Different Effects of Lard and Vegetable Blend Oil on Intestinal Microorganisms, Enzyme Activity and Blood Routine in Mice

Bo Qiao, Xiaoya Li, Tao Zheng*, and Zhoujin Tan*

Hunan University of Chinese Medicine, Changsha, Hunan, CHINA

Abstract: The intake of moderate oils and fats is necessary to maintain the body’s energy balance, and the fatty acid composition of different oils and fats varies in their nutrition and function. The study aimed to investigate the effects of lard and vegetable blend oil on gut microbiota, intestinal enzyme activities, and blood routine. Kunming mice were assigned to the three groups: (1) Control group (CK) was gavage administration with distilled water, (2) Plant oil group (ZWY) was gavage administration with edible vegetable blend oil, (3) Lard group (DWY) was gavage administration with lard. After 42 days, microbiological, digestive enzymes, and blood routine were performed. Compared with the CK group, Escherichia coli, Lactobacilli, and Bifidobacteria were significantly decreased (p < 0.05), the activities of protease, cellulase, amylase, and xylanase were markedly reduced (p < 0.05), the hemoglobin was significantly increased (p < 0.05) in the ZWY group and DWY groups, and the hematocrit was increased in the ZWY group (p < 0.05), while other routine blood indices were increased (p > 0.05). Compared to the ZWY group, the activity of cellulase and amylase were significantly increased (p < 0.05), the intestinal microbiome and the routine blood indexes had no significant difference in the DWY group. Lard and vegetable blend oil diet affected the composition of the intestinal microorganisms, and the functions of digestive enzymes. Meanwhile, the levels of digestive enzymes may be correlated with the intestinal microbiota.

Key words: lard, vegetable blend oil, intestinal microorganism, enzyme activity, blood routine

1 Introduction

The dietary oils and fats is an important nutrient, which provides energy, essential fatty acids, and a variety of fat-soluble vitamins for the human. With the change in diet and the continuous improvement of living standards in recent years, the demand for edible oils and fats is increasing and diverse. These include olive oil, which is rich in monounsaturated fatty acids (MUFAs), soybean oil, which is rich in polyunsaturated fatty acids (PUFAs), and lard, which is rich in saturated fatty acids (SFAs)1. According to the Chinese nutrition society, it has been reported that the consumption of edible oil has increased to 40.8 g/d, which is far beyond the recommendation that should be taken per day (25 g/d)2. Researchers3–5 have found that high-fat diets (HFD) induce a variety of metabolic diseases, such as diabetes, hypertension, and hyperlipidemia. However, the occurrence of these diseases is closely correlated with the intestinal micro-ecosystem6–8.

The gut microbiota that inhabits the gastrointestinal tract is a complex microbial community, and plays an important role in maintaining human health and micro-ecological balance9. The specificity of gut microbiota in response to the internal and external environment of the host, particularly the effect of dietary pattern on the structure of intestinal microorganisms10. Researches11,12 indicated that the composition and content of edible oils strongly affect intestinal microorganisms’ composition and function. HFD caused intestinal microorganisms dysbiosis, which in turn led to a spectrum of diseases. In healthy people, the majority of fatty acids consumed in the diet are accessible to the bloodstream through the efficient process of digestion and absorption13,14. Therefore, blood routine could be applied on early diagnosis of disease and the nutritional evaluation of diet.

In a preliminary experiment15, we confirmed that proper intake of vegetable blend oil contributed to promoting the...
growth of prebiotics and increasing enzyme activities. Therefore, this research aimed to investigate the different effects of lard (mainly SFA) or vegetable blend oil (mainly UFA) on blood routine, gut enzyme activities, and intestinal microorganisms through the in vivo animal experiment. To explore the correlation between different oils and health, as well as to provide the scientific basis for a food guide.

2 Materials and Methods

2.1 Animals

Eighteen specific pathogen-free (SPF) Kunming mice (half male and half female, 20 ± 2 g) were obtained from Slakes experimental Co., Ltd (license number: SCXK (Xiang)2019-0009). The mice were raised under stable conditions (temperature 23–25°C, relative humidity 50-70%) in the laboratory animal center of Hunan University of Chinese Medicine. The process of animal experiments was conducted under animal protocols approved by the Animal Ethics and Welfare Committee of the Hunan University of Chinese Medicine.

2.2 Diets

The compositions of diet are corn starch 30%, soybean meal 29%, wheat 26%, salt 1%, bone meal 1%, lysine 1%, and water 12%, which provided by the laboratory animal center of the Hunan University of Chinese Medicine. Lard and vegetable blend oil (brand: Arowana Golden Ratio Edible Blended Oil, manufacturer: Yihai Jiali Yueyang) grain and oil industry Co., Ltd, license number: SC10243060200170 were purchased in Wal-Mart, Changsha, Hunan. Vegetable blend oil is composed of soybean oil 49.0%, corn oil 9.0%, rapeseed oil 23.5%, sunflower seed oil 14.0%, peanut oil 0.5%, sesame oil 0.6%, flaxseed oil 0.4%, and rice oil 3.0%. In our experiment, the ratio of SFAs and UFAs for vegetable blend oil is 1:6.2, and lard is 1:0.95.

2.3 Animal groups

After the adaptation period, Kunming mice were randomly divided into three groups (n = 6 per group): (1) Control group (CK) was treated with distilled water, (2) Plant oil group (ZWY) was treated with edible vegetable blend oil, (3) lard group (DWY) was treated with lard. Dose gavage was 0.2 mL twice a day for 42 consecutive days. As shown in Fig. 1, experimental design and general conditions of the animals.

2.4 Extraction of intestinal contents

After 42 days, the intestinal contents (jejunum to the rectum) of all the mice in each group were collected in a sterile environment and stored at 4°C.

2.5 Determination of microorganisms in intestinal contents

Bacteria: beef extract-peptone agar medium; E. coli: eosin-methylene blue agar medium; Lactobacillus spp.: deMan Rogosa Sharpe agar medium; Bifidobacteria spp.: Bifidobacteria agar medium. The number of gut microorganisms was measured by the plate count method. Under sterile conditions, a certain amount of intestinal content was weighed into sterile water bottles containing glass beads. After shaking at 120 rpm for 30 min, tenfold dilutions were made and cultures were inoculated by the smearing. Total numbers of bacteria and E. coli were detected after being cultured at 37°C for 24 h, the numbers of Lactobacillus and Bifidobacteria were determined after being anaerobically cultured at 37°C for 48 h. Each dilution was repeated three times, averaged and the number of colonies per gram of gut contents was calculated.

2.6 Analysis of enzyme activity in intestinal contents

Under sterile conditions, a certain amount of the intestinal contents were taken hereafter, placed in triangular bottles containing sterile water, and heated in a water bath at 40°C for 30 min to completely release the enzymes from the intestinal contents. Then the contents were centrifuged at 3000 rpm for 10 min and the enzyme activities in the supernatant were analyzed by UV spectrophotometer. The activities of amylase, xylanase, and cellulase were determined by the DNS colorimeter method and the protease activity was determined by the Feline-phenol method.

2.7 Routine blood examination

Blood samples (1.5-2.0 mL) were taken from mice. The blood was injected into EDTA-K2 anticoagulant tubes and mixed evenly. Blood samples were sent to the laboratory of the First Affiliated Hospital of Hunan University of Chinese Medicine for testing within 2 h.

2.8 Statistical analysis

Data were expressed as mean ± standard deviation (mean ± SD). Statistical analysis was performed by
3 Results

3.1 Observation of mice performance

All animals had similar body weights when the experiments started. After 42 days of the experiment, the weight of all groups had no significant difference (Fig. 2). There were no deaths and abnormalities throughout the experiment.

3.2 Effects of different edible oil intake on the intestinal microorganisms of mice

The intestinal microorganisms play an important role in human energy regulation, nutrient absorption, and immunity\textsuperscript{16}. However, the intestinal microorganisms composition and relative abundance usually change in response to the external environment, particularly the dietary pattern\textsuperscript{17}. From Fig. 3, it appears that the intestinal microorganisms of mice were altered to various degrees after intake of plant oil and lard. Compared with the CK group, *Escherichia coli*, *Lactobacilli*, and *Bifidobacteria* were significantly decreased in the ZWY group and DWY group (\(p < 0.05\)). Compared to the ZWY group, the number of *Lactobacilli* and *Bifidobacteria* were decreased in the lard group (\(p > 0.05\)). The above results suggested that lard and plant oil intake had a large effect on the intestinal microorganisms of mice. Among them, the effect of lard intake on intestinal microorganisms is more significant, which inhibits the growth of probiotics such as *Lactobacillus* and *Bifidobacterium*, induced intestinal microorganisms dysbiosis.

3.3 Effects of different edible oil intake on the intestinal enzymatic activity of mice

Most nutritional components are too complex for immediate use and must be broken down into simpler compounds, which can then be absorbed by the body\textsuperscript{18}. This

---

**Fig. 2** Change of body weight in mice. Data were expressed as (mean ± SD). Statistical analysis was used by one-way ANOVA followed by Tukey’s test.

**Fig. 3** Effect of edible oil intake on intestinal microorganisms. Data were expressed as (mean ± SD). Statistical analysis was used by one-way ANOVA followed by Tukey’s test (*\(p < 0.05\)).
The digestive process is catalyzed by enzymes that are either endogenous or produced by the host’s microbial population. As shown in Fig. 4, the activities of amylase, protease, xylanase, and cellulase in the ZWY group and the DWY group were significantly lower than in the CK group ($p < 0.05$). The activities of amylase and cellulase in the DWY group were significantly higher than the ZWY group ($p < 0.05$), and there was no significant difference in the activities of protease and xylanase between these two groups ($p > 0.05$). Intestinal enzyme activity affects the body’s absorption of nutrients and intestinal vitality. Edible oil intake would reduce the activities of those four intestinal enzymes, and plant oil was more serious than lard in terms of reduced amylase and cellulase activities.

3.4 Effects of different edible oil intake on the blood routine of mice

Blood routine examination contains three systems including white blood cells, red blood cells, and platelets, and is one of the basic clinical examination items. White blood cells were increased, suggesting inflammation $^{19, 20}$. In Fig. 5, the white blood cell count in the ZWY group was a little higher than the CK group and DWY group, but there was no significant difference between the three groups ($p > 0.05$).

Platelets are key in promoting intravascular thrombus formation in infection, a process termed ‘immunothrombosis’, which contributes to containing pathogens, but also potentially damages the host $^{21, 22}$. We can know from Table 1, plant oil and lard intake could increase the platelet count, mean platelet volume, and platelet distribution width ($p > 0.05$), and the DWY group was higher than the ZWY group ($p > 0.05$). It was suggested that lard could promote platelet activity.

The main function of red blood cells is to transport...
Table 1  Effect of edible oil intake on platelet.

|                | CK group | ZWY group | DWY group |
|----------------|----------|-----------|-----------|
| PLT ($10^9$·L$^{-1}$) | 787.00 ± 525.19 | 829.00 ± 130.32 | 865.75 ± 255.69 |
| PCT (mL·L$^{-1}$)    | 0.66 ± 0.18  | 0.59 ± 0.08  | 0.63 ± 0.16  |
| MPV (Fl)             | 6.85 ± 0.34  | 7.20 ± 0.36  | 7.38 ± 0.33  |
| PDW (%)              | 7.52 ± 0.52  | 8.12 ± 0.28  | 8.42 ± 0.42  |

Data were expressed as (mean ± SD). Statistical analysis was used by one-way ANOVA followed by Tukey’s test. PLT, platelet; PCT, platelet crit; MPV, mean platelet volume; PDW, platelet distribution width.

Table 2  Effect of edible oil intake on red blood cell.

|                | CK group | ZWY group | DWY group |
|----------------|----------|-----------|-----------|
| RBC ($10^{12}$·L$^{-1}$) | 10.15 ± 0.42 | 10.84 ± 0.77 | 10.38 ± 0.73 |
| HCT (L·L$^{-1}$) | 48.22 ± 0.36$^a$  | 52.88 ± 2.98$^b$  | 50.12 ± 1.13$^a$  |
| MCV (fL)      | 47.60 ± 2.03  | 48.82 ± 1.04  | 48.52 ± 4.30  |
| RDW (%)       | 28.20 ± 1.63  | 29.02 ± 1.13  | 29.45 ± 0.70  |

Data were expressed as (mean ± SD). Statistical analysis was used by one-way ANOVA followed by Tukey’s test. Different letters indicated significant differences at $p<0.05$ among groups. RBC, red blood cell count; HCT, hematocrit; MCV, mean corpuscular volume; RDW, red cell distribution width.

Table 3  Effect of edible oil intake on hemoglobin.

|                | CK group | ZWY group | DWY group |
|----------------|----------|-----------|-----------|
| HGB (g·L$^{-1}$) | 150.75 ± 1.50$^a$ | 166.25 ± 8.06$^b$ | 159.00 ± 1.41$^b$ |
| MCH (pg)       | 14.88 ± 0.52  | 15.35 ± 0.37  | 15.38 ± 1.04  |
| MCHC (g·L$^{-1}$) | 312.50 ± 5.07 | 314.50 ± 4.65 | 317.25 ± 7.93 |

Data were expressed as (mean ± SD). Statistical analysis was used by one-way ANOVA followed by Tukey’s test. Different letters indicated significant differences at $p<0.05$ among groups. HGB, Hemoglobin; MCH, Mean red blood cell hemoglobin content; MCHC, mean corpuscular hemoglobin concentration.

Oxygen, and a decrease in red blood cells, hemoglobin, and hematocrit is suggestive of anemia, and an increase is suggestive of polycythemia. The red blood cell hemoglobin content and mean corpuscular hemoglobin concentration had no significant difference within these three groups ($p > 0.05$). Table 2 showed that compared with the CK group, the ZWY group was significantly higher in hematocrit ($p < 0.05$). Meanwhile, the ZWY group and the DWY group in red blood cell count, hematocrit, mean corpuscular volume, and the red cell distribution width were higher than the CK group ($p > 0.05$). Compared with the ZWY group, the DWY group had a lower red blood cell count, hematocrit, mean corpuscular volume, and higher red blood cell distribution width, but there was no significant difference ($p > 0.05$). The result was illustrated in Table 3, compared with the CK group, plant oil and lard intake could increase the hemoglobin content of the mice ($p < 0.05$), but there was no significant difference in the hemoglobin content between the ZWY group and the DWY group ($p > 0.05$).

3.5 Correlation between intestinal enzyme activities and intestinal microorganisms

Pearson correlation analysis was performed. Intestinal enzyme activity is closely related to intestinal microorganisms, so the correlation between the two was analyzed. As can be seen in Fig. 6, the total bacteria count was significantly correlated with amylase, cellulase, and xylanase; *Escherichia coli* and *Bifidobacteria* showed significant positive correlations with amylase, protease, cellulase, xylanase; *Lactobacilli* showed a significant positive correlation with amylase, protease, xylanase. These suggested that intake of lard and vegetable blend oil could affect the function of intestinal digestive enzymes.
Dietary oils and fats are the important macronutrients for humans and in terms of consumption, they are one of the three main classes of food, besides carbohydrates and proteins. They are necessary for energy supplying and maintaining the normal functions of the human body. HF diet easily induces lipid metabolism disorders and oxidative stress injury, which are risk factors for CVDs and metabolic syndrome\(^{24, 25}\). Besides, the long-term HFD intake causes internal environment change of the body, and part of the flora is inhibited, thereby causing dysbacteriosis\(^{26}\). The normal dynamic balance of the intestinal flora is broken, causing the clinical symptom. The type and quality of fatty acids play an important role in human health, and the contents of SFA, MUFA, and PUFA in different oils are not the same\(^{27, 28}\) (Fig. 7). The content of SFA is higher in lard than in plant oil, so lard diet-induced accumulation of body fat, liver and serum lipids, which can increase the risk of obesity, non-alcoholic fatty acid liver disease, and atherosclerosis. The mixed oil diet-induced body fat accumulation, but did not cause lipid accumulation in the liver and serum\(^{29}\). In this study, mixed oil SFA: UFA was 1:6.3, lard was 1:0.95. Aimed to investigate the effects of edible oils of different compositions on blood routine, intestinal enzyme activities, and intestinal microorganisms in mice.

The intestinal microorganisms can be altered by the in-

**Fig. 6** Correlation coefficient matrix. Correlation between intestinal enzyme activities and gut microbiota used Pearson correlation analysis. Blue represented a positive correlation. Significant correlations were noted by \(*p<0.05\).

**Fig. 7** The relative content of fatty acids between plant and animal oil (%). Note: SFA, saturated fatty acid; MUFA, saturated fatty acid; PUFA, saturated fatty acids; UFA, unsaturated fatty acid.

### 4 Discussion
Dietary oils and fats are the important macronutrients for humans and in terms of consumption, they are one of the three main classes of food, besides carbohydrates and proteins. They are necessary for energy supplying and maintaining the normal functions of the human body. HF diet easily induces lipid metabolism disorders and oxidative stress injury, which are risk factors for CVDs and metabolic syndrome\(^{24, 25}\). Besides, the long-term HFD intake causes internal environment change of the body, and part of the flora is inhibited, thereby causing dysbacteriosis\(^{26}\). The normal dynamic balance of the intestinal flora is broken, causing the clinical symptom. The type and quality of fatty acids play an important role in human health, and the contents of SFA, MUFA, and PUFA in different oils are not the same\(^{27, 28}\) (Fig. 7). The content of SFA is higher in lard than in plant oil, so lard diet-induced accumulation of body fat, liver and serum lipids, which can increase the risk of obesity, non-alcoholic fatty acid liver disease, and atherosclerosis. The mixed oil diet-induced body fat accumulation, but did not cause lipid accumulation in the liver and serum\(^{29}\). In this study, mixed oil SFA: UFA was 1:6.3, lard was 1:0.95. Aimed to investigate the effects of edible oils of different compositions on blood routine, intestinal enzyme activities, and intestinal microorganisms in mice.

The intestinal microorganisms can be altered by the in-

**Fig. 6** Correlation coefficient matrix. Correlation between intestinal enzyme activities and gut microbiota used Pearson correlation analysis. Blue represented a positive correlation. Significant correlations were noted by \(*p<0.05\).

**Fig. 7** The relative content of fatty acids between plant and animal oil (%). Note: SFA, saturated fatty acid; MUFA, saturated fatty acid; PUFA, saturated fatty acids; UFA, unsaturated fatty acid.
ternal and external environments, and the intestinal microorganisms are associated with pathology, physiology, and various diseases. HFD induces inflammation by increasing endotoxin levels in the intestinal lumen as well as in the plasma by altering the composition of the intestinal microorganisms and increasing its intestinal permeability. Excessive fat intake by the organism, which leads to overload of the mitochondrial electron transport chain, easily causes oxidative stress in the intestinal tissue and changes the microenvironment in the intestine, resulting in a disturbance of the intestinal microorganism. Bifidobacteriales and Lactobacillales are probiotics of the intestinal tract, which inhibit the growth of pathogenic microorganisms and protect the intestinal mucosa by producing bacteriocins or organic acids. Conversely, reduction of probiotics impairs intestinal homeostasis and the intestinal mucosal barrier, promotes endotoxin production and growth, and leads to intestinal inflammation. In terms of Bifidobacteriales, gram-positive bacteria are associated with inflammation development. SFA and high in unsaturated fats diets have negative correlations with the abundance of Bifidobacteriales. The abundance of Lactobacillales decreased after intake of HFD. A previous study revealed that Lactobacillales have anti-obesity activities, and HFD has harmful effects on Lactobacillales. Our results suggested that Escherichia coli, Lactobacillales, and Bifidobacteriales were significantly decreased in the ZWY group and the DWY group. As opposed to passer-by bacteria, most of the gut flora are long-term host microorganisms. The cycle of this experiment was only 42 days, while the impact of edible oil may be long-term chronic, which explains the small differences in intestinal microorganisms diversity and abundance after ingestion of lard and vegetable blended oils.

Enzymes control all metabolic processes in the human system from simple digestion of food to highly complex immune response. Physiological reactions occurring in healthy individuals are disturbed when enzymes are deficient or absent. Bacillus is the main gut microbes producing proteases and xylanase, as well as cellulase producing Bacteroides, Clostridium, prebacteroides. In addition, the gut can also produce digestive enzymes. In this study, we found that the plant oil or lard continued intake reduced the activities of proteases, xylanases, amylases, and cellulases. Correlation analysis by intestinal microorganisms and enzyme activity, we found that the main reason was a decrease in the number of enzyme-producing gut microbes. The reason may be due to the accumulation of acid metabolites produced by intestinal microorganisms associated with intestinal oil metabolism that lower the pH of the gut environment. Plant oils significantly reduced the activities of amylase and cellulase, the reason may be that plant oils contain more PUFA, and PUFA can be transformed into isomerized PUFA (trans-fatty acids) by Lactobacillus, Escherichia, Bacteroides. When the trans-fatty acids accumulate to a certain extent, they may exert opposite effects, inhibiting the growth of amylase and cellulase-producing gut microbes. And the higher amount of dietary carbohydrates in plant oils than that of lard.

A variety of fatty acids exists in the diet of humans, in the bloodstream of humans, and the cells and tissues of humans. It was obvious that within any fatty acid class, different members have different actions and effects. It was reported that a high intake of SFA increases the risks of coagulation, inflammation, and insulin resistance. However, moderate amounts of UFA are good for health and can replace common saturated fatty acids. The blood routine can be sensitive to pathological alterations and related conditions that occur in human function, which has a positive reference value for multiple disease tests. RBC parameters included RBC count, RBC cloth width, the mean volume of RBC, and hematocrit. Research has indicated that elevated HCT levels may be positively associated with cardiovascular risk factors. Thus, the combination of HCT values and cardiovascular risk factors may enable the early diagnosis of cardiovascular diseases (CVDs). The erythrocyte count, hematocrit, mean corpuscular volume, and red cell distribution width in the lard and plant oil groups were higher than those in the control group in this study. Hemoglobin is one of the markers for assessment of the occurrence, development, and prognosis of cardiovascular disease, and it can monitor the condition better in combination with other indicators. We found a significant increase in hemoglobin after ingestion of lard and plant oil, which was more pronounced with lard. The parameters of platelets consist of platelet count, platelet specific volume, mean platelet volume, and platelet volume distribution width, where platelet volume distribution width is a relatively specific indicator of platelet activation. Platelet activation is essential for thrombus formation. Through this study, we found that the platelet count, platelet mean volume, and platelet distribution width was slightly higher in the lard and plant oil groups than in the control group, and higher in the lard group than in the plant oil group. If an abnormally elevated white blood cell count is present, patients may present with conditions such as uremia, pyogenic infections, and leukemia, whereas patients with an abnormally decreased white blood cell count may present with associated conditions such as cirrhosis, anemia, and hyperthyroidism. The impact of edible oil may be long-term chronic. Research has found that a high-fat diet containing lard accelerated lipid and glucose metabolism for 4 weeks. We found that the values of indicators such as hematocrit, hemoglobin, platelet distribution width, and white blood cell count had an elevated trend after the intake of lard and plant oil in mice. Whereas these indicators are closely associated with several CVDs, such as coronary heart disease, hypertension, and slow coronary reflow.
suggesting that regardless of the type of fats and oil, a long-term high-fat diet will have a negative impact.

5 Conclusion
In summary, short-term fats and oil intake affected the composition of the gut microbiota, and the functions of digestive enzymes. Meanwhile, multiple indicators related to CVDs appear abnormal in blood routine, which indicates that long-term excessive intake of saturated fatty acids is detrimental to human health. This study initially explored the effects of different edible oils on intestinal microorganisms and health, and we will focus on the different effects of the length of fatty acid chains and the degree of fatty acid saturation on intestinal microorganisms. Therefore, reasonable and effective fats and oils intake may be a key factor in disease prevention.

Acknowledgments
We thank all the scholars who provided relevant guidance for the study.

Contributions
Z. T. and T. Z. accomplished conceptualization. B. Q. was responsible for writing the manuscript. B. Q., X. L., and T. Z. performed experiments and data curation mainly. All authors approved the manuscript.

Availability of Data and Materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

Human and Animal Rights Statement
The study was approved by the Animal Ethics and Welfare Committee of Hunan University of Chinese Medicine.

References
1) Heidari, M.; Talebpour, Z.; Abdullahpour, Z.; Adib, N.; Ghanavi, Z.; Aboul-Enein, H.Y. Discrimination between vegetable oil and animal fat by a metabolomics approach using gas chromatography-mass spectrometry combined with chemometrics. J. Food Sci. Technol. 57, 3415-3425 (2020). doi: 10.1007/s13197-020-04375-9
2) Yang, Y.X.; Wang, X.L.; Leong, P.M.; Zhang, H.M.; Yang, X.G. et al. New Chinese dietary guidelines: healthy eating patterns and food-based dietary recommendations. Asia Pac. J. Clin. Nutr. 27, 908-913 (2018). doi: 10.6133/apjcn.072018.03
3) Yu, H.; Bi, Y.; Ma, W.; He, L.; Yuan, L. et al. Long-term effects of high lipid and high energy diet on serum lipid, brain fatty acid composition, and memory and learning ability in mice. Int. J. Dev. Neurosci. 28, 271-276 (2010). doi: 10.1016/j.ijdeneu.2009.12.001
4) Wang, J.; Yan, S.; Xiao, H.; Zhou, H.; Liu, S. et al. Anti-obesity effect of a traditional Chinese dietary habit-blending lard with vegetable oil while cooking. Sci. Rep. 7, 14689 (2017). doi: 10.1038/s41598-017-14704-2
5) Hidalgo, M.; Prieto, I.; Abriouel, H.; Cobo, A.; Benomar, N. et al. Effect of virgin and refined olive oil consumption on gut microbiota. Comparison to butter. Food Res. Int. 64, 553-559 (2014). doi: 10.1016/j.foodres.2014.07.030
6) Knip, M.; Veijola, R.; Virtanen, S.M.; Hyötty, H.; Vaarala, O.; Akerblom, H.K. Environmental triggers and determinants of type 1 diabetes. Diabetes 54(Suppl.2), S125-S136 (2005). doi: 10.2337/diabetes.54.suppl_2.s125
7) Dethlefsen, L.; McFall-Ngai, M.; Relman, D.A. An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature 449, 811-818 (2007). doi: 10.1038/nature06245
8) Turnbaugh, P.J.; Gordon, J.I. The core gut microbiome, energy balance and obesity. J. Physiol. 587, 4153-4158 (2009). doi: 10.1113/jphysiol.2009.174136
9) Tremaroli, V.; Bäckhed, F. Functional interactions between the gut microbiota and host metabolism. Nature 489, 242-249 (2012). doi: 10.1038/nature11552
10) Rothschild, D.; Weisbrod, O.; Barkan, E.; Kurilshikov, A.; Korem, T. et al. Environment dominates over host genetics in shaping human gut microbiota. Nature 555, 210-215 (2018). doi: 10.1038/nature25973
11) Liu, H.; Zhu, H.; Xia, H.; Yang, X.; Yang, L. et al. Different effects of high-fat diets rich in different oils on lipids metabolism, oxidative stress and gut microbiota. Food Res. Int. 141, 110078 (2021). doi: 10.1016/j.foodres.2020.110078
12) Netto Candido, T.L.; Bressan, J.; Alfenas, R. Dysbiosis and metabolic endotoxemia induced by high-fat diet.
Lard and Vegetable Blend Oil

J. Oleo Sci. 35, 1432-1440 (2018). doi: 10.20960/nh.1792

13) Calder, P.C. Functional roles of fatty acids and their effects on human health. J. Parenter. Enter. Nutr. 39, 18S-32S (2015). doi: 10.1177/0148607115595980

14) Yang, X.Y.; He, Y.S.; Tan, Z.J.; Xiao, D.; Zeng, Y.T.; Zeng, C. Effects of different doses of vegetable oil on intestinal microorganism, enzyme activity and blood routine in mice. Food and Fermentation Industries 46, 101-106 (2020). doi: 10.13995/j.cnki.11-1802/ts.023375

15) Zhu, J.; Cao, K.M. Biochemical Experiments. Shanghai Science and Technology Press, Shanghai (1981).

16) David, L.A.; Maurice, C.F.; Carmody, R.N.; Gootenberg, D.B.; Button, J.E. et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505, 559-563 (2014). doi: 10.1038/nature12820

17) Rininnella, E.; Cintoni, M.; Raoul, P.; Lopetuso, L.R.; Scaldaferrì, F. et al. Food components and dietary habits: Keys for a healthy gut microbiota composition. Nutrients 11, 2393 (2019). doi: 10.3390/nu11022393

18) Janiak, M.C. Digestive enzymes of human and nonhuman primates. Evol. Anthropol. 25, 253-266 (2016). doi: 10.1002/eavan.21498

19) Hoffman, R.; Gerber, M. Can rapeseed oil replace olive oil as part of a Mediterranean-style diet? Br. J. Nutr. 112, 1882-1895 (2014). doi: 10.1017/S0007114514002888

20) Wirth, M.D.; Sevayan, M.; Hofseth, L.; Shivappa, N.; Hurley, T.G.; Hebert, J.R. The Dietary Inflammatory Index is associated with elevated white blood cell counts in the National Health and Nutrition Examination Survey. Brain Behav. Immun. 69, 296-303 (2018). doi: 10.1016/j.bbi.2017.12.003

21) Nicolai, L.; Massberg, S. Platelets as key players in inflammation and infection. Curr. Opin. Hematol. 27, 34-40 (2020). doi: 10.1097/MOH.0000000000000551

22) Koupenova, M.; Clancy, L.; Corkrey, H.A.; Freedman, J.E. Circulating platelets as mediators of immunity, inflammation, and thrombosis. Circ. Res. 122, 337-351 (2018). doi: 10.1161/CIRCRESAHA.117.310795

23) Yin, Y. Utility and implications of routine blood tests in clinical disease. Guide of China Medicine 18, 74-75 (2020). doi: 10.15912/j.cnki.gcm.2020.01.065

24) Rasmussen, B.M.; Vessby, B.; Uusitupa, M.; Berghlund, L.; Pedersen, E. et al. KANWU Study Group. Effects of dietary saturated, monounsaturated, and n-3 fatty acids on blood pressure in healthy subjects. Am. J. Clin. Nutr. 83, 221-226 (2006). doi: 10.1093/ajcn/83.2.221

25) Siri-Tarino, P.W.; Sun, Q.; Hu, F.B.; Krauss, R.M. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am. J. Clin. Nutr. 91, 535-546 (2010). doi: 10.3945/ajcn.2009.27725

26) Saiyasit, N.; Chunchai, T.; Prus, D.; Suparan, K.; Pittayapong, P. et al. Gut dysbiosis develops before metabolic disturbance and cognitive decline in high-fat diet-induced obese condition. Nutrition 69, 110576 (2020). doi: 10.1016/j.nut.2019.110576

27) Yang, C.Y.; Liu, X.M.; Chen, Z.Y. Determination of fatty acid profiles in fifteen kinds of edible vegetable oil by gas chromatography-mass spectrometry. Food Science 34, 211-214 (2013). doi: 10.7506/spxks1002-6630-201306047

28) Wei, Y.S.; Zheng, M.Y.; Geng, W.; Liu, J. Fatty acid composition analysis of common animal fats and vegetable oils. Food Science 33, 188-193 (2012). doi: CNKI:SUN:SPKX.0.2012-16-038

29) Al-Othman, A.A. Growth and lipid metabolism responses in rats fed different dietary fat sources. Int. J. Food Sci. Nutr. 51, 159-167 (2000). doi: 10.1080/0963748005002956

30) Kim, K.A.; Gu, W.; Lee, I.A.; Joh, E.H.; Kim, D.H. High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. PloS One 7, e47713 (2012). doi: 10.1371/journal.pone.0047713

31) Wallace, J.P.; Johnson, B.; Padilla, J.; Mather, K. Postprandial lipaemia, oxidative stress and endothelial function: a review. Int. J. Clin. Pract. 64, 389-403 (2010). doi: 10.1111/j.1742-1241.2009.02146.x

32) Lacroix, S.; Rosiers, C.D.; Tardif, J.C.; Nigam, A. The role of oxidative stress in postprandial endothelial dysfunc- tion. Nutr. Res. Rev. 25, 288-301 (2012). doi: 10.1017/S0954422412000182

33) Alcock, J.; Lin, H.C. Fatty acids from diet and microbiota regulate energy metabolism. F1000Res. 4 (F1000 Faculty Rev), 738 (2015). doi: 10.12688/f1000research.6078.1

34) Amabebe, E.; Robert, F.O.; Agbalalah, T.; Orubu, E. Microbial dysbiosis-induced obesity: Role of gut microbiota in homeostasis of energy metabolism. Br. J. Nutr. 123, 1127-1137 (2020). doi: 10.1017/S0007114520000380

35) Anitha, M.; Reichardt, F.; Tabatabavakili, S.; Nezami, B.G.; Chassaing, B. et al. Intestinal dysbiosis contributes to the delayed gastrointestinal transit in high-fat diet fed mice. Cell. Mol. Gastroenterol. Hepatol. 2, 328-339 (2016). doi: 10.1016/j.jcmgh.2015.12.008

36) Ye, Z.; Cao, C.; Li, R.; Cao, P.; Li, Q.; Liu, Y. Lipid composition modulates the intestine digestion rate and serum lipid status of different edible oils: a combination of in vitro and in vivo studies. Food Funct. 10, 1490-1503 (2019). doi: 10.1039/c8fo01290c

37) UmaMaheswari, T.; Hemalatha, T.; Sankaranarayanan, P.; Puvanakrishnan, R. Enzyme therapy: Current perspectives. Indian J. Exp. Biol. 54, 7-16 (2016). PMID: 26891548

38) Li, A. Mechanism of thermally induced isomerization of edible oils. Food and Fermentation Industries 39, 101-106 (2020). doi: 10.13995/j.cnki.11-1802/ts.023375

J. Oleo Sci.
of unsaturated fatty acids in soybean oil and safety analysis of the products. Diploma Thesis, Chinese Academy of Agricultural Sciences, Beijing (2013).

39) Ge, Y.; Liu, W.; Tao, H.; Zhang, Y.; Liu, L. et al. Effect of industrial trans-fatty acids-enriched diet on gut microbiota of C57BL/6 mice. *Eur. J. Nutr.* **58**, 2625-2638 (2019). doi: 10.1007/s00394-018-1810-2

40) Jin, Y.Z.; Zheng, D.H.; Duan, Z.Y.; Lin, Y.Z.; Zhang, X.Y. et al. Relationship between hematocrit level and cardiovascular risk factors in a community-based population. *J. Clin. Lab. Anal.* **29**, 289-293 (2015). doi: 10.1002/jcla.21767

41) Li, Y.; Dai, Z.Y.; Zhang, D.C. Evaluation on platelet distribution width as a novel specific marker of platelet activation. *Journal of Chongqing Medical University* **36**, 200-202 (2011). doi:10.13406/j.cnki.cyxb.2011.02.011

42) Hashimoto, Y.; Yamada, K.; Tsushima, H.; Miyazawa, D.; Mori, M. et al. Three dissimilar high fat diets differentially regulate lipid and glucose metabolism in obesity-resistant Slc:Wistar/ST rats. *Lipids* **48**, 803-815 (2013). doi: 10.1007/s11745-013-3805-3

---

CC BY 4.0 (Attribution 4.0 International). This license allows users to share and adapt an article, even commercially, as long as appropriate credit is given. That is, this license lets others copy, distribute, remix, and build upon the Article, even commercially, provided the original source and Authors are credited.