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Dietary fish oil affects food intake, growth and hematologic values of weanling rats

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SUMMARY. The object of this study was to evaluate the effect of increasing amounts of dietary fish oil on growth and hematological variables of the weanling male Sprague-Dawley rat. Animals were fed diets containing either fish oil (FO) or sesame oil (SO) at 5, 10 or 15% (w/w) for 31 d. Growth retardation and reduced food intake was noted in groups fed FO. Hemoglobin (Hb) concentration diminished when the dietary FO was above 5% (w/w). FO is a poor source of (n-6) fatty acids. We postulate that a partial deficiency in (n-6) polyenic family, is a consequence of the increasing amounts of FO in the diets, that may affect growth and erytropoiesis. In this report we show evidence supporting the hypothesis that diets enriched with fish oil can alter normal growth and induced hematological changes in the male weanling rat. Key words: Fish oil, low (n-6)/(n-3) ratio, growth, erytropoiesis, male Sprague-Dawley rats.

RESUMEN. El aceite de pescado afecta el consumo de alimento, el crecimiento y los valores hematológicos de las ratas recién destetadas. El objetivo de este estudio fue evaluar el efecto de dietas con cantidades crecientes de aceite de pescado sobre el crecimiento y las variables hematológicas de ratas machos de la cepa Sprague-Dawley, recién destetadas. Los animales fueron alimentados por un período de 31 días con dietas que contenían aceite de pescado (AP) o aceite de ajonjolí (AA) incorporado al 5,10 'o 15% (pxp). Se observó una reducción tanto del crecimento como de la ingesta de alimento en los grupos que consumieron AP. La concentración de hemoglobina (Hb) disminuyó en relación al incremento (>5% pxp) del AP dietario. El AP es una fuente pobre en ácidos grasos de la serie (n-6). Postulamos que la deficiencia parcial de la serie poliénica (n-6), que resultó al incrementar las cantidades de AP dietario, puede afectar el crecimiento y la eritropoyesis.

INTRODUCTION

Cardiovascular diseases (CD) are one of the most important factors determining mortality and morbidity in industrialized western countries. The control of CD is leaning heavily on primary prevention through changes in lifestyle, among which dietary habit modifications are most relevant. (1-4). Many reports have shown that populations subsisting on diets rich in marine foods show a low prevalence of cardiovascular disease (5-7). The action of marine foods has been attributed mainly to the protective metabolites (TAX_3, PGI_3, LTB_5) derived from eicosapentaenoic acid (EPA) 20:5(n-3) (8-15).

Additionally, polyunsaturated fatty acids themselves have shown a gene suppression effect on the expression of the hepatic fatty acid synthetase, displaying polyenic (n-3) family the greatest inhibitory potency (16).

Evidence exists showing that marine foods (fish or fish oil) can induce hematologic changes, involving both the erythrocyte and megacariocyte lines (17-21). Decreased hemoglobin (Hb) concentration after fish intake, diminished platelet counts and at least one case of severe anemia has been reported (22-24).

We hypothesize a negative modulation of the erythrocyte production, when bone marrow microenvironment and erythropoietic related organs, are enriched in (n-3) PUFA. We reported here the effects of increasing dietary fish oil on growth, food intake and hematologic values of weanling Sprague-Dawley rats.

MATERIALS AND METHODS

Animals.- The experimental protocol was adhere to the Guide for Care and Use of laboratory Animals (25). Forty eight weanling Sprague-Dawley male albino rats. (Instituto de Medicina Experimental, Facultad de Medicina UCV. Caracas, Venezuela) weighing 40-60 g were randomly assigned to six groups (F5, F10, F15, S5, S10, S15) each containing eight

animals wich were placed individually in stainless steel cages with wire-mesh floors.

Diets.- Six experimental diets were prepared based on AIN recommendations (26) and formulated to contain (w/w) 5, 10 and 15% fish oil (FO) or 5, 10 and 15% sesame oil (SO). The composition of the basal diet is shown in Table 1. (27). Dry components were mixed and stored at -20°C just prior to the addition of dietary oils. After oils addition, diets were stored at -20°C under N_2 and used within 7 d. The peroxide values of the oils, measured by peroxide index (28) were 71 and 42 meq/L for FO and SO respectively. All diets were supplemented with vit E (d-alpha tocopherol plus dl-alpha tocopheryl, Sundown Vitamins) 2 mg/g polyunsaturated fatty acid as an antioxidant.

TABLE 1
COMPOSITION OF THE BASAL DIET (%)

Ingredients	g/100 g diet
[1]Casein	15
[2]D-L Methionine	0.3
[2]Fiber (cellulose)	3
[2]Choline bitartrate	0.2
[3]Vitamin mix AIN-76A	1
[4]Mineral mix AIN-76	3.5
[5]Corn starch	q.s
[6]Oil	

1 Vitamin-Free Test Casein, (Teklad, Madison, WI) 89% crude protein measured by Kjeldahl (26).

2 Teklad, Madison, WI.

3 Vitamin mix AIN-76A (Teklad, Madison, WI) in g/kg mixture: Thiamin HCL, 0.6; Riboflavin, 0.6; Pyridoxine HCl, 0.7; Niacin, 3.0; Biotin, 0.02; Calcium Pantothenate, 1.6; Folic acid, 0.2; Vitamin B12 (0.1% trituration in mannitol), 1.0; Dry Vitamin A palmitate (500,000 U/g), 0.8; Dry Vitamin E acetate (500 U/g), 10.0; Vitamin D trituration (400,00 U/g), 0.25; Menadione sodium bisulfite complex, 0.15; Sucrose, fine powder, 981.08.

4 Mineral Mix AIN-76 (Teklad, Madison, WI) in g/Kg mixture: Calcium phosphate, dibasic, 500.0; sodium chloride, 74; potassium citrate, 220.0; potassium sulfate, 52.0; magnesium oxide, 24; manganous carbonate, 3.5; ferric citrate, 6.0; zinc carbonate, 1.6; cupric carbonate 0.3; potasium iodate, 0.01; sodium selenite, 0.01; cromium potasium sulfate, 0.55; sucrose finely powdered, 118.03.

5 Corn starch, adjusted (w/w) at expense of the dietray oils (Pandock, Caracas, Venezuela).

6 Fish Oil (Rp Scherer co., Caracas, Venezuela) and Sesame oil (Industrias El Rey, Caracas, Venezuela) Oils were added 5,10 or 15% (w/w) into the basal diet at expense of the corn starch.

Fatty Acid composition. The fatty acid composition of the dietary oils is shown in Table 2. Analytical grade solvents (Merck, Darmstadt) were used for the transmethylation (29) of lipids, and the fattuy acid methyl ester were separated using gas chromatography (Hewlett Packard Model 5880A) on a polyethylene adipate 4% Cromosorb column. Retention time of highly purify standars were used to identified fatty acids methylesther.

TABLE 2
FATTY ACID COMPOSITION OF DIETARY OILS

Fatty acid	Fish oil (%)	Sesame oil
< 16:0	35.29	.12
16:0	16.80	10.15
18:0	2.77	3.32
18:1 n-9	9.78	38.27
18:2 n-6	1.97	48.14
18:3	3.05	—
20:4 n-6	1.61	—
20:5 n-3	21.03	—
22:6 n-3	7.8	—

Single determinations

Experimental design.
Six groups of animals were fed for 31 d two different sources of dietary oils, groups F5, F10, F15 consumed 5, 10 and 15% (w/w) fish oil and groups S5, S10, S15 consumed 5, 10 and 15% (w/w) sesame oil respectively. Food and fresh tap water were provided adlibitum. Food was changed daily to prevent rancid diet intake, individual food intakes were registered daily and body weights increments recorded every 3 d. A 12 h dark-light cycle was maintained in the room.

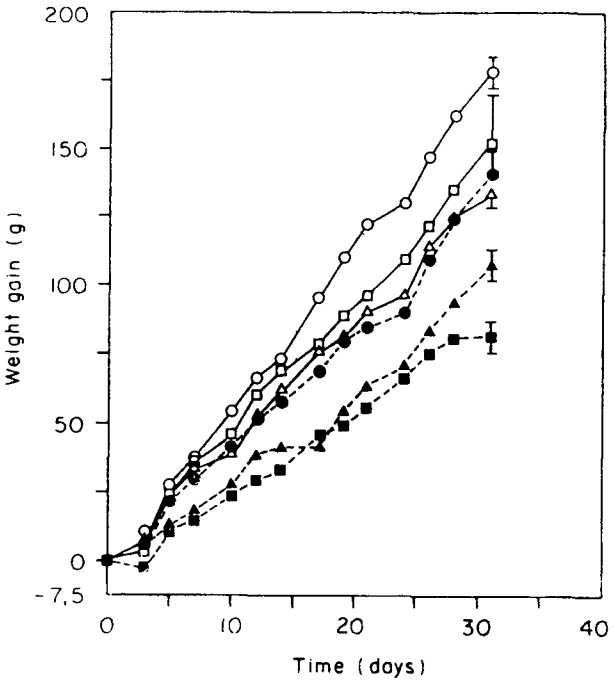
Hematologic parameters.
At the end of the experiment, blood samples were obtained by cutting the tip of previously warmed tails. Hb concentration was determined using the cyanomethahemoglobin method (30), hematocrit (packed red cell volume) was read on a microcapillary reader after centrifugation (Internationl IEC micropaillary centrifuge) for 5 min.

Statistical analysis
We used a completly randomized design with six treatments and eight repetitions. Results are reported as mean +/- standard error (SEM). Mean differences intra-treatments were compared using a one-way ANOVA. To compare means, Duncan's multiple range test was used. Differences intertreatments were assessed by two-tailed Student's t-test. The level of significance for all test was $p<0.05$.

RESULTS

Weight gain and food intake. Figure 1 shows the differences in growth curves during the experimental days. Weight gain for groups fed S10 and S15 were below that of the S5 (25 and 14% respectively), which may reflect the effects of the lower food intake among these groups (Table 3) although food efficiency of SO diets show no differences. However, in groups fed FO diiets growth was markedly affected. Negative growth was detected in F15 as early as the first three days of treatment. In general, feeding FO diets resulted in a signficant growth impairment that is 20% less for groups F5 and F10 and 46% less for group F15 when compared to the corresponding groups S5, S10 and S15 respectively ($p<0.05$). Mean differences for growth in FO treatment were statistically significant ($P<0.05$). The total food intake in groups fed FO diets (Table 3) decreased with increasing FO concentration affecting both, protein intake and food efficiency ($P<0.05$).

FIGURE 1



Weight gain of 24.d -old rats. Groups FO -fish oil -and SO -sesame oil- at various concentrations:
5% Ö———O 10%; D ————D 15%———— —— (w/w) in the basal diet during 31 d. Filled symbols for fish oil, open symbols for sesame oil. Values are means, at 0, 10 and 20 days. Mean +/- SEM at 30 d n=8 rats/group.
When FO was used above 5% a marked negative effect on growth was observed $p<0.05$. Feeding SO above 5% have a less marked effect.

TABLE 3
EFFECTS OF INCREASING AMOUNTS OF DIETARY OILS ON FOOD INTAKE AND FOOD EFFICIENCY

	Dietary Oil	Concentration of dietary oils g/100g diet 5	10	15
Total Food Intake (g)	FO	319.5+/-17.7^{a}	264.6+/-4.5^{b}	201.5+/-13.6^{c}
	SO	397.3+/-3.1^{A*}	341.5+/-31.2^{B*}	326.8+/23.5^{c*}
Protein (g)	FO	48+/-2.7^{a}	38+/-2.0^{b}	30+/-2.2^{c}
	SO	58+/-1.9^{A*}	51+/-4.7^{B*}	49+/-3.5^{C*}
Fat (g)	FO	16+/-0.9^{a}	25+/-1.2^{b}	30+/-2.2^{c}
	SO	20+/-1.9^{A*}	34.6+/-3.1^{B*}	49+/-3.5^{C*}
Food Efficiency (%)	FO	44.32+/-0.9^{a}	40.51+/-1.5^{ab}	38.1+/-2.7^{b}
	SO	44.96+/-1.3^{A}	43.29+/-3.3^{A}	45.98+/-3.6^{A*}

FO: Fish oil; SO: sesame oil.
Values are means +/- SEM, n=8. The intratreatment effects of the increasing amounts of dietary oil were tested by Duncan's multiple range test. Means not sharing same superscript are significantly different, $p<0.05$. Upper-case letter were used fo SO treatment, lower-case letter were used for FO treatment. Intertreatment effects at each dietary oil concentration were tested by Student's t-test. Values with and asterisk are signficantly different $p<0.05$.

$$\text{Fodd Efficiency} = \frac{\text{Total growth (g)}}{\text{Total food intake (g)}} \times 100$$

Hematologic parameter.- Hb concentration among groups fed SO diets showed no differences Table 4. Data from FO on the contrary, showed an Hb concentration markedly reduced when fish oil was fed above 5%, which resulted into significant changes ($p<0.05$) when comparing F10 vs S10 and F15 vs S15. Packed red cell volume (PRCV) (Table 4) consistently reflected the Hb results inter and intra treatments. In general, the hematologic values for groups fed FO diets were lower compared to control oil (SO). Mean corpuscular concentration (MCHC) index (Table 4) decreased with increasing amounts of dietary oils, this effect was remarkable in F10 and F15 groups.

TABLE 4
EFFECTS OF INCREASING AMOUNTS OF DIETARY OILS ON HB, PRCV, AND MCHC

	Dietary Oil	Concentration of dietary oils g/100g diet 5	10	15
Hb	FO	12.1+/-0.2^{a}	9.8+/-0.4^{b}	10.1+/-0.3^{c}
(g/100mL)	SO	13.1+/-0.7^{A}	13.1+/-1.1^{A*}	13.9+/-0.3^{A*}
Protein	FO	38.3+/-0.6^{a}	32.7+/-1.3^{b}	32.8+/-1.8^{b}
(%)	SO	40.0+/-3.1^{A*}	38.4+/-1.2^{A*}	42.3+/-0.7^{A*}
MCHC	FO	16+/-0.9^{a}	25+/-1.2^{b}	30+/-2.2^{c}a
(%)	SO	35.0+/-0.9^{A*}	33.9+/-1.0^{A*}	33.1+/-0.3^{A*}

FO: Fish oil; SO: sesame oil.
Values are means +/- SEM, n=8. The intratreatment effects of the increasing amounts of dietary oil were tested by Duncan's multiple range test. Means not sharing same superscript are significantly different, $p<0.05$. Upper-case letter were used fo SO treatment, lower-case letter were used for FO treatment. Intertreatment effects at each dietary oil concentration were tested by Student's t-test. Values with different an asterisk are signficantly different $p<0.05$.

DISCUSSION

Rats adjust energy input by reducing food intake when caloric density is increased (31). In agreement with this, food intake decreases significantly ($P<0.05$) when dietary SO increases above 5% level, however the rate of growth was persistently normal as well as the food efficiency. On the contrary, the FO diets caused a reduction in food intake, lowered food efficiency (Table3) ($P<0.05$) and a considerable lower growth rate. Growth rate showed two groups of curves, one with a normal growth rate and similar food efficiency (S5, S10, S15 and F5) and another group with clearly low growth rate (F10 and F15) (Fig. 1). In general, growth retardation results from dietary restriction or chronic ingestion of unbalanced diets. In this study protein ingestion was reduced in the F10 and F15 groups but not in the others (Table 3), the low growth rate showed by F10 and F15 could reflects poor protein synthesis as judge by impaired food efficiency.

The unbalance in essential fatty acids in the experimental FO diets [low (n-6)/(n-3 ratio]could explain the lower intake and the poor food efficiency showed by F10 and F15 groups. In this study the anemia syndrome produced by diets containing FO above 5% (w/w) shows that FO, when present as a major caloric source, interferes with erythropoiesis. Our hematologic findings rule out hemolysis, evidenced by normal reticulocyte count (data not shown) and are more indicative of a hypocromic anemia. Moreover, others investigators have shown an increase in red cell deformability after FO treatments (17-18).

Since, on the other hand, bleeding has not occurred, iron bioavailability should be considered carefully. In this context, considering that gastrointestinal absorption of non-heme iron is subjected to the interactions of macronutrients present in the diet (32), thus FO could conceivably interfere with its absorption.

However, the degree of fatty acid unsaturation does not have a marked effect on iron absorption, at least on diets with more than 5% dietary fat (33). Moreover, increasing the amounts of dietary fat enhanced non-heme iron absorption regardless of the type (34).

In view of these considerations, we can't reject some degree of inhibition of iron absorption by FO because ferrokinetics was not assessed in our study.

We can't attribute the diminished Hb concentration in our reuslts entirely to the reductioj of protein intake, since anemia of protein malnutrition is observed only with more restricted protein ingestion.

On the other hand, we should take into account that our experimental diets marginally covered the essential nutrient requirement for (n-6) fatty acid of weanling rats. In fact, they provided 0.22; 0.42 and 0.60% of total dietary energy as (n-6) fatty acid in diets F5, F10 and F15 formulations respectively, which is below the near 1% requirement value found by Pudelkewicz (35). Therefore, it is possible that the anemia may be due to a general inhibition of new tissue formation in growing animals, as a consequence of the partial (n-6) fatty acid deficiency. In fact, phospholipid structure in the red cell membrane is rich in linoleic and araquidonic acids. In addition, it has been shown that FO due to its high content of 20:5(n-3) and 22:6(n-3) fatty acids inhibits $\Delta 6$ and $\Delta 5$ desaturases, which are the enzymes responsible for the reactions (36-37). Thus, even a partial restriction on linoleic intake, in diets high in (n-3) fatty acids might hamper growth and new tissue formation. This is specially true in growing animals when a considerable mass of cell membranes is being formed. Besides erythropoiesis, other hematopoietic cell lines are affected under these conditions since thrombocytopenia has been repeatedly reported by several workers (19-22).

Because growth of rats was markedly retarded in groups fed FO above 5% the implication are that a low (n-6)/(n-3) ratio might impair protein synthesis, as well as affect hematopoietic function. Whether supplementation of the diets with SO enough to avoid a low (n-6)/(n-3) ratio could have functional consequences leading to restore functions needs further investigation. The link between hematopoietic function and dietary (n-6)/(n-3) ratio deserves more attention and further studies to test this possibility.

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