Comparative Effect between Sardine Oil and Fish Oil Rich in Omega-3 Fatty Acids on Hypertension and the Membrane Composition of Adipocytes in SHR Rats

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Summary Omega-3 polyunsaturated fatty acids, have an important role in reducing hypertriglyceridemia, these acids decrease the mortality for Coronary Heart Disease. Very important is the relationship between fatty acid biosynthesis and distribution in organs and tissues involved in insulin resistance and hypertension due to its role in the production of vasoactive eicosanoids and their effects on insulin sensitivity; which is estimated with the HOMA-IR index, which relates the physiological and metabolic behavior of glucose and insulin in the body. The aim of this project was to compare the effect of sardine oil and omega-3 oils rich in polyunsaturated fatty acids: EPA (≈30%) and DHA (≈50%) administered for 6 to 8 wk respectively; on the lipid composition of the plasma membrane of epididymal adipocytes in spontaneously hypertensive rats (SHR) and their relation to obesity, insulin resistance and hypertension. The administration of omega-3 enriched oil significantly decreased the HOMA criteria as an insulin resistance indicator compared to the sardine oil.

Key Words fish oil, hypertension, membrane composition, omega-3 fatty acids, sardine oil

Hypertension is an important risk factor for cardiovascular and cerebrovascular morbidity and mortality (1). Significant collaborative efforts to combat hypertension in low- and middle-income countries are urgently needed (2). Different factors have an effect on deficient control of hypertension, for example poor adherence to treatment, unhealthy lifestyles and bad eating habits (3).

There is a permanent interest in knowing how dietary fat affects the alterations associated with insulin resistance and its related cardiovascular disorders such as hypertension.

Long-chain (omega-3) polyunsaturated fatty acids (*n*-3 PUFAs) from some marine products such as fish oil have attracted important attention in recent years. Fish oil, a rich source of *n*-3 PUFA, has an important role in reducing hypertriglyceridemia, improves several determinant metabolic factors of cardiovascular risk, and decreases mortality from coronary heart disease (4). The interrelationship between the cellular synthesis of fatty acids and their distribution in organs and tissues related to insulin dependence and hypertension is of considerable importance because of their role in the biosynthesis of vasoactive eicosanoids and their effects on membrane properties that regulate insulin sensitivity (5).

With respect to the above, the amount of *n*-3 PUFA that needs to be in the diet to provide health effects is still unknown. All fish contain EPA and DHA in amounts that vary depending on species, the ratio in fish ranges between 0.22 and 1.25. There is controversy about the amounts of *n*-3 PUFAs, in particular DHA or EPA, that can have a positive effect on the prevention of metabolic disorders and cardiovascular disease (CVD). FAO/WHO, the American Dietetic Association and American Heart Association now recommend dietary intakes for total *n*-3 PUFA of 1.4 to 2.5 g/d, with EPA and DHA ranging from 140 to 600 mg/d, depending on the authority issuing the guidelines. It is very important to know the impact of increasing the concentration of dietary *n*-3 fatty acids on the composition of the plasma membrane of adipocytes and their relationship with insulin resistance and hypertension; since the plasma membrane provides fatty acids for different cellular functions, including the production of eicosanoids related to vascular tone and hypertension (6).

In relation to the above, it is not clear whether the increase in the dietary consumption of *n*-3 PUFA improves the concentration in the plasma membrane of adipocytes and proportionally reduces blood pressure.

The present study evaluated the comparative effect of two oils (sardine oil and fish oil rich in *n*-3 PUFAs) on hypertension and insulin resistance, and their relation with the fatty acid composition of the membrane of adi-

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Table 1. Composition of diets administered to rats.

Ingredients	Fish oil diet (g)	Sardine oil diet (g)	Sunflower oil diet (g)
Casein	440	440	440.0
DL-Methionine	3.1	3.1	3.1
Cellulose	40	40	40
Cornstarch	658	658	658
Vitamin mix ¹	20	20	20
Mineral mix ²	80	80	80
BHT ³	0.4	0.4	0.4
Sunflower oil	—	—	100
Fish oil	100	—	—
Sardine oil	—	100	—

¹ Vitamin mix (Teklad 40060).² Mineral mix (Teklad IIN-76 170915).³ Butylated hydroxytoluene.

pocytes in spontaneously hypertensive rats (SHRs).

MATERIALS AND METHODS

Materials. The sardine oil and fish oil rich in *n*-3 PUFAs used were obtained from Sonora University, Mexico; and Ocean Nutrition (San Diego, CA) respectively. Male Kyoto Wistar (KW) rats, spontaneously hypertensive rats (SHR) and dietary feed components were purchased from Harlan Teklad Inc. (Mexico City, Mexico).

Experimental design. Utilized were 40 male SHR rats and 20 male KW rats (21 d old). The rats were individually housed, and maintained on a 12-h light/dark cycle at 25°C. Animal handling and maintenance was realized in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (1985) (7). We worked two experimental designs; the first one consisted of three groups: a sunflower oil group (SF-SHR; *n*=10), receiving a diet with 7.5% of this oil as the lipid source; a sardine oil group (SO-SHR; *n*=10) receiving 7.5% of this oil; and a control group (KW; *n*=10) receiving a diet with 7.5% sunflower oil. Rats ingested the diets ad libitum during 6 wk. The second experimental design consisted of three groups: The SF-SHR group in which SHR rats received a diet with 7.5% sunflower oil as the lipid source; the FO-SHR group in which SHR rats received a diet with 7.5% of fish oil rich in omega-3 fatty acid ([EPA 27.22±0.15% and DHA 50.55±0.30%] Ocean Nutrition); and the control group (KW; *n*=10) in which KW rats received the 7.5% sunflower oil diet. For 8 wk, the rats had ad libitum access to the diets which were prepared with 0.02% BHT as an antioxidant and stored under refrigeration until used. Tables 1 and 2 show the ingredients of diets and their fatty acid composition.

At the end of the experimental treatments, body weight and blood pressure were measured; after 18 to 20 h of fasting, the animals were sacrificed. Serum was obtained by centrifugation of blood at 3,000 rpm, for 15 min, at 4°C. Epididymal fat pads were weighed and immediately frozen at 70°C until their use.

Table 2. Fatty acid composition of oils used in diet preparations.

Fatty acid	<i>n</i> -3 enriched fish oil	Sardine oil	Sunflower oil
14:0	0.37±0.01 ^a	7.28±1.09 ^b	0.55±0.01 ^c
16:0	1.28±0.01 ^a	26.99±3.90 ^b	12.80±0.07 ^c
18:0	1.66±0.14 ^a	8.20±1.20 ^b	Nd
Total	3.31±0.16 ^a	42.47±6.37 ^b	13.35±0.08 ^c
16:1	0.76±0.01 ^a	6.34±0.91 ^b	Nd
18:1	3.11±0.01 ^a	15.1±2.26 ^b	50.70±0.28 ^c
Total	3.87±0.02 ^a	21.64±3.24 ^b	50.70±0.28 ^c
18:2 (<i>n</i> -6)	5.18±0.90 ^a	0.48±0.07 ^b	32.80±0.12 ^c
20:4 (<i>n</i> -6)	1.20±0.03 ^a	0.36±0.05 ^b	Nd
Total	6.38±0.93 ^a	0.84±0.12 ^b	32.80±0.12 ^c
18:3 (<i>n</i> -3)	0.28±0.04 ^a	2.85±0.42 ^b	3.50±0.03 ^c
20:5 EPA	27.22±0.15 ^a	20±3.00 ^b	Nd
22:5 DPA	9.92±0.10	Nd	Nd
22:6 DHA	50.05±0.30 ^a	13±1.95 ^b	Nd
Total	87.47±0.59 ^a	35.85±5.37 ^b	3.50±0.03 ^c

^{a,b,c} *p*<0.05.

Values are expressed as percentage of total fatty acids.

Nd: not detected.

Blood pressure measurement. Systolic blood pressure was measured by a tail-cuff method (IITC noninvasive blood pressure system, model 29; Life Science Instruments, Woodland Hills, CA) while animals were conscious. The resultant blood pressure value is the mean of five systolic measurements. These measurements were made after 6 and 8 wk of treatment with the experimental diets.

Glucose and insulin measurement. Serum insulin levels was determined by a commercial double-antibody solid-phase radioimmunoassay (Coat-A-Count, DPC), and serum glucose concentration was measured by the glucose oxidase method.

HOMA-IR. The homeostasis model assessment of insulin resistance (HOMA-IR) index was obtained with the following formula: [insulin in blood in fasting (μU/mL)]×[blood glucose in fasting (mmol/L)]/22.5 (8).

Isolation of adipocytes and preparation of plasma membranes. Epididymal adipocytes were isolated from fat tissue using 1–2 g of fresh tissue, which was homogenized in Bouskella–Ringer–Tris buffer, pH 7.4 at 4°C with a Teflon pestle homogenizer (Daigger) and centrifuged at 40,000 ×*g* for 40 min at 4°C. Later, the plasma membranes were separated from the precipitates obtained by a Percoll gradient at 10,000 ×*g* for 15 min (9).

Lipid extraction and fatty acid composition determination. Lipids from diets and plasma membrane preparations were extracted according to the Folch method (10). Fatty acids were methylated (1 N HCl-methanol) and analyzed by gas chromatography (Hewlett-Packard gas chromatograph model 6890-Andover, MA), equipped with a 60-m Supelcowax-10 capillary column and a flame ionization detector. Injection and detector

Fig. 1. Total body (^{a,b} $p<0.05$) and adipose tissue weight after 6 wk of sardine oil administration (^{a,b} $p<0.01$). KW group ($n=10$), SF-SHR group ($n=10$), SO-SHR ($n=10$) group. Values represent the mean $\pm$ SD.

Fig. 2. Total body (^{a,b,c} $p<0.05$) and adipose tissue weight after 8 wk administration of fish oil rich in $n-3$ PUFA (^{a,b,c} $p<0.01$). KW group ($n=10$), SF-SHR group ($n=10$), FO-SHR ($n=10$) group. Values represent the mean $\pm$ SD.

temperatures were maintained at 250°C, nitrogen was the carrier gas, and the column temperature was programmed to rise from 100 to 210°C at a rate of 2°C/min. Fatty acid methyl esters were identified by comparison with the corresponding fatty acid standards (Sigma).

Data analysis. Data is shown as the mean $\pm$ SD. Statistical significance was determined by ANOVA, and Tukey's multiple range test was utilized for mean comparison ($p<0.05$).

RESULTS

Body weight, total adipose tissue

Figures 1 and 2 show the results of body weight and total adipose tissue; as can be seen, SHR groups had a higher body and adipose tissue weight, in comparison with KW control rats. Sardine oil administration during 6 wk did not produce any change in these parameters; however, after 8 wk of consuming fish oil rich in $n-3$ PUFAs a significant reduction in body weight (8%) and adipose tissue weight (38%) were observed, in comparison with SHR rats fed the sunflower oil diet.

Fatty acid composition of adipocytes membranes

The fatty acid profiles of the membrane lipids of epididymal adipocytes of the rats were modified in accordance to the fatty acid composition of the diets administered. Rats fed the sardine oil diet, showed lower levels of monounsaturated and polyunsaturated $n-6$ fatty acids

with respect to KW and SHR rats which were fed the sunflower oil diet, while rats fed the $n-3$ polyunsaturated fatty acids showed a significantly augmented incorporation of EPA and DHA in membranes (3-fold and 4-fold respectively), with respect to SHR rats fed the sunflower oil diet (Table 3).

In a similar way, rats fed with omega-3 enriched fish oil also exhibited lower levels of $n-6$ PUFA in membranes in comparison with SHR rats fed with sunflower oil, and an increase in $n-3$ PUFA. A higher incorporation of EPA and DHA (4-fold and 5-fold respectively) was observed, in comparison with SHR rats fed sunflower oil (Table 4). These changes resulted in a significant modification of the $n-6/n-3$ relation; while SHR rats fed the sunflower oil diet showed elevated $n-6/n-3$ ratios (59.03 ± 11.49 and 44.35 ± 3.88), the SHR rats fed sardine oil and fish oil with a higher concentration of omega-3 presented a decrease in the $n-6/n-3$ relation (2.72 ± 0.01 and 2.35 ± 0.05 , respectively).

Blood pressure, HOMA-IR and $n-6/n-3$ fatty acids ratio

Table 5 depicts the results of the blood pressure and insulin resistance in rats fed with sardine oil and $n-3$ enriched fish oil. As expected, SHR groups fed sunflower oil diets had a significant higher blood pressure with respect to KW control rats (39% and 56%). After consuming sardine oil during 6 wk, blood pressure diminished by 28% in this group, and the rats ingesting $n-3$

Table 3. Fatty acid composition of adipocyte membranes of rats fed sardine oil diet during 6 wk.

	KW	SF-SHR	SO-SHR
14:0	0.70±0.05 ^a	0.97±0.30 ^a	0.74±0.19 ^a
15:0	0.20±0.04 ^a	0.18±0.02 ^a	0.17±0.04 ^a
16:0	22.44±2.10 ^a	22.84±0.96 ^a	19.60±2.38 ^b
17:0	0.40±2.10 ^a	0.79±0.14 ^b	0.38±0.08 ^c
18:0	18.28±2.15 ^a	14.73±2.48 ^a	17.22±0.42 ^a
20:0	0.60±0.11 ^a	0.79±0.21 ^a	0.46±0.01 ^b
16:1	1.78±0.30 ^a	1.35±0.01 ^a	3.17±0.24 ^b
18:1	20.06±7.90 ^a	21.36±4.61 ^a	15.59±1.73 ^b
24:1	1.70±0.21 ^a	1.62±0.23 ^a	1.27±0.06 ^b
18:2 (<i>n</i> -6)	10.20±5.15 ^a	10.48±0.22 ^a	10.77±0.03 ^a
18:3 (<i>n</i> -6)	1.70±0.10 ^a	3.87±0.04 ^b	6.03±0.01 ^c
20:4 (<i>n</i> -6)	20.62±0.20 ^a	20.49±0.44 ^a	14.16±0.06 ^b
18:3 (<i>n</i> -3)	0.08±0.01 ^a	0.08±0.01 ^a	9.70±0.38 ^b
20:5 (<i>n</i> -3)	0.10±0.01 ^a	0.17±0.02 ^a	0.67±0.03 ^b
22:6 (<i>n</i> -3)	0.28±0.02 ^a	0.33±0.04 ^a	0.98±0.12 ^b
Total PUFA	33.56±5.51 ^a	35.42±0.77 ^a	42.31±0.64 ^b
<i>n</i> -6/ <i>n</i> -3	71.95±17.51 ^a	59.03±11.94 ^b	2.72±0.01 ^c

Values are expressed as percentage of total fatty acid (mean of %±SD).

Different letters represent differences between groups, $p<0.01$.

Table 4. Fatty acid composition of adipocyte membranes of rats fed. *n*-3 PUFA fish oil diet during 8 wk.

	KW	SF-SHR	FO-SHR
14:0	0.73±0.04 ^a	0.95±0.21 ^b	0.77±0.13 ^a
15:0	0.17±0.04 ^a	0.22±0.03 ^a	0.17±0.03 ^a
16:0	23.23±1.97 ^a	22.87±0.92 ^a	21.44±2.22 ^a
17:0	0.42±0.01 ^a	0.69±0.11 ^b	0.36±0.09 ^c
18:0	18.82±1.87 ^a	11.89±1.92 ^b	15.67±0.92 ^a
20:0	0.63±0.12 ^a	0.75±0.23 ^a	0.42±0.01 ^b
16:1	1.69±0.35 ^a	1.45±0.03 ^a	2.89±0.01 ^b
18:1	22.12±4.31 ^a	23.67±1.23 ^a	18.79±1.11 ^a
24:1	1.78±0.22 ^a	1.68±0.12 ^b	1.28±0.04 ^c
18:2 (<i>n</i> -6)	10.34±2.01 ^a	11.45±0.65 ^a	10.32±0.45 ^a
18:3 (<i>n</i> -6)	1.56±0.34 ^a	2.81±0.57 ^b	3.78±0.03 ^c
20:4 (<i>n</i> -6)	17.35±0.07 ^a	20.78±0.05 ^b	13.42±0.23 ^c
18:3 (<i>n</i> -3)	0.26±0.02 ^a	0.09±0.02 ^b	7.79±0.12 ^c
20:5 (<i>n</i> -3)	0.55±0.12 ^a	0.37±0.06 ^b	1.79±0.07 ^c
22:6 (<i>n</i> -3)	0.35±0.07 ^a	0.33±0.03 ^b	2.10±0.09 ^c
Total PUFA	30.45±2.63 ^a	35.83±1.38 ^b	39.50±0.99 ^c
<i>n</i> -6/ <i>n</i> -3	25.21±3.32 ^a	44.35±3.88 ^b	2.35±0.05 ^c

Values are expressed as percentage of total fatty acid (mean of %±SD).

Different letters represent differences between groups, $p<0.01$.

Table 5. Blood pressure, HOMA-IR in rats fed with the experimental diets.

	6 wk		
	KW group	SF-SHR group	SO-SHR group
Systolic blood pressure (mmHg)	150.60±10.67 ^a	209.10±2.95 ^b	150.60±10 ^a
HOMA-IR	0.95±0.01 ^a	1.30±0.01 ^b	0.75±0.01 ^c
	8 wk		
	KW group	SF-SHR group	FO-SHR group
Systolic blood pressure (mmHg)	142.00±6.32 ^a	222.88±12.32 ^b	164.53±11.15 ^c
HOMA-IR	2.71±0.02 ^a	4.88±0.04 ^b	1.58±0.03 ^c

KW: Kyoto-Wistar, SHR: spontaneously hypertensive rats.

KW group, $n=10$; SF-SHR group, $n=10$; SO-SHR group, $n=10$ and FO-SHR group, $n=10$.

Values represent the mean±SD. Means in the same row sharing a superscript are statistically similar ($p<0.05$).

HOMA-IR = (Insulin [μ U/mL]) (glucose [mmol/L])/22.5.

enriched fish oil during 8 wk decreased their blood pressure by 26%.

With respect to insulin resistance, HOMA-IR was higher in SHR groups fed sunflower oil (36% and 80%), thus confirming the presence of metabolic syndrome in SHR rats.

This parameter was lower in SHR rats fed sardine oil (42%) or fish oil enriched with omega-3 (67%) with respect to rats of the SF-SHR groups, the rats consuming fish oil showed even lower levels in comparison to

rats of the group control group.

DISCUSSION

Hypertension and other chronic diseases (obesity and type 2 diabetes) are associated with insulin resistance (IR) and with environmental determinants like the type of dietary acids (11, 12). For this reason, fatty acids have gained nutritional importance, particularly omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) such as EPA and DHA. There are numerous studies using diets high

in fat or sucrose in which their beneficial effect on insulin sensitivity has been demonstrated (13–15); however, it is unknown if the excessive increase in *n*-3 PUFA intake and the time of consumption have a greater or equal dietary effect on insulin resistance and hypertension.

In this work, the administration of a sardine oil diet for 6 wk did not generate any change in body weight or adipose tissue weight. In contrast, a decrease in body (8%) and adipose tissue weight (38%) was observed after the administration of the *n*-3 enriched fish oil diet for 8 wk. The cellular mechanisms related with the results above mentioned have been realized by some researchers who study the effect of *n*-3 PUFA on weight, for example, the *n*-3 PUFA increase in the expression of many genes involved in lipid metabolism (16), and transcription factors like PPAR- γ 2 remodeling the adipose tissue in adult animals, driving the formation of small insulin-sensitive white adipocytes (17).

On the other hand, with respect to blood pressure, feeding white sardine oil or *n*-3 PUFA enriched fish oil showed a similar impact on blood pressure levels; conversely, insulin resistance, evaluated by the HOMA-IR index, diminished to a greater percentage with *n*-3 PUFA enriched fish oil (67%) compared to sardine oil (42%).

In connection with the above results, several studies in humans and animals have demonstrated the beneficial effects of *n*-3 PUFA supplementation on insulin sensitivity and blood pressure; feeding with *n*-3 PUFA improves insulin sensitivity (18), while *n*-6 PUFAs deteriorate the sensitivity to this hormone (19). In addition, numerous epidemiologic studies support these findings in animals and demonstrate that habitual fish intake is inversely associated with the incidence of impaired glucose tolerance and type 2 diabetes (20).

With respect to the results obtained on the increase in the sensitivity to insulin and its relation with the composition of fatty acids of the plasma membrane of adipocytes, we can mention that according to other authors, the *n*-3 PUFAs change the fatty acid composition of membrane phospholipids and modify membrane-mediated processes such as insulin transduction signals, and activities of any enzymes for example lipases and the biosynthesis of eicosanoids (21). On the other hand, *n*-3 PUFAs compete for the same metabolic enzymes as *n*-6 PUFAs (22), so that decreasing the dietary *n*-6/*n*-3 ratio increases the concentration of (*n*-3) into membrane phospholipids, resulting in a decrease of the deleterious eicosanoids and thromboxane formation, while increasing the synthesis of eicosanoids from *n*-3 PUFAs, which promote vasodilatation and are anti-atherogenic.

In relation to the above, the ideal *n*-6/*n*-3 ratio is approximately 5 : 1 (23) in order to have a beneficial equilibrium of substrates to synthesize pro-atherogenic and vasoconstrictive versus anti atherogenic and vasodilators eicosanoids. In our case, sardine and *n*-3 PUFA enriched fish oils had *n*-6/*n*-3 ratios lower than 1, while the sunflower oil *n*-6/*n*-3 ratio was 9.3.

Finally, with respect to the composition of fatty acids of the plasma membrane of the adipocyte, and the effect of the concentration of dietary polyunsaturated fatty

acids *n*-6 and *n*-3, we found no drastic changes in the concentration of EPA or DHA in the membrane of the adipocyte of rats that consumed sardine oil for 6 wk or oil rich in *n*-3 PUFA for 8 wk (Tables 3 and 4). The above results are similar to those of other authors, who reported that EPA and DHA are not stored in adipose tissue in similar proportion to their dietary concentrations (24, 25). We found that the incorporation of DHA and EPA in membranes was in lesser proportions than those found in the diet, which suggests that these fatty acids may be preferentially oxidized and not stored (26–29), favoring the production of antihypertensive and anti-atherogenic compounds.

CONCLUSIONS

The results of this study demonstrate that increasing the concentration of *n*-3 PUFAs by utilizing dietary fish oil rich in EPA and DHA, and a longer intake (8 wk) significantly improved insulin sensitivity in comparison with a lower consumption of *n*-3 PUFAs and shorter feeding time (6 wk). In the same way, the antihypertensive effect was similar, regardless of the level of intake and time consumption of *n*-3 PUFAs. These effects could be related to changes in the lipid composition of plasma membrane of adipocytes, oxidation of *n*-3 PUFA, or the type of substrates available for the production of active lipid metabolites acting on insulin resistance and hypertension.

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