Effect of different commercial fat sources on brain, liver and blood lipid profiles of rats in growth phase[1]

Michel Cardoso de Angelis-Pereira[I], Maria de Fátima Píccolo Barcelos[II], Juciane de Abreu Ribeiro Pereira[I], Rafaela Corrêa Pereira[III], Raimundo Vicente de Souza[IV]

[I]Associate Professor, Department of Nutrition, Universidade Federal de Lavras (UFLA), Minas Gerais, Brazil. Conception and design of the study, acquisition of data, manuscript preparation and manuscript writing, critical revision.
[II]Associate Professor, Department of Food Science, UFLA, Lavras-MG, Brazil. Conception and design of the study, manuscript preparation and writing, critical revision.
[III]Fellow PhD degree, Postgraduate Program in Food Science, Department of Food Science, UFLA, Lavras-MG, Brazil. Analysis and interpretation of data, statistics analysis, manuscript preparation and manuscript writing.
[IV]Associate Professor, Department of Veterinary Medicine, UFLA, Lavras-MG, Brazil. Conception and design of the study, analysis and interpretation of data, critical revision.

Abstract

Purpose: To investigate the fatty acid content of different fat sources and evaluate the effect of them on plasma and hepatic lipids and on the fatty acid profile of the brain tissue of Wistar rats.
Methods: Thirty male albino Wistar rats received for 59 days, the following diets: diet added of margarine with low content of polyunsaturated fatty acids (PUFA); diet added of margarine with high content of PUFA; diet added of butter; diet added of hydrogenated vegetable fat; diet added of soybean oil. Fatty acid profile of the lipid sources, blood and hepatic lipids fractions and fatty acid profile of the brain tissue were determined.
Results: Margarine consumption of provided different responses as to concentrations of blood and hepatic lipid fractions. Intake of butter and hydrogenated increased LDL-c/HDL-c ratio, being the steepest increase promoted by hydrogenated vegetable fat, which also raised LDL-c levels expressively. All fats used in the treatments reduced the cerebral concentration of docosahexaenoic acid when compared to soybean oil (control).
Conclusion: The different fat sources commonly consumed by population provided different responses in vivo. This is particularly relevant considering the role of these lipids in the incidence and prevention of cardiovascular diseases.
Key words: Dyslipidemias. Lipid Metabolism. Rats.

■ Introduction

According to the World Health Organization (WHO), cardiovascular disease (CVD) is the leading cause of death worldwide, accounting for 30% of global deaths[1]. In general, the pathophysiological basis for cardiovascular events is atherosclerosis, a chronic inflammatory disease closely associated with certain cardiovascular risk factors such as hypercholesterolemia, hypertriacylglyceridemia, lowering of HDL cholesterol (HDL-c), hypertension, diabetes mellitus and obesity[2].

Hypercholesterolemia, in particular, stands out for being one of the main responsible for metabolic disorders of lipids, and considered the greatest risk factor for CVD[3]. It is evident, therefore, the role of different dietary patterns on the modulation of atherosclerosis and cardiovascular risk factors, especially with regard to the presence of lipids in the diet. These standards, however, do not relate only to the quantity, but also the type of ingested lipids[4]. Features such as fatty acid chain length, degree of unsaturation and double bonds configuration, related to variations observed in fatty acids present in food (saturated, monounsaturated, polyunsaturated, *trans),* strongly influence the metabolism of these nutrients as well as blood concentration of cholesterol and its distribution in lipoproteins[2,5].

It is noteworthy, in this case, the high consumption of saturated fatty acids (SFA) and *trans* fatty acids (*t*FA) by western populations, since they are present in many industrial products such as biscuits, cookies, ice cream, pies, chips among others. However, the high consumption of these fat sources are classically associated with elevation of plasma LDL cholesterol (LDL-c) and increased cardiovascular risk[6]. Other authors also state the consumption of hydrogenated fat with high content of *t*FA is related to the increase of inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) in humans with moderately increased LDL-c levels, which also contributes strongly for atherosclerotic effect, considering the different effects of these pro-inflammatory cytokines on lipid metabolism and inflammatory responses of the vascular system[7]. In addition, changes in plasma cholesterol levels induced by *t*FA can also affect immune responses, as evidenced by Simons and Ikonen[8]. Lee *et al*.[9] in turn showed SFA induced activation of nuclear factor κB (NF-kB) and the expression of cyclooxygenase-2 (Cox-2) by the receiver Toll-like-4 (Tlr4), while polyunsaturated fatty acids (PUFA) inhibit their expression, when it is mediated by SFA.

Allied with the high intake of SFA and *t*FA, with consequent adverse effects reported, currently it is also noted further trend of reducing the consumption of essential PUFA. These fatty acids play important role in numerous physiological and biochemical functions in the body, and substitution of SFA by monounsaturated (MUFA) and PUFA fat sources in the diet is considered an effective strategy for the control of hypercholesterolemia and consequent reduction in the incidence of CVD. Numerous evidence also emphasize PUFA may influence the improvement of autonomic function, antiarrhythmic, decreased platelet aggregation and blood pressure, improved endothelial function, stabilization of atheromatous plaque and triacylglycerols levels[3]. Furthermore, deficiencies in these fatty acids may have various health damages[10], especially on the cognitive function of the individual, related, for example, to the docosahexaenoic acid (DHA, 22:6 n-3) deficiency[11]. SFA are also related to the development of neurological disorders. Some studies indicate high intake of saturated fats and cholesterol are associated with loss of cognitive ability and increased incidence of Alzheimer.[12]

Given the above, and knowing the diversity of lipid sources available on the market, the differences between them in terms of fatty acid composition and the effects of these sources on biochemical parameters of the body related to lipid metabolism, this study aimed to analyze the content of fatty acids in commercial fat sources highly consumed by the population (margarine low in PUFA, margarine high in PUFA, butter and hydrogenated vegetable fat) and evaluate the effect of consumption of these sources on blood and liver lipid profiles, and on fatty acid profile of the brain tissue of Wistar rats.

■ Methods

The study was conducted according to the principles recommended by the Brazilian Society of Sciences in Experimental Animals (SBCAL) and was approved by the Internal Committee on Bioethics, Department of Veterinary Medicine, Universidade Federal de Lavras.

For the *in vivo* test 30 male albino Wistar rats (*Rattus norvegicus*), in the growth phase, initially weighing between 70 and 80g were used. The animals were from the central animal facility of the Universidade de Alfenas (Alfenas-MG, Brazil). The animals received for 59 days, standard AIN-93G diet[13] modified as to the fat source and total lipid content. The animals were divided into five groups and treated as follows: Group 1 (MGL) - diet added of margarine low in PUFA; Group 2 (MGH) - diet added of margarine high in PUFA; Group 3 (BT) - diet added of butter; Group 4 – (HVF) diet added of hydrogenated vegetable fat; Group 5 (Control) – diet added of soybean oil. All diets were given increment of 4% fat in substitution of starch, totaling 12% of lipids (Table 1).

Table 1 - Experimental diet composition.

Ingredient	AIN-93G modified ($g.100g^{-1}$)
Corn starch	38.60
Casein	14.00
Dextrin	15.50
Sucrose	10.00
Lipid source *	12.00
Celulose	5.00
Minerals mix	3.50
Vitamins mix	1.00
L-cistine	0.15
Choline bitartrate	0.25
Total	100.00

*Group 1 (MGL) - diet added of margarine with low content of PUFA; Group 2 (MGH) - diet added of margarine with high content of PUFA; Group 3 (BT) - diet added of butter; Group 4 (HVF) - diet added of hydrogenated vegetable fat; Group 5 (Control) – diet added of soybean oil.

Weight gain and feed intake of the animals were followed every 3 days to obtain the growth curve. These parameters were used to calculate the average daily intake (ADI), average daily gain (ADG) and feed efficiency coefficient (FEC).

At the end of the experimental period, the animals were fasted for 16 hours and then anesthetized with intraperitoneal injection of ketamine hydrochloride and 2% aqueous xylazine hydrochloride at doses of 60 and 20 mg. Kg^{-1}, respectively, for removal of blood from the large abdominal vessels through the thoracic and abdominal opening. Subsequently, livers and brains were removed for cholesterol and triacylglycerols analysis and chromatographic analyzes, respectively.

Analytical methods

Fatty acid profile of the lipid sources

The fatty acid profile of the lipid sources under study was determined by gas

chromatograph Varian® (model 3800) equipped with flame ionization detector, injector type split/split-less, software for monitoring the analysis, capillary column of polyethylene glycol (Ohio Valley®) with 30 m long and 0.25 mm internal diameter, stationary phase non-bonded, thickness 0.25µm (Carbowax 20M). The identification of fatty acids was performed using standard fatty acids (Supelco, template 37 mix components FAME), comparing the retention times of the fatty acids of the sample with the retention times of the standards.

Determination of blood lipid fractions

Blood samples were centrifuged at 3000 rpm for 5 minutes in centrifuge Eppendorf/ Centrifuge 5415® to obtain the serum. The analysis of lipid fractions was performed by the colorimetric-enzymatic method, using the commercial kit (In Vitro Diagnóstica®) for total cholesterol (TC) (code CAT 10552), triacylglycerol (TG) (code CAT 10724) and high density lipoprotein (HDL-c) (code CAT 044).

Total cholesterol was determined after enzymatic hydrolysis and oxidation of serum samples for quantification of quinoneimine indicator, formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. Triacylglycerols were determined after enzymatic hydrolysis with lipases. Readings were performed in spectrophotometer at 500 nm. HDL-c fraction was determined by precipitation of chylomicrons. Very low density lipoprotein (VLDL-c) and LDL-c were determined by phosphotungstic acid and magnesium chloride.

Determination of hepatic lipid fractions

Liver samples were grinded and subjected to lipid extraction with chloroform: methanol (2:1) as described by Folch *et al*.[14] for the determination of total cholesterol and triacylglycerols, following the same procedures for blood tests described above.

Fatty acids in brain tissue

The lipid extraction of the brain was performed according to the method described by Folch *et al*.[14], with adaptations. The fatty acids profile of the tissue was determined by gas chromatograph, following the same procedures adopted for analysis in lipid sources, as described above.

Statistical analysis

Results were expressed as the mean ± standard deviation. For comparisons between treatments, the numerical values were subjected to analysis of variance (ANOVA) followed by Tukey test. In all experiments the level of significance adopted was $p < 0.05$.

■ Results

Fatty acid profile of the lipid sources

Table 2 shows the fatty acid profile of the fat sources analyzed. Among the SFA, C16:0 (palmitic) and C18:0 (stearic) were the most abundant ones in the samples tested, except in the margarine with high content of PUFA, whose concentration of C18:0 was low. In addition to presenting the highest concentration of C16:0 compared to other sources, butter stood out by the high concentration of C14:0 (myristic), which, according to Santos *et al*.[2], it is a major contributor to the rise in cholesterol concentration. As for MUFA, the highest concentrations were observed in butter and hydrogenated vegetable fat, being C18:1 (oleic) the largest fraction obtained.

Table 2 - Mean content of fatty acid in the lipid sources in study.

Fatty acid	Lipd source * (g.100g^{-1})			
	Margarine with low content of PUFA	Margarine with high content of PUFA	Butter	Hydrogenated vegetal fat
C 8:0	-	-	0.23 ± 0.03	-
C 10:0	-	-	1.38 ± 0.15	-
C 12:0	0.03 ± 0.04	2.10 ± 0.31	2.20 ± 0.31	-
C 14:0	0.18 ± 0.08	1.03 ± 0.11	9.31 ± 1.02	0.14 ± 0.01
C 14:1		-	0.93 ± 0.16	-
C 15:0		-	1.29 ± 0.27	-
C 16:0	16.37 ± 2.59	11.84 ± 1.78	31.58 ± 0.17	13.53 ± 0.60
C 16:1 ω-7	0.07 ± 0.06	-	0.47 ± 0.12	0.06 ± 0.01
C 17:0	-	-	0.59 ± 0.02	-
C 18:0	11.86 ± 11.42	0.83 ± 0.82	11.43 ± 5.47	33.28 ± 3.78
C 18:1	25.84 ± 5.39	29.21 ± 2.39	37.10 ± 8.27	39.10 ± 4.32
C 18:2 ω-6	39.41 ± 7.94	45.92 ± 1.79	1.80 ± 0.80	12.97 ± 1.21
C 18:3 ω-3	4.59 ± 0.84	5.32 ± 0.25	0.63 ± 0.44	0.11 ± 0.03
C 18:3 ω-6	0.12 ± 0.22	0.13 ± 0.10	-	-
C 20:0	-	0.13 ± 0.26	0.54 ± 0.06	0.21 ± 0.13
C 20:1	0.59 ± 0.36	0.94 ± 0.35	-	-
C 20:2 ω-6	-	0.09 ± 0.08	-	-
C 20:4 ω-6	0.49 ± 0.33	0.91 ± 0.56	0.22 ± 0.18	-
C 20:5 ω-3	-	1.29 ± 1.16	-	-
C 22:0	-	-	0.15 ± 0.22	-
C 22:2 ω-6	-	0.17 ± 0.33	0.17 ± 0.14	-
C 22:6 ω-3	-	0.09 ± 0.18	-	-
C 24:0	0.46 ± 0.54	-	-	0.59 ± 0.40
SFA *	28.90	15.93	58.70	47.75
MUFA **	26.50	30.15	38.50	39.16
PUFA ***	44.61	53.92	2.82	13.08
Total ω-6	39.90	47.09	2.17	12.97
Total ω-3	4.59	6.70	0.80	0.11
ω-6:ω-3	9:1	7:1	3:1	118:1

* SFA – saturated fatty acids; ** MUFA – monoinsaturated fatt acids; *** PUFA – poli-insaturated fatty acids

PUFA, in turn, especially C18:2 ω-6 (linoleic acid) and C18:3 ω-3 (linolenic) were present in high concentrations in margarine samples. C18:2 ω-6 was found at intermediate amounts in hydrogenated vegetable fat. In addition, this product presented the highest concentration of C18:1. However, a large portion of these fractions possibly is in the *trans* form, as is characteristic of this product.

From the results presented for SFA, total MUFA and PUFA, clear differences are noted between the samples tested. While

butter and hydrogenated vegetable fat were the sources with higher fractions of SFA and MUFA, both margarines stood out by larger fractions of PUFA, and these fractions are even larger in margarine high in PUFA. As for fatty acids ω-6 and ω-3, higher concentrations were present in margarines.

In vivo assay

Table 3 shows the daily average values during the experimental phase for feed intake (ADI), weight gain (ADG) and feed efficiency coefficient (FEC) of the experimental animals.

From data presented, it is noted that the type of dietary fat did not change significantly ($p > 0.05$) ADI and ADG of the animals during the experimental period. Values found for FEC, an index of the amount of feed used for weight gain, also did not differ significantly ($p > 0.05$) among the five types of diets tested.

Table 3 - Values (mean ± standard deviation) for average daily intake (ADI), average daily gain (ADG) and feed efficiency coefficient (FEC) of the animals treated with different fat sources during 59 days of experiment.

Experimental group*	ADI (g)	ADG (g)	FEC
MGL	15.83 ± 2.33 a	3.50 ± 2.23 a	0.217 a
MGH	15.82 ± 1.44 a	3.43 ± 2.05 a	0.217 a
BT	15.35 ± 2.79 a	3.18 ± 3.56 a	0.207 a
HVF	16.65 ± 3.21 a	3.39 ± 3.44 a	0.204 a
Control	14.39 ± 3.13 a	3.09 ± 3.00 a	0.214 a

* MGL - diet added of margarine with low content of PUFA; MGH - diet added of margarine with high content of PUFA; BT - diet added of butter; HVF - diet added of hydrogenated vegetable fat; Control – diet added of soybean oil.
** Means followed by the same letters within a column do not differ from each other by Tukey test at significance level of $p < 0.05$.

Table 4 shows the mean values of serum lipids (total cholesterol, HDL-c, VLDL-c, LDL-c and triacylglycerols) of the experimental animals. Total cholesterol values ranged from 74 to 92 mg/dL among the groups and, despite the observed variations were not significant ($p > 0.05$), numerically it can be seen the lowest average for this parameter were found in the groups treated with fat sources high in PUFA (Control and MGH).

Table 4 - Values (mean ± standard deviation) for serum lipids of the animals treated with different fat sources during 59 days of experiment.

Experimental group*	Lipid fraction ** ($mg.dL^{-1}$)				
	Total cholesterol	HDL-c	VLDL-c	LDL-c	Triacylglycerol
MGL	92.97 ± 24.19 a	57.49 ± 25.68 a	21.16 ± 8.07 a	14.32 ± 12.79 ab	105.79 ±40.33 a
MGH	76.42 ± 22.06 a	50.23 ± 16.06 a	13.91 ± 4.67 ab	12.28 ± 10.62 ab	69.54 ± 23.34 ab
BT	87.61 ± 17.87 a	51.91 ± 9.95 a	13.71 ± 1.90 ab	22.48 ± 22.92 ab	68.55 ± 10.63 ab
HVF	87.90 ± 12.42 a	40.39 ± 9.41 a	12.38 ± 2.64 b	35.13 ± 17.14 a	61.89 ± 14.75 b
Control	74.28 ± 10.32 a	53.54 ± 8.97 a	14.80 ± 3.65 ab	9.26 ± 4.63 b	73.87 ± 18.11 ab

* MGL - diet added of margarine with low content of PUFA; MGH - diet added of margarine with high content of PUFA; BT - diet added of butter; HVF - diet added of hydrogenated vegetable fat; Control – diet added of soybean oil.
** Means followed by the same letters within a column do not differ from each other by Tukey test at significance level of $p < 0.05$.

Values obtained for HDL-c were not significantly different ($p > 0.05$) among the groups. However, values obtained for group treated with hydrogenated vegetable fat was numerically smaller compared to the other groups, indicating its tendency to reduce this parameter, which is a negative factor as the concentration of HDL-c is directly related to the protective effect on cardiovascular disease[15].

For VLDL-c and triacylglycerols, no group differed significantly from control, however, significant differences were found for groups treated with margarine low in PUFA and hydrogenated vegetable fat, with the latter presenting the lowest values for both parameters.

Regarding LDL-c, significant differences ($p \leq 0.05$) were found between the control group and the group treated with hydrogenated vegetable fat. In this case, hydrogenated vegetable fat increased significantly this parameter (379%).

For other groups, values found did not differ significantly when compared to values obtained for control and hydrogenated vegetal fat group.

LDL-c/HDL-c ratio for the experimental groups of this study are illustrated in Figure 1. Although LDL-c/HDL- ratio ideal for rodents has not yet been determined and the value stipulated for humans cannot be used directly, it is agreed this relationship should be as small as possible to indicate beneficial feature.[16] Therefore, from data presented, it can be seen the group treated with hydrogenated vegetal fat shown the highest ratio of LDL-c/HDL-c (0.93). This is probably due to high levels of saturated fatty acids presented (47.75%), mainly in *trans* configuration than the other lipid sources evaluated in this study.

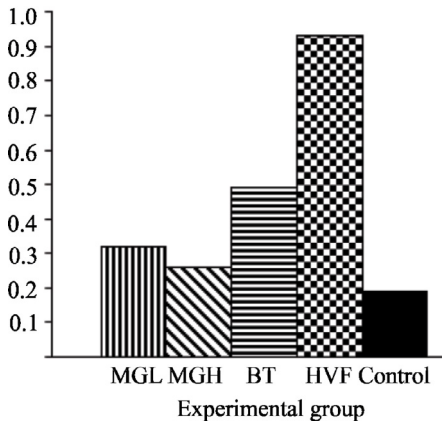

Figure 1 - Serum LDL-c/HDL-c ratio of the animals treated with different fat sources during 59 days of experiment. MGL - diet added of margarine with low content of PUFA; MGH - diet added of margarine with high content of PUFA; BT - diet added of butter; HVF - diet added of hydrogenated vegetable fat; Control – diet added of soybean oil.

Followed by hydrogenated vegetable fat, butter also presented high LDL-c/HDL-c ratio (0.49), which may be explained by the higher content of saturated fatty acids (58.70%) such as myristic and palmitic acids, as well as presence of cholesterol. In the present study, it is also evident that, accompanied the elevation of LDL-c (significant for hydrogenated vegetable fat), the groups treated with hydrogenated vegetable fat and butter had decreased HDL-c values, which explains the high values found for this index.

On the other hand, lower levels were observed in the groups treated with margarine low in PUFA (0.32), margarine high in PUFA (0.26) and soybean oil (0.19), whose PUFA content was higher. It is worth mentioning although the hydrogenated vegetable fat presented high content of C18:1 in the tested product, its *trans* form is the most abundant, being therefore, a negative aspect of the product.

The low concentration of the essential fatty acids linoleic acid (ω-6) and linolenic acid (ω-3) found in butter, and of the linolenic acid found in hydrogenated vegetable fat may have contributed to the increase of LDL-c of the animals since these fatty acids.

Table 5 shows the levels of cholesterol and hepatic triacylglycerols of the experimental

groups. The concentration of hepatic cholesterol was similar between the animals treated with different fat sources. However, triacylglycerols levels were higher in groups fed margarines.

Table 5 - Values (mean ± standard deviation) of hepatic cholesterol and triacylglycerols of the animals treated with different fat sources during 59 days of experiment.

Experimental group*	Hepatic lipid fraction ** (mg.g^{-1})	
	Total cholesterol	Triacylglycerol
MGL	5.06 ± 1.47 a	38.36 ± 9.35 a
MGH	4.70 ± 1.35 a	31.56 ± 7.90 a
BT	4.79 ± 0.50 a	20.00 ± 2.95 b
HVF	4.94 ± 0.34 a	16.30 ± 3.89 b
Control	4.18 ± 0.49 a	20.30 ± 1.80 b

* MGL - diet added of margarine with low content of PUFA; MGH - diet added of margarine with high content of PUFA; BT - diet added of butter; HVF - diet added of hydrogenated vegetable fat; Control – diet added of soybean oil.
** Means followed by the same letters within a column do not differ from each other by Tukey test at significance level of $p < 0.05$.

The animals fed margarine with high level of PUFA had hepatic triacylglycerols levels increased ($p \leq 0.05$) compared to the group treated with soy oil. This elevation where even more higher ($p \leq 0.01$) for those fed margarine with low content of PUFA. As for the other fats (hydrogenated vegetable fat and butter) no effects were noted ($p > 0.05$) in the concentration of these hepatic lipids.

Regarding profile of fatty acids in the brain tissue, it was noted in this study, despite the difference in the fats ingested, the profile of the majority of fatty acids in brain remained uniform, as shown in Table 6. However, docosahexaenoic acid (DHA) (C22:6 ω-3) was at varying concentrations in the different groups.

Table 6 - Values (mean ± standard deviation) for fatty acid content in the brain tissue of the animals treated with different fat sources during 59 days of experiment.

Fatty acid	Lipid source ** (g.100g^{-1})				
	MGL*	MGH*	BT*	HVF*	Control*
C 16:0	17.36 ± 2.90 a	18.50 ± 7.31 a	23.46 ± 4.47 a	19.97 ± 2.11 a	19.40 ± 1.48 a
C 16:1 ω-7	0.01 ± 0.01 a	0.05 ± 0.02 a	0.01 ± 0.01 a	0.15 ± 0.26 a	0.78 ± 1.15 a
C 18:0	9.96 ± 2.63 a	16.37 ± 3.57 a	9.81 ± 4.25 a	14.79 ± 1.94 a	15.09 ± 2.98 a
C 18:1	34.42± 6.50 ab	31.42 ±14.51 ab	40.23 ± 7.53 a	27.71 ± 3.24 b	29.38 ±2.70 ab
C 18:2 ω-6	2.55 ± 1.12 a	1.66 ± 0.36 a	0.94 ± 0.60 a	2.45 ± 1.76 a	2.21 ± 0.72 a
C 20:1 ω-9	2.25 ± 0.26 a	1.4 ± 2.26 a	2.29 ± 1.40 a	3.40 ± 3.64 a	1.75 ± 0.76 a
C 20:2 ω-6	1.19 ± 0.34 a	1.14 ± 0.41 a	1.09 ± 0.82 a	2.92 ± 1.95 a	1.24 ± 1.61 a
C 20:3 ω-6	0.14 ± 0.30 a	0.01 ± 0.01 a	0.01 ± 0.05 a	0.56 ± 0.98 a	1.27 ± 1.75 a
C 20:4 ω-6	15.80 ± 3.75 a	15.67 ± 4.83 a	17.35 ± 1.12 a	14.18 ± 1.91 a	12.45 ± 1.02 a
C 24:0	4.98 ± 5.61 a	7.38 ± 9.01 a	0.95 ± 1.27 a	6.85 ± 6.18 a	2.77 ± 2.38 a
C 22:6 ω-3	11.37 ± 2.91 ab	6.96 ± 3.24 bc	4.35 ± 1.98 c	6.75 ± 3.89 bc	13.55 ± 3.02 a
AGS	32.30	42.25	34.22	41.61	37.26
AGMI	36.68	32.88	45.53	31.26	31.91
AGPI	31.05	25.43	23.74	26.86	30.82

* MGL - diet added of margarine with low content of PUFA; MGH - diet added of margarine with high content of PUFA; BT - diet added of butter; HVF - diet added of hydrogenated vegetable fat; Control – diet added of soybean oil.
** Means followed by the same letters within a line do not differ from each other by Tukey test at significance level of $p < 0.05$.

Eicosapentaenoic acid (EPA) and DHA (n-3 PUFA) improve or prevent some psychiatric and neurodegenerative diseases both in experimental and clinical studies. As important membrane components, these PUFA benefit brain health by modulating neuroimmune and apoptotic pathways, changing membrane function and/or competing with n-6 PUFA, the precursors of inflammatory mediators[37].

The group BT, presented concentration of DHA in the brain significantly lower (4.35%) to the group MGL (11.37%), with an even greater difference when compared to the control group (soybean oil) (13.55%). The group MGH also showed significantly lower percentage (6.96%) than soybean oil, with values close to the HVF group (6.75%).

The values for total SFA, MUFA and PUFA were not different between the study groups. It is noteworthy, however, the concentration of MUFA in the brain was relatively close to the concentration present in the diet.

Although DHA synthesis is induced by the presence of α-linolenic acid, in the present study, the reduction of DHA is probably due to the absence of α-linolenic acid, or another fatty acid from ω-3 series. This was expressively noted in the brain of animals treated with butter, which presents α-linolenic acid in trace level (Table 2).

■ Discussion

In the present study, the consumption of margarines of different brands shown the margarine with high content of PUFA content had increased hepatic triacylglycerol level. This increase was even more pronounced in the group treated with margarine with low content of PUFA, which also showed the highest levels of blood triacylglycerols and VLDL-c. Consumption of butter and hydrogenated vegetable fat increased the LDL-c/HDL-c ratio and the steepest rise was promoted by the hydrogenated vegetable fat, which also raised LDL-c levels separately.

The ability of hydrogenated vegetable fat in increase LDL-c, several mechanisms have been proposed for this change, such as: reduction of hepatic LDL-c receptors, greater activity acilcolesterilaciltransferase (ACAT), increasing esterification of cholesterol in lipoproteins containing apo B17 and increased amount of esterified cholesterol transported in LDL-c, due to rectilinear chemical conformation of the fatty acid[18]. Additionally, as high plasma LDL-c levels are among the main cardiovascular risk factors, even more discrete reductions in LDL-c levels, as observed in the present study must be considered. This fact is evidenced by Baigent *et al*.[19] in their meta-analysis of 26 trials. The authors found even small reductions in LDL-c can contribute significantly to the reduction of deaths from coronary heart disease.

The I Guideline on Fats Consumption and Cardiovascular Health published by the Brazilian Society of Cardiology[2] also reinforces this point by stating the first lipid target for cardiovascular prevention is the reduction of LDL-c. The researchers report several epidemiological and intervention studies, especially with statins, which show unequivocally that the reduction in levels of LDL-c decreases the chance of cardiovascular events, either in the case of those who already had an event (secondary prevention), or in who never showed it (primary prevention).

Furthermore, according to Santos *et al*.[2,] although epidemiological studies report strong association between high intake of cholesterol and higher incidence of atherosclerosis, the dietary cholesterol has little effect on plasma concentration of cholesterol and atherosclerosis. Actually, SFA exert greater effect on blood cholesterol. However, because of the controversy over colesterolemiant effect of dietary cholesterol[20], several international

guidelines recommend the restriction of total fat and dietary cholesterol, aiming to reduce and control cholesterol and LDL-c levels[2].

According to Das[21], however, along with other long chain PUFA, linoleic and linolenic fatty acids have the property of reducing serum cholesterol, by the ability to block the β-hydroxy-methylglutaryl coenzyme A reductase, reducing, thus, its activity.

Some studies, however, reported that exaggerated consumption of PUFA can lead to undesirable metabolic changes, providing, for example inflammatory conditions due to excessive formation of pro-inflammatory eicosanoids such as prostaglandins and leukotrienes[22] besides formation of reactive oxygen species (ROS) and consequent oxidative damage to cellular components[23].

Regarding triacylglycerols data, according to Hosomi *et al*.[24] two factors are related to changes in triacylglycerols levels in the body: lipid absorption in the intestine and stimulation of fatty acid oxidation in the liver, mediated by CPT1a.

However, according to Murray *et al*.[25], hepatic triacylglycerols are immediate precursors of triacylglycerols contained in plasma VLDL-c. This process can be evidenced in the present study, since the group treated with margarine with high content of PUFA had significantly higher levels of liver triacylglycerols and, therefore, higher serum VLDL-c (Table 4). Such behavior is also confirmed by the group treated with hydrogenated vegetal fat, which conversely showed lower levels of liver triacylglycerols and VLDL-c.

The accumulation of lipids in the liver tissue suggests possible occurrence of oxidative damage and disruption of homeostasis in glucose and lipid metabolism[17]. Thus, results regarding the increase in triacylglycerols levels in liver shows susceptibility of the tissue of these animals to peroxidation, with possible effect on increasing end products of lipid peroxidation.

In contradiction to the results of this study, Asadi *et al*.[20] investigated the effect of intake of different lipid sources for ten weeks in Wistar rats, using canola oil, corn, grapeseed oil and lactic acid derivative (yoghurt). Results showed significant decrease in triacylglycerol levels in the liver after grapeseed oil and yoghurt intake compared to other oils and the control group, treated with water.

All fats used in the treatments reduced the cerebral concentration of DHA when compared to soybean oil, and this reduction increased according to the following order: margarine with low content of PUFA, margarine with high content of PUFA, hydrogenated vegetal fat and butter.

DHA, together with EPA, is one of the most important lipids for brain due to their numerous physiologic properties, since they can assist in prevention of psychiatric and neurodegenerative diseases through neuroprotective effects involving the modulation of membrane flow, anti-inflammatory responses and antioxidant activity[26].

Although fatty acid uptake by the brain is selective, favoring PUFA entrance and excluding cholesterol, SFA and MUFA, the relative amount of fatty acids in blood can affect the degree of penetration of them in the central nervous system[27].

Greiner *et al*.[28], analyzing the profile of fatty acids in the brain of rats consuming diet deficient in ω-3 fatty acids, identified significant reductions of 22:4 ω-6, 22:5 ω-3 and 22:6 ω-3 in the tissue evaluated. Ikemoto *et al*.[29], in turn, administering sunflower oil as lipid source in the diet of mice, demonstrated a reduction of DHA and increased content of arachidonic acid in the brains of the animals.

In the same way fatty acid deficiency in the diet can alter the lipid composition of cells, altering thus, organ function, the presence of different fatty acids in the diet can also promote such changes. Weber *et al*.[30]

demonstrated different types of oils in the animal diet during growth and development phase at the concentration of 12%, may alter the composition of brain fatty acids such as linoleic acid, arachidonic acid and DHA. In the study, the authors cite the conventional sunflower oil is rich in linoleic acid and deficient in the α-linolenic acid. Consequently, the animals fed with this fat source had the lower concentration of DHA on brain when compared with other types of oil (sunflower oil rich in oleic acid, olive oil), while arachidonic acid content was higher in this tissue.

The lipids present in brain and other neural tissues are more resistant to change than other organs, especially in adults. However, it is incorrect to say the fatty acid composition in the brain cannot be affected by diet. The biggest changes in the concentrations of DHA in the brain are obtained with diets deficient in ω-3 during pregnancy, in the early stages of prenatal development and in numerous generations submitted to diet deficient in ω-3[27].

■ Conclusions

The fatty acid profile of the fat sources analyzed indicated, in general, the fatty acids found in greater abundance were C16:0, C18:0, C18:1 and C18:2 ω-6. The *in vivo* experiment showed consumption of margarines of different brands provided different answers as to concentration of serum and hepatic lipid fractions. The animals treated with margarine with high content of PUFA content had increased hepatic triacylglycerol level. This increase was even more pronounced in the group treated with margarine with low content of PUFA, which also showed the highest levels of blood triacylglycerols and VLDL-c.

Consumption of butter and hydrogenated vegetable fat, in turn, did not alter the concentration of blood and hepatic cholesterol and triacylglycerols. However, these fat sources increased the LDL-c/HDL-c ratio. The steepest rise was promoted by the hydrogenated vegetable fat, which also raised LDL-c levels separately. All fats used in the treatments reduced the cerebral concentration of DHA when compared to soybean oil, and this reduction increased according to the following order: margarine with low content of PUFA, margarine with high content of PUFA, hydrogenated vegetal fat and butter.

■ References

1. Organização Mundial da Saúde (OMS). Cardiovacular Diseases (CVDs). Fact Sheet nº 317; 2011. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/index.html.
2. Santos RD, Gagliardi ACM, Xavier HT, Magnoni CD, Cassani R, Lottenberg AMP, Casella Filho A, Araújo DB, Cesena FY, Alves RJ, Fenelon G, Nishioka SAD, Faludi AA, Geloneze B, Scherr C, Kovacs C, Tomazzela C, Carla C, Barrera-Arellano D, Cintra D, Quintão E, Nakandakare ER, Fonseca FAH, Pimentel I, Santos JE, Bertolami MC, Rogero M, Izar MC, Nakasato M, Damasceno NRT, Maranhão R, Cassani RSL, Perim R, Ramos S. I Diretriz sobre o consumo de gorduras e saúde cardiovascular. Arq Bras Cardiol. 2013;100(1, Suppl. 3): 1-40.
3. Al Rasadi K, Almahmeed W, Al Habib KF, Abifadel M, Farhan HA, AlSifri S, Jambart S, Zubaid M, Awan Z, Al-Waili K, Barter P. Dyslipidaemia in the Middle East: current status and a call for action. Atherosclerosis. 2016;252:182-7. doi: 10.1016/j.atherosclerosis.2016.07.925.
4. Pavlisova J, Bardova K, Stankova B, Tvrzicka E, Kopecky J, Rossmeisl M. Corn oil versus lard: metabolic effects of omega-3 fatty acids in mice fed obesogenic diets with different fatty acid composition. Biochimie. 2016;(124): 150-62. doi: 10.1016/j.biochi.2015.07.001.
5. Raclot T, Groscolas R. Differential mobilization of white adipose tissue fatty acids according to carbohydrates in mixed meals. Am J Clin Nutr. 1997;(65):1427-33.

6. Brouwer IA, Wanders AJ, Katan MB. Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans--a quantitative review. PLoS One. 2010;5(3):e9434. doi: 10.1371/journal.pone.0009434.
7. Han SN, Leka LS, Lichtenstein AH, Ausman LM, Schaefer EJ, Meydani SN. Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. J Lipid Res. 2002;43(3):445-52.
8. Simons K, Ikonen E. How cells handle cholesterol. Science. 2000;290:1721–6.
9. Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptors 4. J Biol Chem. 2001;276:16683–9.
10. Valenzuela R, Barrera C, Espinosa A, Llanos P, Orellana P, Videla LA. Reduction in the desaturation capacity of the liver in mice subjected to high fat diet: Relation to LCPUFA depletion in liver and extrahepatic tissues. Prostaglandins Leukot Essent Fatty Acids. 2015;98:7-14.
11. McNamara RK, Able J, Jandacek R, Rider T, Tso P, Eliassen JC, Alfieri D, Weber W, Jarvis K, DelBello MP, Strakowski SM, Adler CM. Docosahexaenoic acid supplementation increases prefrontal cortex activation during sustained attention in healthy boys: a placebo-controlled, dose-ranging, functional magnetic resonance imaging study. Am J Clin Nutr. 2010;91(4):1060-67.
12. Grant WB, Campbell A, Itzhaki RF, Savory J. The significance of environmental factors in the etiology of Alzheimer's disease. J Alzheimer's Dis. 2002;4:179–89.
13. Reeves PG, Nielsen FH, Faheey GC. AIN-93 Purified diets for laboratory rodents: Final report of the American Institute of Nutriction ad Hoc Writing Committe on the reformulation of the AIN-76A rodent diet. J Nutr. 1993;123(11):1939-51.
14. Folch J, Lees M, Stanley S. A sample method for isolation and purification of total lipids from animal tissues. J Biol Chem. 1957;226(2):479-503.
15. Kitamura A, Iso H, Naito Y, Iida M, Konishi M, Folsom AR, Sato S, Kiyama M, Nakamura M, Sankai T. High-density lipoprotein cholesterol and premature coronary heart disease in urban Japanese men. Circulation. 1994;89:2533–9.
16. Chiattoni LM, Machado MRG, Rodrigues R da S, Soares LA de S. Influência do consumo de diferentes níveis de Spirulina no desenvolvimento e perfil lipídico de ratos. Rev Ceres. 2015;62(2):142-8.
17. Shinagawa FB, Santana FC de, Mancini-Filho J. Efeito do óleo de semente de uva prensado a frio nos marcadores bioquímicos e perfil inflamatório de ratos. Rev Nutr. 2015;28(1):65-76.
18. Lee JY, Carr TP. Dietary fatty acids regulate acyl-CoA: cholesterol acyltransferase and cytosolic cholesteryl ester hydrolase in hamsters. J Nutr. 2004;134(12):3239-44.
19. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N; Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomized trials. *Lancet.* 2010;376(9753):1670-81.
20. Asadi F, Shahriari A, Charhardah-cheric M. Effect of long-term optional ingestion of canola oil, grape oil, corn oil and yogurt butter on serum, muscle and liver cholesterol status in rats. Food Chem Toxicol. 2010;48(8-9):2454-7.
21. Das UN. Long-chain Polyunsaturated fatty acids n the growth and development of the brain and memory. Nutrition. 2003;16:62-5.
22. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polynsaturated fatty acids. J Nutr Metabol. 2012;2012(1):1-16.
23. Williams C, Buttriss J. Health benefits of Polyunsaturated Fatty Acids (PUFAs). Improving the fat content of foods. Boca Raton: CRC Press; 2006.
24. Hosomi R, Fukunaga K, Arai H, Nishiyama T, Yoshida M. Effects of dietary fish protein on serum and liver lipid concentrations in rats and the expression of hepatic genes involved in lipid metabolism. J Agricult Food Chem. 2009;57:9256–62.
25. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper: bioquímica. 7ed. São Paulo: Atheneu; 1994.
26. Song C, Shieh CH, Wu YS, Gaikwad AKS, Su KP. The role of omega-3 polyunsaturated fatty acids eicosapentaenoic and

docosahexaenoic acids in the treatment of major depression and Alzheimer's disease: acting separately or synergistically? Prog Lipid Res. 2016;62:41-54.
27. Freitas JJS, Kietzer KS. Ácidos graxos e sistema nervoso. In: Pompeia C, Procopio J, Myasaka CK, Curi R. Entendendo a gordura – Os ácidos graxos. São Paulo: Editora Manole; 2002.
28. Greiner RS, Moriguchi T, Slotnick BM, Hutton A, Salem N. Olfactory discrimination deficits in n-3 fatty acid-deficient rats. Physiol Behav. 2001;72(3):379-85.
29. Ikemoto A, Ohishi M, Sato Y, Hata N, Misawa Y, Fujii Y, Okuyama H. Reversibility of n-3 fatty acid deficiency-induced alterations of learning behavior in the rat: level of n-6 fatty acids as another critical factor. J Lipid Res. 2001;42(10):1655-63.
30. Weber N, Irmgard K, Mukherjee KD. Modulation of brain lipids of rats by various dietary oils: sunflower, high-oleic sunflower, olive, rapessed or coriander oil. Nutr Res. 1999;19(7):997-1007.

Correspondence:
Michel Cardoso de Angelis-Pereira
Departamento de Nutrição, UFLA
Caixa Postal 3037
37200-000 Lavras – MG Brasil
Tel.: (55 35)3829 1992
deangelis@dnu.ufla.br

Received: Aug 16, 2017
Review: Oct 18, 2017
Accepted: Nov 20, 2017

Conflict of interest: none
Financial sources: CNPq, CAPES

[1]Research performed at laboratories of Veterinary Science and Food Science, Universidade Federal de Lavras (UFLA), Minas Gerais, Brazil.