Shortening tends to increase aortic foam cell count and wall thickness in male Wistar rats

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BACKGROUND
Shortening is widely used as raw material for bread and other foods. Hypercholesterolemia increases aortic arch foam cell formation and abdominal aortic wall thickness. This study aimed to determine the effect of shortening on the number of aortic arch foam cells and abdominal aortic wall thickness in rats.

METHODS
This study was of experimental posttest control group design. Twenty four male Wistar rats were randomized into 4 groups. The negative control group (C-) received standard feed, the positive control group (C+) standard high-fat feed, group T1 shortening and standard feed at a ratio of 1:5 and group T2 shortening and standard feed at a ratio of 1:10. The interventions were given for 6 weeks through gavage. The foam cell count in the aortic arch and the thickness of the abdominal aortic wall were measured. One-way ANOVA test was used to analyze the data.

RESULTS
There was no significant difference in the mean foam cell count of the aortic arch between the four groups C- (7.17 ± 4.17), C+ (9.33 ± 7.01), T1 (11.83 ± 4.88) and T2 (9.33 ± 6.80) (p=0.598). The mean thickness of the abdominal aortic wall between the four groups C- (741.98 ± 60.67μm), C+ (714.29 ± 90.59μm), T1 (838.90 ± 75.86 μm), and T2 (749.88 ± 99.37μm) also was not significantly different (p=0.110).

CONCLUSION
Shortening tends to increase the foam cell count of the aortic arch and the thickness of the abdominal aortic wall of rats.

Keywords: Shortening, histopathology, aortic arch, abdominal aorta, rats

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INTRODUCTION

Cardiovascular disease is one of the causes of mortality and disability in cases of non-communicable disease (NCD) in various countries.\(^1\)\(^2\) Non-communicable disease, such as cardiovascular disease, is a main problem not only in developed countries but also in developing countries. In 2014, Russia and the US had the highest mortality rates from ischemic heart disease.\(^3\) According to the Global Status Report on Non-communicable Disease, in 2008 more than 36 million persons died (63% of total mortality) as a result of non-communicable disease in countries with lower to middle per capita incomes.\(^4\) Cardiovascular disease may be caused by a number of factors, one of them being hyperlipidemia that may occur as a result of consumption of a high fat diet.\(^5\)

Hypercholesterolemia is a condition characterized by a high cholesterol concentration in the blood. Hypercholesterolemia may cause the accumulation of fat and oxidized low density lipoprotein (LDL) cholesterol in blood vessel walls,\(^6\) leading to the formation of plaques in the blood vessels and the occurrence of ischemic heart disease. Hypercholesterolemia may also increase oxidative stress in various organs, such as the liver, heart, and kidneys.\(^6\)\(^8\)

Several studies on animal models of hypercholesterolemia have been conducted, in which the condition was induced by means of intravenous adrenalin, duck egg yolk, and lard. However, these studies yielded less significant and consistent results in causing hypercholesterolemia in rats. In addition, the use of lard is constrained by ethical considerations, while cost is also a problem with standard high fat feeds.\(^9\)\(^10\) In this connection, shortening may also be used as inducer of hypercholesterolemia, since it is of relatively low cost and easily available.\(^11\)

Fat is widely used as raw material of bread and other foods.\(^12\)\(^13\) According to Nurmasitoh and Pramaningtyas, shortening may increase total cholesterol concentration, triglycerides and LDL in the blood of male Wistar rats.\(^11\) Shortening, which is made by hydrogenation, has a high trans fat content.\(^12\)\(^13\) Trans fat increases the LDL concentration and decreases the high density lipoprotein (HDL) concentration in the blood, so increasing the risk for atherosclerosis. Oxidized LDL may induce the formation of atherosclerotic plaques that ultimately lead to cardiovascular disorders, one of them being acute coronary syndrome. Hypercholesterolemia may promote the formation of atherosclerotic lesions, the onset of which is marked by an increase in foam cell count. Hypercholesterolemia may also cause an increase in the thickness of blood vessel walls.\(^6\)\(^14\)\(^15\)

To date there are still few studies on the effect of shortening on foam cells and aortic wall thickness. Therefore the purpose of the present study was to evaluate the effects of stepped doses of shortening on foam cell count and abdominal aortic wall thickness in male Wistar rats.

METHODS

Study design

This was an experimental post-test only controlled study, that was conducted in the Physiology Laboratory and the Integrated Research Laboratory, Faculty of Medicine, Universitas Islam Indonesia, from May to December 2016.

Animals and experimental procedure

The study involved 24 male Wistar rats aged 2-3 months and weighing 200-250 grams that were obtained from the Integrated Research and Testing Laboratory (LPPT) Gadjah Mada University. The sample size was calculated using the Federer formula \((n-1) (t-1) \geq 15\), where \(n=\)sample size for each intervention and \(p=\)number of interventions. Previous to the intervention, the rats were adapted for 7 days. The rats were put in cages of 40 cm x 20 cm x 20 cm and subjected to 12-hour light and dark cycles. The rats were randomly divided into 4 groups, i.e. a negative control group (C-), a
positive control group (C\(_{-}\)) and two intervention groups (T\(_{1}\) and T\(_{2}\)). Group C\(_{-}\) received AD II standard rat feed containing 51% carbohydrates, 15% crude protein, 3-7% crude fat, 6% crude fiber, 7% ash, 0.9-11% calcium, 0.6-0.9% phosphor, 12% water, antibiotics, and a coccidiostat. Group C\(_{+}\) received standard high fat feed, while groups T\(_{1}\) and T\(_{2}\) received shortening and standard feed at a ratio of 1:5 and 1:10, respectively. The intervention was administered via the oral route by gavage for 6 weeks in the Physiology Laboratory. Upon completion of the intervention, the animals were terminated.

Preparation of histological slides

After the rats were terminated, their aortic arch and abdominal aorta were removed, fixed in formalin for 2 days, dehydrated in alcohol 70%, and transported to the Integrated Research Laboratory, Faculty of Medicine, Universitas Islam Indonesia, where they were made into histological slides. The aortic arch and abdominal aorta of each rat were block paraffinized, microtomed, and stained with hematoxylin-eosin (HE). The histological slides were evaluated with respect to foam cell count, using an Olympus CX41 binocular microscope connected to an Optilab camera, with which images were taken of 10 fields of view at 40x magnification. The foam cell count was counted using a cell count application. The foam cells that were counted were those located in the tunica intima and tunica media. The thickness of the abdominal aortic wall was measured from the lamina intima up to the lamina media in 8 fields of view in a clockwise direction (at 12.00, 13.30, 15.00, 16.30, 18.00, 19.30, 21.00, 22.30) using Optilab and micrometer scale.\(^{(10)}\)

Data analysis

The test used was one-way Anova, with the level of significance set at p<0.05, followed by a post-hoc Tukey test to determine between-group differences.\(^{(16)}\)

Ethical clearance

The present study obtained ethical clearance from the Ethics Committee Faculty of Medicine Universitas Islam Indonesia under no. 03/Ka.Kom.Et/70/KE/II/2016.

RESULTS

The results of the present study shows that the greatest increase in foam cell count was in the intervention group T\(_{1}\) receiving the combination of shortening and standard feed at the ratio of 1:5, followed by groups C\(_{+}\), T\(_{2}\) and C\(_{-}\) (Figure 1). Although T\(_{1}\) had the greatest increase in foam cell count as compared to group C\(_{-}\), the results of the statistical analysis were still not significant (p=0.598).

In agreement with the increase in foam cell count, the greatest abdominal aortic wall thickness was in intervention group T\(_{1}\), followed by groups T\(_{2}\), C\(_{-}\), and C\(_{+}\) (Figure 2).

Although T\(_{1}\) had increased abdominal aortic wall thickness as compared to group C\(_{-}\), the results of the statistical analysis were not yet significant (p=0.110). The complete set of data on mean aortic arch foam cell count and abdominal aortic wall thickness is shown in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>C(_{-}) (n=6)</th>
<th>C(_{+}) (n=6)</th>
<th>T(_{1}) (n=6)</th>
<th>T(_{2}) (n=6)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foam cell count</td>
<td>7.17 ± 4.17</td>
<td>9.33 ± 7.01</td>
<td>11.83 ± 4.88</td>
<td>9.33 ± 6.80</td>
<td>0.598</td>
</tr>
<tr>
<td>Aortic wall thickness (µm)</td>
<td>741.98 ± 60.67</td>
<td>714.29 ± 90.59</td>
<td>838.90 ± 75.86</td>
<td>749.88 ± 99.37</td>
<td>0.110</td>
</tr>
</tbody>
</table>

C\(_{-}\): negative control (AD II standard feed), C\(_{+}\): positive control (standard high fat feed), T\(_{1}\): shortening +AD II standard feed (1:5 ratio), T\(_{2}\): shortening +AD II standard feed (1:10 ratio)
DISCUSSION

The present study shows that the use of high fat feeds comprising a combination of shortening and standard feed at a ratio of either 1:5 or 1:10 did not yet show a significant increase in foam cell count. These results are in line with previous studies reporting that administration of high fat feeds in consisting of 0.2% egg yolk, 2% cholic acid, fat 5% goat and 92.8% standard feed in amounts of 20 gram/day was proved to be able to form foam cells but did not yet show a significant increase in foam cell count in the control group and the groups receiving the aforesaid high fat feeds. These results differ from those of a study on the administration of lard, which showed an increase in foam cell count. The differing results may very well be the result of a difference in the induction period of the test animals. In the latter study the induction period was 8 weeks, whereas in our study the induction period was 6 weeks. An induction period of less than 8 weeks may cause less than optimal foam cell formation. This is also referred to in the study of Nurmasitoh and Pramaningtyas who found that administration of shortening for 14 days to Wistar rats already showed a significant increase in lipid profile. However, as a result of the shorter induction period, the increase in lipid profile could not yet lead to significant structural vascular changes, i.e. an increase in wall thickness and foam cell count.

Atherosclerosis occurs as a result of the conversion of low density lipoprotein (LDL) to oxidized LDL, causing endothelial lesions. Oxidized LDL and plasma molecules also undergo extravasation into the endothelial space where they are trapped so that they may become...
cytotoxic, proinflammatory, chemotactic, and proatherogenic. Endothelial injury facilitates the entry of monocytes that adhere in the subendothelial space and subsequently become macrophages. Active macrophages will secrete an oxygen species toxin that increases LDL oxidation, leading to the development of foam cells. In addition, the endothelium becomes more active, with a decrease in NO synthesis and development of endothelial stiffness.$^{18,19}$

Our study also showed that the use of a combination of shortening in standard feed at a ratio of 1:5 or 1:10 also does not yet show an increase in vessel wall thickness. This supports the results of a previous study where administration of daun katuk (Sauropus androgynus or sweetleaf), cholic acid and cholesterol for 8 weeks, also did not have significant effects on vessel wall thickness.$^{20}$

Another study also states that fat induction in Wistar rats significantly increases the lipid concentration in the 22nd week.$^{21}$

A limitation of the present study is the relatively short intervention period so that the results were less than maximal. Therefore there is a need for further studies using higher induction doses and longer intervention periods, so that the results will be according to theory.

**CONCLUSION**

Shortening tend to increase the aortic arch foam cell count and abdominal aortic wall thickness in male Wistar rats.

**CONFLICT OF INTEREST**

There was no conflict of interest.
ACKNOWLEDGEMENT

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CONTRIBUTORS

RL and MDP contributed to the design of the study and drafting of the manuscript. TN and RHD contributed to data collecting and analysis. AD contributed to revising the manuscript. All authors read and approved the final manuscript.

REFERENCES