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The Journal of Biomedical Research, 2017 31(2): 122–129

JBR

Original Article

Comparative effects of plant oils and *trans*-fat on blood lipid profiles and ischemic stroke in rats

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Abstract

Since plant oils are believed to be better than animal fats for cerebrovascular and cardiovascular diseases, the effects of various plant oils and *trans*-fat on blood lipid profiles and ischemic stroke were investigated. Sprague-Dawley rats were fed a diet containing the oils or *trans*-fat, and then body weights, blood lipids, and effects on brain infarction and physical dysfunction induced by middle cerebral artery occlusion (MCAO) were analyzed. All the oils and *trans*-fat, except perilla oil, significantly increased body fats and body weight gain. Sesame oil and *trans*-fat specifically increased blood cholesterols and triglycerides, respectively, while perilla oil decreased both cholesterols and triglycerides. Perilla oil not only attenuated cerebral infarction, but also restored locomotor activity and rota-rod performances of MCAO rats. It is suggested that perilla oil among oils and fats could be the first choice to reduce the risk of metabolic syndrome and ischemic stroke.

Keywords: plant oil, *trans*-fat, blood lipid, body fat, ischemic stroke, physical activity

Introduction

Cerebral stroke is one of the most devastating diseases worldwide, leading to sudden death or serious disability in humans[1-2]. Stroke causes loss of brain function due to disturbance in supplying blood to certain brain regions following cerebral ischemia or hemorrhage[3].

In modern society, diet-induced metabolic syndrome is one of the major causes of obesity, atherosclerosis, hypertension, and stroke[4-5]. The major risk factor of stroke may be hyperlipidemia which is characterized by increased blood levels of total cholesterol (TC) and low-density lipoproteins (LDL), in addition to decreased high-density lipoproteins (HDL)[6-7]. In the blood vessels, oxidized LDL (OxLDL) is a triggering molecule of endothelial injury and atheroma formation, whereas HDL is a transporter of cholesterol for metabolism in the liver[8-11]. Accordingly, suppression of blood TC and LDL and augmentation of HDL are believed to be front-line strategies for management of atherosclerosis and stroke[10-11].

Since excessive consumption of fats and oils cause hyperlipidemia, selection of dietary fats and oils might be very important. In contrast to harmful effects of saturated fatty acids (SFA), unsaturated fatty acids

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Received 06 August 2016, Revised 23 August 2016, Accepted 19 September 2016, Epub 20 November 2016
CLC number: R332, Document code: A
The authors reported no conflict of interests.

doi:10.7555/JBR.31.20160095

(UFA) properly regulate blood lipid profiles, and thereby improve cerebrovascular and cardiovascular diseases[12-15]. Actually, fish oil omega-3 (ω-3) poly-unsaturated fatty acids (PUFA) affected both platelets and endothelial cells that play a crucial role in the regulation of thrombosis and hemostasis[16]: i.e., they prevented vasoconstriction[17] and suppressed vascular inflammatory response by decreasing production of reactive oxygen species (ROS)[18-19]. Notably, α-linolenic acid, a well-known ω-3 PUFA, improved insulin sensitivity and hyperlipidemia, and prevented cardiovascular disease[20-21]. In our recent studies, perilla oil, containing a high level of α-linolenic acid, not only inhibited platelet aggregation and lipid accumulation, but also improved atherosclerosis and blood flow[22-23].

Notably, however, many favorite plant oils including canola (rapeseed) oil, soybean oil, corn oil, and olive oil containing high levels of ω-6 PUFA facilitated brain hemorrhage of stroke-prone spontaneously hypertensive rats (SHR-SP), shortening their longevity[13,24-25]. On the contrary, perilla oil possessing a low linoleic acid/α-linolenic acid (ω-6/ω-3) ratio improved lipid profiles and delayed and attenuated brain hemorrhage in SHR-SP, and thereby extending their life span[26].

Such controversial results of plant oils on the blood flow and cerebral hemorrhagic stroke led us to investigate the effects of perilla oil on ischemic stroke, in comparison with canola oil, sesame oil, and *trans*-fat (shortening).

Materials and methods

Materials

Perilla oil was obtained from Anydoctor Healthcare Co. Ltd. (Cheonan, Korea). Perilla oil was extracted under a cold-pressed method at 30°C-48°C, and analyzed with Varian 3800 gas chromatograph (Varian Inc., Walnut Creek, CA, USA) equipped with a Supelcowax 10 fused-silica capillary column (Supelco, Bellefonte, PA, USA). From the fatty acid analysis, it was found that perilla oil contained 72.12% PUFA, 19.1% monounsaturated fatty acids (MUFA), and 8.49% SFA. Especially, among PUFA, 57.47% was ω-3 α-linolenic acid[22,26]. Separately, shortening, a hydrogenation product of palm oil, contained a high concentration (47.15%) of saturated palmitic acid[26].

Animals

Seven-week-old male Sprague-Dawley rats (body weight ranged from 260 to 280 g) were purchased from Daehan Biolink (Eumseong, Korea). Rats were housed in a room with a constant temperature (23°C±2°C), relative humidity (55%±10%), and a 12 hours light/dark cycle. Rats were fed standard rodent diet (Hanlan #2018) and purified water *ad libitum*. All experimental procedures were carried out in accordance with the Standard Operation Procedures of Laboratory Animal Center, Chungbuk National University (CBNU), Korea. The protocol was approved by the Institutional Animal Care and Use Committee of CBNU.

Measurement of plasma lipids

After 1-week acclimation to the laboratory environment, the rats (8 weeks old, n = 8/group) were fed a powdered diet (Hanlan #2018) containing 10% plant oils (perilla, canola or sesame oils) or *trans*-fat for 5 weeks.

Body weights and daily feed consumption were recorded every week immediately from starting the experiment. After 16-hour fasting at the end of the experiment, the animals were sacrificed under deep anesthesia with diethyl ether. Blood samples were collected from abdominal artery, and lipids including TC, LDL, HDL, and triglycerides (TG) were measured in sera using a blood chemistry analyzer (Hitachi-747; Hitachi Korea, Seoul, Korea). Epididymal, perirenal, and mesenteric fats were removed and weighed.

Establishment of a rat ischemic stroke model

The rats (8 weeks old, n = 8/group) were fed a powdered diet (Hanlan #2018) containing 10% plant oils (perilla, canola or sesame oils) or *trans*-fat for 2 weeks before induction of cerebral ischemia.

Silcone-coated threads were prepared based on the method described previously[27], using a 4/0 monofilament nylon suture (Ailee, Busan, Korea) and polyethylene tubing with an internal diameter of 0.28 mm (Intramedic, Batavia, IL, USA). Focal cerebral ischemia, through middle cerebral artery occlusion (MCAO) surgery, was produced as described previously[28], with slight modification[27,29]. In brief, under anesthesia of the rats with isoflurane, a midline incision was made on the ventral side of the neck, exposing the left common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA). The ICA and lower part of the CCA were blocked by clips, and the upper part of the ECA was ligated. An opening was made in the middle of the ECA, and a silicone-coated thread was introduced through the opening. The thread was advanced by 18 mm *via* the ICA up to the origin of the middle cerebral artery. The thread was secured in place by ligature, and the incision was sutured. Two hours later, the silicone thread was removed for reperfusion.

Evaluation of neurologic function

Spontaneous locomotor activity was evaluated using a video tracking system (Smart v2.5; Panlab, Barcelona, Spain), connected to a CCTV monitor (Samsung, Changwon, Korea) 48 hours after MCAO surgery. Rats were placed in a square activity chamber (50 × 50 × 30 cm), and the equipment was kept in a quiet testing room. Each time of the movement types, i.e., resting, slow-moving, and fast-moving times, was recorded for 5 minutes following 15-second adaptation time, and the ratio was analyzed[30-32].

Rota-rod performance was measured to analyze the motor coordination and balance of animals after the locomotor activity test. Rats were placed on a rotating rod at an accelerating mode (from 4 rpm to 40 rpm over a period of 5 minutes), and the latency time of 3 consecutive trials was recorded[30-32].

2,3,5-Triphenyltetrazolium chloride (TTC) staining

At the end of the physical activity tests, the rats were sacrificed under deep anesthesia with diethyl ether. The rat brains were carefully dissected in 2-mm coronal sections using a stainless matrix. The serial sections were stained with 2% TTC for 15 minutes at 37°C, and fixed with 10% neural buffered formalin[27,29]. The infarction area of each section was determined using a computerized image analysis system (ImageJ; National Institutes of Health, Bethesda, Maryland, USA). Total infarct volume was calculated by summing up the infarction areas in each section and multiplying by slice thickness (2 mm).

Statistical analysis

The results were presented as mean±standard error. The significance of differences of all results was analyzed by one-way analysis of variance followed by Dunnett's multiple-range test correction, using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set a priority at $P < 0.05$.

Results

During 5 weeks, the rats fed a normal diet gained 54.7 g of body weight by ingesting 23.3 g/day of diet, leading to 0.235 of feed efficiency rate (FER) (***Table 1***). By comparison, long-term feeding diets containing a high concentration (10%) of plant oils or *trans*-fat increased the body weight gain, in spite of reduced feed consumption. Notably, the degree of body weight gain was lower in rats fed perilla oil than animals given canola, sesame oils, or especially, *trans*-fat exhibiting a marked increase.

In parallel with the body weight increase, there were increases in the body fats: i.e., plant oils, excepting perilla oil, and *trans*-fat significantly increased the epididymal, perirenal, and mesenteric fats, although there were differences in the location of accumulation among plant oils and *trans*-fat (***Fig. 1***). It is of interest to note that perilla oil did not cause remarkable deposition of abdominal fats.

Plant oils and *trans*-fat affected the blood lipid profiles (***Fig. 2***). Sesame oil predominantly increased blood cholesterols, including TC, LDL, and HDL, without affecting TG. By comparison, *trans*-fat specifically increased only TG level. Interestingly, perilla oil decreased both cholesterols and TG, while canola oil did not influence the blood lipids.

MCAO for 2 hours produced 112.2 mm^3 of infarct, reaching 11.8% of whole brain sections (***Fig. 3***). Notably, brain injury was significantly attenuated by feeding perilla oil. Similar effects were not shown in rats fed canola oil, sesame oil or *trans*-fat.

In normal animals, moving times (slow- and fast-moving) were about 45-50% in locomotor activity analysis (***Fig. 4A***). However, the resting time greatly increased in MCAO rats, leading to significant decreases in slow- and fast-moving times. However, 2-week feeding of perilla oil significantly recovered the physical activity. By comparison, such a beneficial effect was not achieved by feeding other oils or *trans*-fat.

Table 1 **Body weight gain and feed efficiency rate during 5-week feeding of plant oils or *trans*-fat**

Treatment in diet (%)	Body weight gain (g)	Food intake (g/day)	FER
Normal diet	54.7±2.3	23.3±0.6	0.235±0.108
Perilla oil (10)	60.7±5.5	16.7±0.5*	0.362±0.097
Canola oil (10)	62.0±3.3*	18.8±0.5*	0.305±0.085
Sesame oil (10)	62.1±4.3*	20.0±0.8*	0.311±0.091
Tans-fat (10)	70.3±4.9*	19.8±0.4*	0.355±0.095

*Significantly different from normal control ($P < 0.05$).

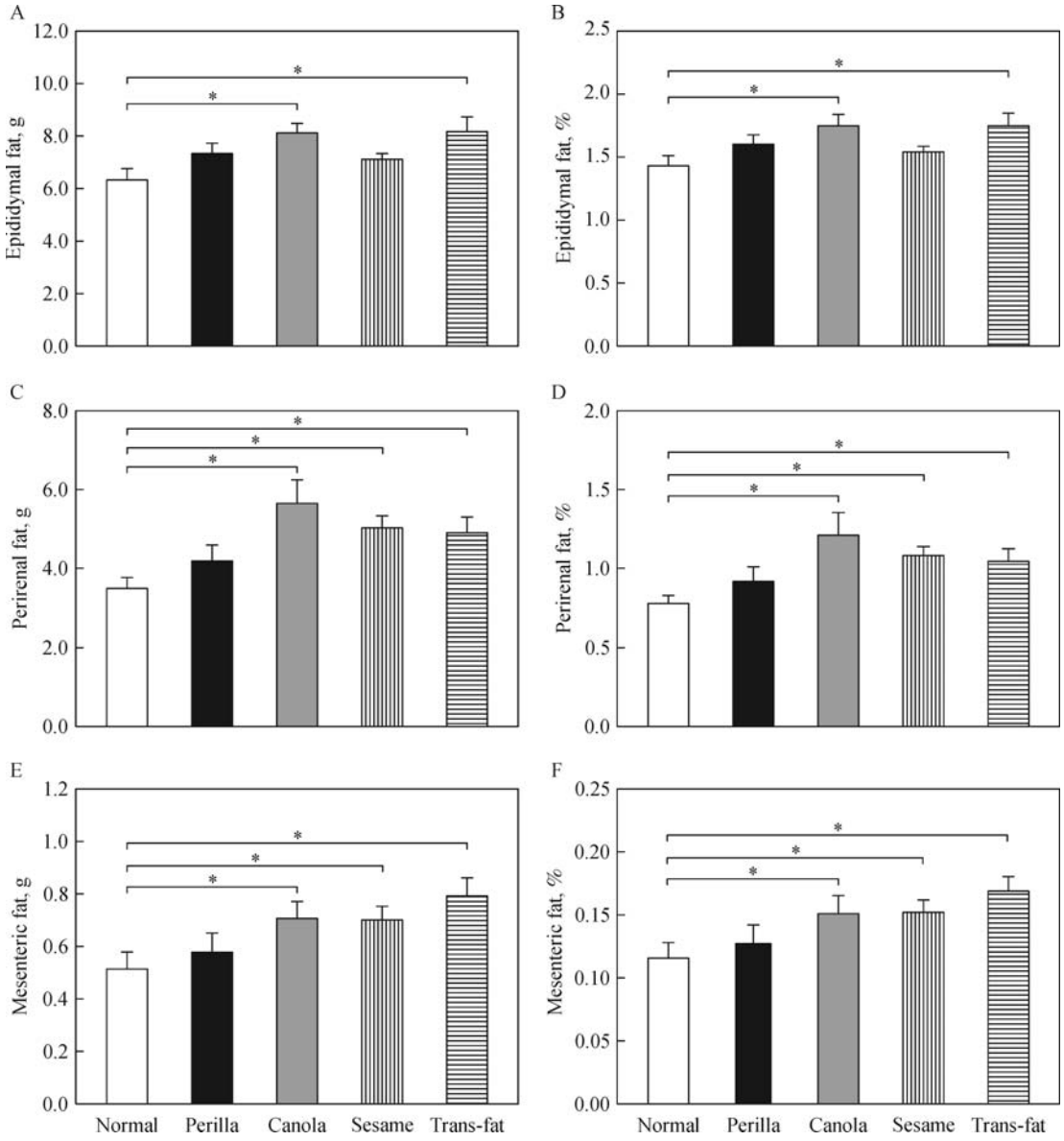

Fig. 1 **Body fat weights after 5-week feeding of plant oils or *trans*-fat.** Absolute weights (A, C, and E) of epididymal (A and B), perirenal (C and D), and mesenteric (E and F) fats and their relative weights to body weights (B, D, and F) were measured and calculated. *$P < 0.05$.

The latency time of MCAO-challenged animals on an accelerating rota-rod significantly decreased to one-third of normal rats (***Fig. 4B***). Such disorders in motor coordination and balance were significantly restored by feeding perilla oil. Such movement-improving effects were not attained by canola oil, sesame oil or *trans*-fat.

Discussion

In the present study, a long-term administration of perilla oil led to decreases in blood lipids and relatively low fat accumulation and body weight gain, in comparison with marked hyperlipidemia and over-weight in the rats fed other plant oils and *trans*-fat. In addition, only perilla oil significantly reduced brain lesion and physical dysfunction of MCAO rats.

Whereas excessive consumption of fats and oils, especially containing high levels of SFA, is known to be harmful for vascular diseases, UFA is believed to be beneficial. In the previous and present studies, short-ening, a *trans*-fat contained a high concentration (47.15%) of SFA, not only significantly increased blood TG level and body fats, leading to overweight, but also exacerbated hemorrhagic stroke[26]. On the contrary, it has been reported that ω-3 PUFA and perilla oil containing a high level (72.12%) of PUFA decreased blood TG, TC, and LDL, the causative factors for fat accumulation, obesity, and atherosclerosis, in animals

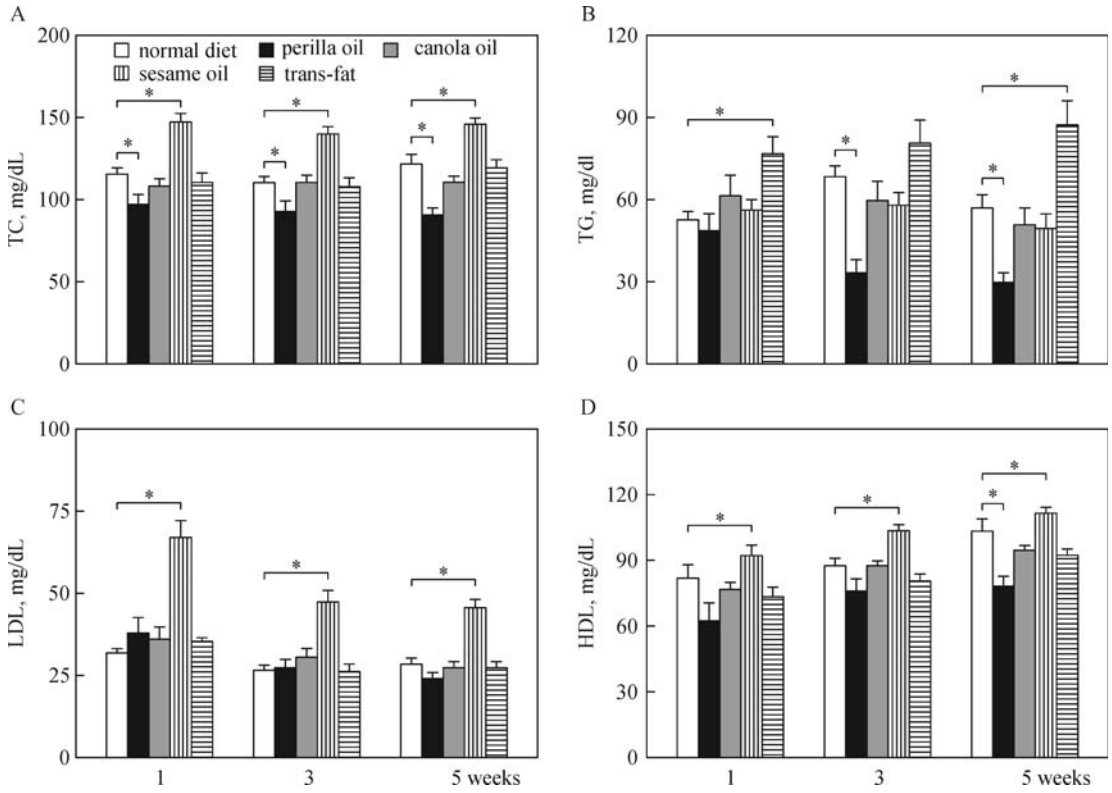

Fig. 2 **Time-courses of blood lipids during 5-week feeding of plant oils or *trans*-fat.** A: total cholesterol (TC), B: triglycerides (TG), C: low-density lipoproteins (LDL), D: high-density lipoproteins (HDL). $*P < 0.05$.

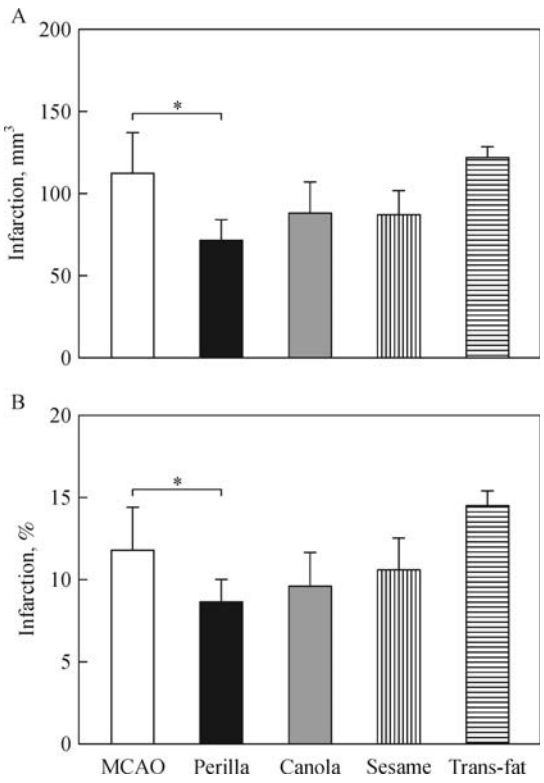

Fig. 3 **Effects of plant oils and *trans*-fat on brain infarction.** The plant oils and *trans*-fat were pretreated for 2 weeks, and then focal cerebral infarction was induced by middle cerebral artery occlusion (MCAO) surgery. The infarct volume (A) and ratio to the whole brain volume (B) were calculated from serial 2-mm coronal sections. $*P < 0.05$.

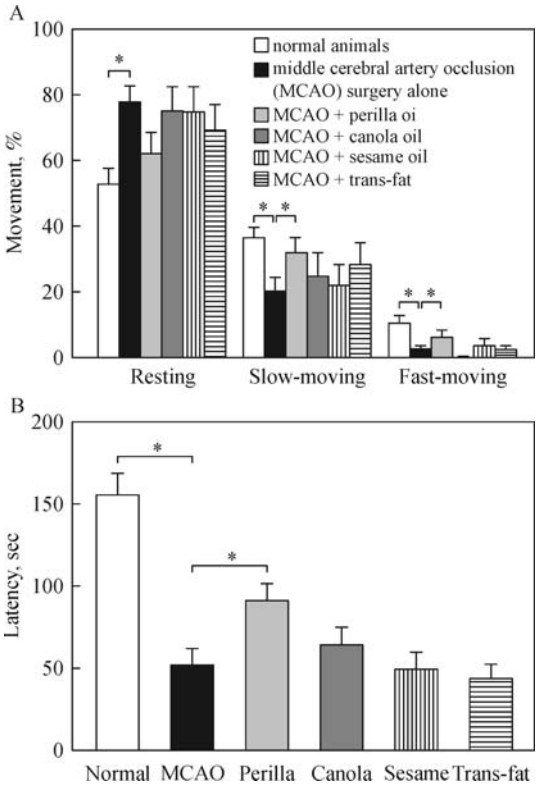

Fig. 4 **Effects of plant oils and *trans*-fat on the physical activities of rats with ischemic stroke.** A: Locomotor activity. B: Rota-rod performance. $*P < 0.05$.

and humans[23,26,33-34]. ω-3 PUFA reduces hepatic lipogenesis not only by suppressing sterol regulatory element binding protein (*SREBP-1c*) gene transcription, but also by inhibiting diacylglycerol acetyl-transferase and phosphatidic acid phosphohydrolase[35]. However, there are controversial results in the effects of ω-3 PUFA and perilla oil on anti-atherogenic HDL: i.e., HDL decreased in normal and SHR-SP rats[26], but increased in rats and rabbits fed an HCD[23,33]. Interestingly, it was suggested that ω-6 and ω-3 PUFAs affect HDL metabolism: i.e., blood HDL level is increased *via* upregulation of hepatic apoA-1 and ATP binding cassette transporter A1 (ABCA-1) with suppression of apoA-II by ω-6 PUFA[36], explaining the decreased HDL level after feeding perilla oil containing a low ω-6/ω-3 ratio in the present study. Therefore, in human studies, ω-3 PUFA and perilla oil did not significantly alter blood HDL[34,37]. Collectively, ω-3 and perilla oil reduced the risk of cardiac infarction by improving blood lipids, inflammatory responses, and function of arterial endothelial cells[19,34,37]. In addition, we demonstrated that perilla oil improved the blood lipid profile and fat accumulation in tissues, and thereby remarkably attenuated ischemic and hemorrhagic cerebral strokes[23,26].

More importantly, a high concentration of ω-6 PUFA, compared to ω-3 PUFA, in plant oils plays a critical role for aggravation of hypertension-mediated hemorrhagic stroke[26]. It has been demonstrated that most of the plant oils with high ω-6/ω-3 fatty acid ratios including canola oil, safflower oil, olive oil, corn oil, and soybean oil increased hemorrhagic stroke in SHR-SP and shortened their life span[13-14,24-25]. Actually, it was reported that ω-6 PUFA has pro-inflammatory properties, increasing C-reactive protein expression[19,38-39], and that canola oil reduced antioxidant status in the vessels[40]. By comparison, ω-3 PUFA exerts antioxidative and anti-inflammatory activities, attenuating atherosclerosis[19,23,41-42]. Therefore, the ω-6/ω-3 ratio was suggested to be a key factor governing the integrity and health status of blood vessels, because safflower oil (ω-6/ω-3 ratio of 20), soybean oil (10), and canola oil (2.5-2.7) shortened the longevity of SHR-SP animals, whereas perilla oil (0.25) and fish oils rich in ω-3 fatty acids prolonged it[13-14,24-26].

Plant oils and *trans*-fat differently affected the blood lipid profiles, although they all increased fat accumulation and body weight gain. *Trans*-fat containing a high level of SFA specifically increased blood TG, a well-known risk factor of obesity. Actually, *trans*-fat significantly facilitated body fat accumulation, resulting in overweight, and advanced the onset time of

hemorrhagic stroke[27]. By comparison, sesame oil increased blood cholesterols including TC, LDL, and HDL. Although TC and OxLDL are believed to be key factors for atherosclerosis and thrombosis, leading to stroke, it is suggested that the increased HDL eliminated the harmful effects of TC and LDL[9,11]. Furthermore, it is believed that antioxidant polyphenols such as luteolin in sesame oil attenuated brain injury in this ischemic stroke model[43-45]. Canola oil did not affect the blood lipids and ischemic brain injury, in spite of its increasing potential on body fat accumulation. However, blood TC and LDL were enhanced by canola oil in SHR-SP, implying that canola oil affects lipid metabolism and the integrity of hypertensive vessel walls *via* increased lipids and/or ω-6 PUFA. As aforementioned, perilla oil decreased both cholesterols and TG. In addition, it is not excluded that luteolin in perilla oil prevented brain injury caused by ischemic stroke[43-45].

In fact, statins such as lovastatin and simvastatin have been preferentially prescribed to suppress hepatic cholesterol synthesis for the improvement of blood lipid profiles[46]. However, it is well known that long-term administration of high-dose statins causes severe adverse effects including hepatotoxicity, and that low doses could not effectively control blood cholesterol from diets[47]. Notably, management of risk factors in preventive mode, rather than therapeutic mode, of cardiovascular and cerebrovascular diseases is extremely important, because the time to death or irreversible injuries after outbreak of stroke is very short[48]. Accordingly, dietary restriction and appropriate choice of fats or oils are strongly recommended.

On the basis of our serial studies, the beneficial effects of perilla oil should be emphasized, in comparison with the aggravating potential of other plant oils and fats on lipidemia, fat accumulation, overweight, blood flow, atherosclerosis as well as hemorrhagic and ischemic cerebral strokes. Therefore, it is suggested that perilla oil could be the first choice on modern food tables consuming high amount of fats and oils.

Acknowledgments

This work was supported by the "Food Functionality Evaluation Program" under the Ministry of Agriculture, Food and Rural Affairs and partly Korea Food Research Institute (G2015).

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