, there was a significant increment of LDL and a significant decrement of HDL similarly to the results of Asrullah's study in SD rats fed with high fat diet for 28 days.<sup>21</sup> Male Sprague Dawley rats fed with high-fat lard diet for 24 weeks also showed the significantly increased triglyceride (TG) levels.<sup>22</sup>

In recent study, cardiac pathological alterations were found in rats fed with high fat diet, especially in SD rats. The accumulation of mononuclear cells associated with inflammation and perivascular fat surrounding the blood vessel was evident. The appearance of mononuclear cell infiltration with extravasation of red blood cells in the heart tissue may indicate the inflammatory response.<sup>23</sup> Stemmer suggested that obesity can induce the low-grade inflammation by releasing of inflammatory adipocytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6) from enlarged adipose tissue. Due to these adipocytokines, they can adversely affect various non-adipose target tissues.<sup>24</sup> In addition, perivascular fat surrounding the blood vessel was also observed in this study. Montani<sup>25</sup> proposed that obesity could lead to lipid accumulation in tissues and even organs. Surrounding blood vessels, there are lipid deposits, called perivascular fat, which may affect vascular function at least through the secretion of proatherogenic cytokines. This can contribute to the increased vascular stiffness. Therefore, perivascular fat may be a key factor of the impairment of vascular functions and increasing cardiovascular diseases prevalence.

In this study, rats fed with high fat diet tended to consume higher amount of water. It is possibly because more water is required for the enzymatic reactions in lipid digestion and absorption. Water is essential for the integrity of the structure of protein molecule used for digestion, especially in enzymatic hydrolysis reactions.<sup>26</sup> Lipase enzyme is one of the significant enzymes composed of water. It hydrolyzes triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol.<sup>27,28</sup> In addition, bile salt consists of approximate 95% of water. Its role is to emulsify dietary fats and facilitate their intestinal absorption which is the major route for elimination of cholesterol.<sup>29</sup> For these reasons, rats fed with high fat diet had a tendency to drink more water than those fed standard diet.

## Conclusion

The results of this study showed the enhancement of blood LDL, the low level of HDL concentrations, the infiltration of mononuclear cells, and the high amounts of perivascular fat in cardiac tissue in SD rats fed with high fat diet when compared to Wistar rats fed with the same diet. Therefore, SD rats may be the more suitable model for dyslipidemia induced by high fat diet.

## Conflict of interest

The authors have no conflicts of interest to declare.

## Acknowledgements

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## References

1. Leong XF, Ng CY, Jaarin K. Animal models in cardiovascular research: hypertension and atherosclerosis. *BioMed Res Int*. 2015;1–11. Article ID 528757.
2. Mashimo T, Voigt B, Kuramoto T, Serikawa T. Rat phenome project: the untapped potential of existing rat strains. *J Appl Physiol*. 2005;98:371–9.
3. Bergen WG, Mersmann HJ. Comparative aspects of lipid metabolism: impact on contemporary research and use of animal models. *J Nutr*. 2005;135:2499–502.
4. Otunola GA, Oloyede OB, Oladiji AT, Afolayan AA. Effects of diet-induced dyslipidemia on the lipid profile and some enzyme activities in female Wistar rats. *Afr J Biochem Res*. 2010;4:149–54.
5. Marques C, Meireles M, Norberto S, Leite J, Freitas J, Pestana D, et al. High-fat diet-induced obesity rat model: a comparison between Wistar and Sprague-Dawley rat. *Adipocyte*. 2016;5:11–21.
6. Sasidharan SR, Joseph JA, Anandakumar S, Venkatesan V, Madhavan CNA, Agarwal A. An experimental approach for selecting appropriate rodent diets for research studies on metabolic disorders. *BioMed Res Inter*. 2013;2013:752870.
7. Jia YJ, Liu J, Guo YL, Xu RX, Sun J, Li JJ. Dyslipidemia in rat fed with high-fat diet is not associated with PCSK9-LDL-receptor pathway but ageing. *J Geriatr Cardiol*. 2013;10:361–8.
8. Zaman MQ, Leray V, Le Bloc'h J, Thorin C, Ouguerram K, Nguyen P. Lipid profile and insulin sensitivity in rats fed with high-fat or high-fructose diets. *Br J Nutr*. 2011;106:S206–10.
9. Bhandari U, Kumar V, Khanna N, Panda BP. The effect of high-fat diet-induced obesity on cardiovascular toxicity in wistar albino rats. *Hum Exp Toxicol*. 2011;9:1313–21.
10. National Research Council (US) Committee for the update of the guide for the care and use of laboratory animals. *Guide for the Care and Use of Laboratory Animals*. 8th edition Washington (DC): National Academies Press (US); 2011.
11. Lim AY, Segarra I, Chakravarthi S, Akram S, Judson J. Histopathology and biochemistry analysis of the interaction between sunitinib and paracetamol in mice. *BMC Pharmacol*. 2010;10:14.
12. van der Heijden RA, Sheedfar F, Morrison MC, Hommelberg PP, Kor D, Kloosterhuis NJ, et al. High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice. *Aging (Albany NY)*. 2015;7:256–67.
13. Norazmir MN, Ayub MY, Ummi MMA. Enzyme activities and histology study on high fat diet-induced obese rats by Pink Guava Puree. *Pak J Nutr*. 2010;9:1100–6.
14. Ahmadian M, Duncan RE, Jaworski K, Sarkadi-Nagy E, Sul HS. Triacylglycerol metabolism in adipose tissue. *Future Lipidol*. 2007;2:229–37.
15. Sour S, Belarbi M, Sari N, Benammar CH, Baghdad CH, Visioli F. Argan oil reduces, in rats, the high fat diet-induced metabolic effects of obesity. *Nutr Metab Cardiovasc Dis*. 2015;25:382–7.
16. Park S, Kim YW, Kim JY, Jang EC, Doh KO, Lee SK. Effect of high fat diet on insulin resistance: dietary fat versus visceral fat mass. *J Korean Med Sci*. 2001;16:386–90.
17. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. *Nutr Res Rev*. 2010;23:270–99.
18. Gajda AM, Pellizzon MA, Ricci MR, Ulman EA. Diet-induced metabolic syndrome in rodent models. *Anim LABNEW S*. 2007.

19. Fernandez ML, West KL. Mechanisms by which dietary fatty acids modulate plasma lipids. *J Nutr.* 2005;135:2075–8.
20. Kadir NAAA, Rahmat A, Jaafar HZE. Protective effects of Tamarillo (*Cyphomandra betacea*) extract against high fat diet induced obesity in Sprague-Dawley rats. *J Obes.* 2015;2015:846041.
21. Asrullah M, Lestari LA, Helmyati S, Farmawati A. The effect of mung bean sprouts (*Phaseolus radiatus* L.) to lipid profile of male Sprague-Dawley rats fed with high-fat diet. *AIP Conf Proc.* 2016;1755:140001.
22. Jurgoński A, Juśkiewicz J, Zduńczyk Z. A high-fat diet differentially affects the gut metabolism and blood lipids of rats depending on the type of dietary fat and carbohydrate. *Nutrients.* 2014;6:616–26.
23. Abdelhalim MAK. Gold nanoparticles administration induces disarray of heart muscle, hemorrhagic, chronic inflammatory cells infiltrated by small lymphocytes, cytoplasmic vacuolization and congested and dilated blood vessels. *Lipids Health Dis.* 2011;10:233.
24. Stemmer K, Perez-Tilve D, Ananthakrishnan G, Bort A, Seeley RJ, Tschöp MH, et al. High-fat-diet-induced obesity causes an inflammatory and tumor-promoting microenvironment in the rat kidney. *Dis Model Mech.* 2012;5:627–35.
25. Montani JP, Carroll JF, Dwyer TM, Antic V, Yang Z, Dulloo AG. Ectopic fat storage in heart, blood vessels and kidneys in the pathogenesis of cardiovascular diseases. *Int J Obes Relat Metab Disord.* 2004;28:S58–65.
26. Sattari S, Vahabzadeh F, Aghtaei HK. Performance of Loofa-Immobilized *Rhizopus Oryzae* in the enzymatic production of biodiesel with use of oleic acid in n-hexane medium. *Braz J Chem Eng.* 2015;32:367–76.
27. Houde A, Kademi A, Leblanc D. Lipases and their industrial applications: an overview. *Appl Biochem Biotechnol.* 2004;118:155–70.
28. Páez BC, Medina AR, Rubio FC, Moreno PG, Grima EM. Modeling the effect of free water on enzyme activity in immobilized lipase-catalyzed reactions in organic solvents. *Enzyme Microb Technol.* 2003;33:845–53.
29. Boyer JL. Bile formation and secretion. *Comp Physiol.* 2013;3:1035–78.