The effect of trans fatty acids from repeated heating

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Submission date: 15-Apr-2021 11:11AM (UTC+0800)
Submission ID: 1559618698
File name: The_effect_of_trans_fatty_acids_from_repeated_heating.docx (35.04K)
Word count: 2827
Character count: 15816

The effect of trans fatty acids from repeated heating cooking oil on blood TNF-α

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Abstract

Background: Fried foods are often found in the Indonesian dishes. Heating process can increased of *tran*-fatty acids, affecting lipid metabolism and induces an inflammatory reaction
Objectives: To determine the effect of *trans*-fatty acids of comercial cooking oil on concentration of TNF-α, we studied 5 groups of rats; group I received a standard food as control, group II had additional corn oil, group III had additional palm oil, group IV had additional three times repeted heating of corn oil, and groups V had aditional three times repeted heating of palm oil. TNF-α was examined from the rats blood serum by ELISA.
Results: Increasing of rats body weight between groups were not statistically significant. TNF- α of control group (59.36 ±7.83) was statistically significant lower (p<.0,005) in compare to the group III (72.25 ±5.65), group IV (108.71 ±6.40) and group V (88.70 ± 5, 42), but was not different with group III (54.36 ±6.70), The results showed that repeated heating of corn oil and palm oil increased trans fatty acids and associated with TNF-α concentration
Conclusion: Repeated heating cooking oil increasing trans fatty acid that increase of TNF-α

Keywords: repeated heating cooking oil, *trans*-fatty acid, TNF-α

Background

Epidemiological transition in Indonesia in last 12 years has been going along with the demographic transition, that characterized by a shift in the epidemiology of the cause of mortality from infectious diseases to non-communicable diseases. The data shows that non-communicable diseases such as stroke, hypertension and heart disease encompasses more than a one third the cause of death. Coronary heart disease can occur as it is influenced by several risk factors such as age, gender, genetics, high cholesterol levels, hypertension, smoking, diabetes mellitus, obesity, poor lifestyle (stress, alcohol, lack of physical exercise). National prevalence for population in age more than ten years that consumed less fruits and vegetables was 93,6%, and less physical activity was 48,2% (1).

One of the factors that led to high morbidity and mortality from heart disease is caused by eating habits and lifestyle of most Indonesian community, such as consuming processed foods with cooking oil. It starts from breakfast like fried rice, then lunch and dinner dishes with rice accompanied by fried fish, meat, or egg, and sauteed vegetables. For the snack they like sweet potatoes or banana fries, crackers and fried tempeh or tofu, that may contain trans fat. The commercial baked goods that used cooking oil repeatedly by reason of the saving, can lead to increased levels of *trans*-fatty acids (TFA) in the cooking oil (2).

Natural foods that is, unprocessed foods contain two main types of fatty acids there were saturated and unsaturated. Saturated fatty acids which come from animal fats (meat, lard, dairy products) and tropical oils such as coconut and palm oils - raise the levels of LDL cholesterol. Unsaturated fatty acids which come from vegetable oils, in general do not increase cholesterol levels, and may reduce them. *Trans*-fatty acids are a third form of fatty acids. While TFA do occur in tiny amounts in some foods (particularly foods from animals), almost all the TFA now in our diets come from an industrial process that partially hydrogenates (adds hydrogen to) unsaturated fatty acids. TFA, then, are a form of processed vegetable oils (3).

TFA are unsaturated fats with at least one double bond in the *trans* configuration that are formed during the industrial hydrogenation of vegetable oils for food manufacturing. The consumption of monounsaturated *trans* fatty acids has been associated with a greater risk of cardiovascular disease (CVD). Two dietary sources exist for the TFAs present in the food supply of industrial production, in which TFAs are mainly derived from partially hydrogenated vegetable oils and natural sources, in which TFAs are found in smaller amounts in ruminant-derived food products. Unfortunately, very little scientific research to date has compared the specific health effects of TFAs from industrial and natural sources (4).

The influence of TFA consumption are decreases high-density lipoprotein (HDL) levels and increases low-density lipoprotein (LDL) levels. LDL is a transport protein that takes synthesized and stored cholesterol from the liver and carries it to the bloodstream where it can be used for steroid hormone synthesis, cell synthesis, and other important physiological processes. If too much cholesterol is in the blood, it can begin to deposit in arterial walls leading to inflammation and recruitment of macrophages and other immune cells. Inflammatory responseas measured by CRP, Interleukin-6 (IL-6), Tumour necrosis factor alpha (TNF-α), increased thrombogenesis and increase markers of endothelial dysfunction including E-selectin, ICAM, and impair flow-mediated vasodilation (measure of vascular NO production), all of which in combination or individually contribute to increased cardiovascular risk (5).

The biological mechanisms underlying the adverse effects of *trans* fatty acids on endothelial function are not clear. *Trans* fats are incorporated into endothelial cell membranes and thus could alter cellular and macromolecular components acting at the interface of the blood vessel wall. This could result in changes in the antihemostatic properties, altered vascular tone, hyperadhesiveness to blood leukocytes, and increased cytokine and growth factor production, all of which are characteristics of endothelial dysfunction (6)

An important feature during the development of atherosclerotic lesions is the infiltration of circulating monocytes into the intravascular space. LDL particles, especially the modified forms, increase the expression and secretion of soluble chemotactic compounds and enhance the expression of adhesion molecules such as integrins and selectins, which are exposed on the surface of activated endothelial cells and favor leukocyte (monocyte and T-cell) recruitment, adhesion, and transmigration. Diapedesis of monocytes takes place through the spaces (junctions) between endothelial cells, preferably in areas where the basal lamina is enriched with modified LDL particles. Then, infiltrated monocytes differentiate into macrophages and express scavenger receptors such as CD36 and LOX-1, which internalize many of the cholesterol molecules and cholesterol esters contained in modified LDL particles. LDL aggregates are potent inducers of massive cholesterol accumulation in macrophages; whereas some authors propose phagocytosis as the classic mechanism of LDL aggregates internalization in macrophages (7).

Cholesterol internalization leads to the formation of foam cells, a characteristic cell constituent of atherosclerotic lesions. In turn, foam cells secrete proinflammatory cytokines (TNF-α, IL-1 and IL-6), growth factors, tissue factor, interferon (IFN) q, MMP, and reactive oxygen species (ROS) that maintain the chemotactic stimulus for leukocytes adhered to the vascular endothelium, increase the expression of scavenger receptors, and enhance macrophage replication (8).

Experimental studies suggest that TFA exert their multiple effects by influencing metabolic and signaling pathways in hepatocytes, monocytes, adipocytes and in endothelial cells. The precise molecular pathways through which TFA influence these cell types are unknown (9).

To determined the effect of TFA from repeated heating cooking oil to pro-inflammatory cytokine, we investigated the relations between TFA intake and blood TNF-α of the rat.

Material and Methods

Samples of this study were 8-week-old male *rattus norvegicus* strain wistar weight of 100-150 grams. The number of samples was 25 and there were divided into 5 groups. Group I was the control group that received standard diet for rats, and the other groups received an additional industrial cooking oil. Group II get an additional fresh corn oil, group III get an additional fresh palm oil. Group IV get an additional corn oil were performed three times repeated heating and group V get an additional palm oil performed three times repeated heating. Each rat in the group recieved 0.1 ml/10 g body weight / day for eight weeks.

Values for the *trans*-fatty acid from the repeated heating cooking oil performed at the Agricultural Research and Development Laboratories of the Indonesian Ministry of Agriculture.

After eight weeks of treatment, blood sample was taken from the rats and centrifuged within 30–45 minute of collection, and serum is separated from blood cells. TNF-α concentrations were measured from the serum by enzyme-linked immunoassay (eBioscience). This study was approved by the Research Ethics Committee of Medical Faculty of Andalas University.

Data are expressed as mean ± SD, and value of $p<0,005$ were considered statistically significant. Data were analyzed by one-way analysis of variance using the Bonferoni-post-hoc comparison test when appropriate.

Results, Discussion and Conclusion

The body weights of rats at the end of dietary period were increased in all groups, but the increase of weight did not differ among those consuming the standard diets with different kind of additional oil ($p>0,005$). We suggest that increased of rats body weight was due to increasing of the age, feeding and maintenance.

Fatty acids are characterized as either *saturated* or *unsaturated* based on the presence of double bonds in its structure. During the partial hydrogenation, solidifies and stabilizes of polyunsaturated of vegetable oils the number of double bonds is reduced, while approx. 30-50% of unsaturated fatty acids are transformed from *cis* into *trans*(5). The main fatty acid formed in the process of vegetable oil solidification is elaidic acid (C18:1, *trans*-9). However, the process of frying or baking food in vegetable oils results in the generation of linoelaidic acid (C18:2; *trans*-9,12). The high temperature accompanying this process causes the conversion of the double bond from the *cis* configuration into *trans* fatty acid (2).

In our study, we obtained that the levels of *trans*-fatty acids in repeated heating palm oil was 0.2914mg/100 gr and in repeated heating corn oil was 0.4171mg/100gr, and the type of trans fatty acids in the repeted heating cooking oil was oleic acid. Oleic acid play a role in endithel disfunction. The intake of oleic acid appeared to be more strongly associated with the concentrations of biomarkers as well as marker of inflammatory activation and endothelial disfunction than *trans* palmitoleic acid and *trans* linoleic acid (10).

From the examination of TNF-α from rats blood serum, we obtained that TNF-α concentration in the group I as a control group was 59,36±7,84 ρg/ml, in the group II that get an additional fresh corn oil was 54,34±6,70 ρg/ml, in the group III that get an additional fresh palm oil was 72,25±5,65 ρg/ml, in the group IV that get an additional three times repeated heating corn oil was 108,71±6,39 ρg/ml and in the group V that get an additional three times repeated heating palm oil was 88,69±5,42ρg/ml (table 1).

Table 1: Concentration of TNF-α in control group (I), group that added fresh corn oil (II), and fresh palm oil (III), group that added repeated heating corn oil (IV) and that added repeated heating palm oil (V)

No	Group (n=5)	Mean ±SD
1	I	59,36±7,84
2	II	54,34±6,70
3	III	72,25±5,65
4	IV	108,71±6,39
5	V	88,69±5,42

In this study, the concentration of TNF-α of the groups that get an additional fresh corn oil were not statistically different to the control group ($p>0,005$), but the group that get an additional fresh palm oil, the concentration of TNF-α was higher in compare to the control group ($p<0,005$). The concentration of TNF- α of the group that get an additional three times repeated heating palm oil was higher in compare to control group and of the group that get an additional three times repeated heating corn oil was higher in compare to control group with highly statistically significant differences ($p<0,005$).

Common dietary sources of unsaturated fat include fish and oils derived from olives, soybeans, corn and sunflower seeds. Common sources of saturated fat include fatty meats, whole dairy products and palm oils. Common sources of trans fat include margarine, various types of commercial baked goods and processed or fried foods.

Fresh Corn oil as unsaturated fatty in this study did not contribute to the concentration of TNF-α, otherwise fresh palm oil which is a saturated fatty acid contributes to the concentration of TNF-α. But when heated repeatedly its shown the high concentrations of TNF-α in corn oil than palm oil. Its indicating that heating process saturated fatty acid and unsaturated fatty acid were transformed into TFA may cause endothelial dysfunction.

TNFα is a pleiotropic cytokine that has many proinflammatory actions with negative inotropic effects. It has been implicated in the pathogenesis of many non-infectious disorders, from rheumatoid disease, to multiple sclerosis. This cytokine also affects the heart (11).

Intake TFA, which are come from repeated heating cooking oil, increasing LDL. Circulating LDL particles invade the arterial wall and accumulate in the intima, where they undergo chemical modifications, such as oxidation. Modified LDL can induce endothelial cell activation and expression of adhesion

molecules. Furthermore, intimal macrophages can internalize modified LDL particles through scavenger receptors and become foam cells. Macrophages and lymphocytes release a range of proinflammatory cytokines and chemokines which stimulate the migration of smooth muscle cells from the media. This process is facilitated by cytokines such as IFN-γ and TNF-α secreted by proatherogenic Th1 cells and also IL-12 secreted by macrophages and foam cells (11).

TFAs intake is associated with an elevated risk of coronary artery disease and new-onset diabetes, and activation of the TNF system is a risk factor for diabetes, independent of adiposity. Thus, activation of the TNF system may represent a mediating step between TFA consumption and risks of coronary artery disease and diabetes (9). TFA activate TNF system throughout two mechanisms. First of all TFAs are incorporated into endothelial cell membranes, which have great number of cell-specific pathways associating to TNF system activation. Secondly TFAs may also modulate TNF system via effects on membrane phospholipids of macrophage and signaling pathways, with analogous mechanisms seen in n-3, n-6, and monounsaturated fatty acids. TNF-α is a potent cytokine that induces the production of IL-6, which is the major determinant of the acute phase response and is also produced by adipose tissue (4).

Numerous studies have correlated elevated dietary intake of TFA with increased mortality and morbidity from cardiovascular disease. This study examined the ability of TFA to activate endothelial NF-κB and subsequently reduce NO production on endothelial dysfunction (9). TFA intake is positively associated with markers of systemic inflammation in women (4). Esmaillzadeh and Azadbakh (12) found in Tehrani woman that higher intakes of partially hydrogenated vegetable oils. Partially hydrogenated vegetable oils are associated with elevated concentrations of inflammatory biomarkers, whereas higher intakes of non- hydrogenated vegetable oils are associated with lower plasma concentrations of these biomarker.

In conclusion, this studies demonstrate that repeated heating of two common industrial cooking oil increase *trans*-fatty acid that correlated with the concentration of TNF-α as proinflammatory cytokine in rats blood serum. These study lend further support for the recommendation to minimize the using of the repeated heating cooking oil in food processing.

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