

Research Article

Different Lipid's Sources: Influence on Serum Fatty Acid Profile, in an Experimental Model

Perris PD¹, Fernández I¹, Sanahuja MC¹, Slobodianik NH¹, Feliu MS^{1*}

¹Department of Nutrition, University of Buenos Aires Argentina, Argentina, USA

*Corresponding author: María Susana Feliu, Department of Nutrition, School of Pharmacy and Biochemistry, University of Buenos Aires Argentina, Junin 956- 2nd Floor (PA: 1113) Caba- Argentina, USA, Tel: 54-11-49648242; Fax: 54-11-49648243; Email: msfeliu@ffyb.uba.ar

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Abstract

It is widely accepted and recognized, the importance of diet in maintaining health. Diet lipid profile is important to prevent chronic diseases and improve the quality of life of individuals. The objective of this report is to analyze the effect of different sources of dietary lipids with standard and high concentration, on fatty acid profile of growing rats. Experimental diets contained 15 or 40% kcal of fat, provided by butter (B), olive oil (O), sunflower oil high oleic acid (HO) and sunflower oil (S). Control diet (C) was norm caloric, with 15%kcal of fat provided by soy oil. All diets were complete in the others nutrients and were administered for 40 days. Daily intake was similar in all groups. The administration of these diets provoked changes in serum fatty acid profile in response to the differences sources of dietary lipids used, only some changes observed in G group were in response to the high fat percentage.

Keywords: Nutrition; fatty acids; Lipids; Diets; Rats

Introduction

A balanced and varied diet is important to maintain optimal health status and prevent non-communicable chronic diseases. There are a rapidly increased of the non-communicable diseases, unhealthy diet and lack of physical activity, which are the major causes of cardiovascular disease, type 2 diabetes and certain type of cancers.

The Global Strategy on Diet, Physical Activity and Health proposed that diet must achieve energy balance and a healthy weight; limit energy intake from total fats and change fat intake from saturated red fats to unsaturated fats and towards the elimination of trans fatty acids; increase consumption of fruits and vegetables and legumes, whole grains and nuts; limit de intake of free sugars and limit salt (sodium) ingestion from all sources and ensure that salt is iodized [1].

Children and adolescents who are overweight and obese have a higher risk of cardiovascular disease. There is a close correlation between childhood obesity and obesity throughout life, which increases with age. Preventing obesity in childhood significantly decrease morbidity and mortality in adults [2-4].

A balanced diet formed by the foods that provide an adequate amount of each and every one of the nutrients (carbohydrates, lipids, proteins, vitamins, minerals and water) is that we need to have optimal health. Malnutrition occurs when nutrients are not contributed in the necessary quantities. From a physiological point of view, malnutrition is the result of the imbalance between the specific needs of essential nutrients and energy that the organism demands, and its provision by food.

Dietary lipids have a very important role in nutrition and must be consumed in proper proportion. From a publication made in 1929 by George and Mildred Burr [5], lipids were no longer seen such a simple source of energy; the experimental study performed in rats showed that with the administration of a diet without lipid, animals

fell ill and mostly ended up dying. Then, they followed several studies which allowed learning new features. Today the lipids are recognized as the most concentrated source of energy, source of essential fatty acids, vehicle of fat-soluble vitamins (vitamins A,D,K and E), confer palatability to the food, produce satiety and play an important role immune modulator.

A modified diet, particularly in its lipid profile combined with regular physical activity, can prevent, delay, or reverse the development of Coronary Heart Disease, with subsequent reduction in cardiovascular events. [6-11]

Fatty acids (FA) have different functions: participate in the formation of phospholipids membrane, precursors in the synthesis of prostaglandins, thromboxanes, leukotrienes and prostaciclins, being involved in the regulation of blood pressure, platelet aggregation, modulation of inflammatory processes, etc. The ω 3 FA are cardiovascular health protectors: reduce blood concentrations of triglycerides and cholesterol, produce weak platelet aggregation, prevent arrhythmias, and improve microcirculation. The consumption of ω 6 FA lowers total and low density lipoprotein cholesterol (LDL) [8,12,13].

The FA families: ω 3, ω 6 and ω 9 (oleic acid), share the same biosynthetic route using the same enzymes (desaturases and elongates). These enzymes have greater affinity for the ω 3 series; nevertheless, high levels of linoleic acid (LA) can inhibit the conversion of Alfa-linolenic acid (ALA) in Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Thus, for each unit of ω 3 FA supplied by diet, 5-10 units of ω 6 should be consumed (FAO-WHO). In most Western countries, diets are usually unbalanced, with very high intake of ω 6 FA (ω 6/ ω 3 ratio= 15-20:1), as it is the case in our country, with a high consumption of sunflower oil. On the other hand, the type and quantity of FA from the diet usually consumed, has a direct effect on the concentration of plasma lipids and on the different lipoprotein [14-17]. Taking into account the exposed and the

importance of the quantity and balance of lipids in the diet on the progress of cardiovascular diseases and obesity, the aim of this study is to analyze the effect of diets containing 15 or 40 Kcal of lipids in 100 total Kcal (F%), from different sources, for 40 days, on serum fatty acids profile, in growing rats.

Methods and Materials

Animals

Wistar rats were obtained from a closed colony at the breeding unit from the Food Science and Nutrition Department (Faculty of Pharmacy and Biochemistry, Buenos Aires University). During all the experiments, animals were housed individually in screen-bottom cages and exposed to a 12 hour light-darkness cycle (7.00 AM to 7.00 PM) room temperature was kept at 21°C±1.0. The rats were suckled since birth to weaning (21-23 days) in groups of 6-8 pups per dam. At weaning (35-40g), the animals were fed for 40 days with diets containing 15 or 40 F%. Water and diet were offered "ad libitum". Diet consumption of each rat was calculated every 2 or 3 days.

Each experience was performed in duplicate using 6-8 animals per group and the results are the average of them.

At the end of the experimental feeding period and after a 4 hour fasting, the animals were anesthetized with ketamine/xylazine and were killed.

The protocol was approved by the Ethics Committee of University of Buenos Aires and all procedures were in accordance with the department's guide for the care and use of laboratory animals.

Diets

The sources for both lipids concentrations-expressed as 15 or 40 Kcal of lipids in 100 total Kcal (F %) was: butter (B Diet); olive oil (O Diet); sunflower oil high oleic acid (HO Diet) and sunflower (S Diet).

The control group received a norm caloric diet containing F% 15% fat provided by soy oil (C Diet). Composition of experimental and control diets is presented in Table 1.

Determination of FA profiles of diets

The determination of the fatty acid profile was done by gas chromatography (GC) on a Perkin Elmer chromatograph Claurus 500 equipped with a flame ionization detector. The fat was derivatized

according to IRAM 5650 Part II [18]. FA was identified by retention time and data were expressed as the percent (%) of total fatty acids. $\omega 6/\omega 3$ and PUFA/SFA ratios were calculated.

Serum lipid profile

Serum was obtained from centrifugation of blood samples obtained by heart puncture. FA profiles on serum were determined by GC. Fatty acid methyl esters (FAMES) from serum were prepared according to a modified method of Lepage [19]. Briefly, 2 ml of a methanol-n-hexane (4:1, vol/vol) were added to 200µl of plasma and then 0.2 ml of acetyl chloride was slowly added. After heating at 100°C for 1h, 5ml of a 6% K₂CO₃ solution was added to the tube, mixed on a vortex and centrifuged, and the clear n-hexane top layer containing FAMES was obtained. FAMES were analyzed using a Claurus 500 GC equipped with a flame ionization detector. The serum fatty acid data were expressed as the percent (%) of total fatty acids.

Statistical analysis

The statistical analysis used Bartlett's test, followed by one-way analysis of variance (ANOVA) and Dunnett test [20], considering the differences significant with the control group when $p < 0.01$.

Results

FA profiles of diets

B diet offer a greater quantity of palmitic acid in comparison with the other diets; O and HO diets contains a high percentage of oleic acid and S diet provides high amount of linolenic acid. The $\omega 6/\omega 3$ and polyunsaturated and saturated FA (PUFA/SFA) ratios were calculated (Table 3). O, HO and S diets are above the normal range for $\omega 6/\omega 3$ ratio. B, O and HO diets have distorted ratio PUFA/ SFA (>1.5), the diet B provided a large amount of saturated FA and O and HO provide low proportion of PUFA (Table 2).

Diet intake

Diets had a good acceptance by the rats and no significant differences were found when food intake was compared with control (Table 3).

Serum FA profile

Table 4 shows serum FA profiles of the experimental groups when diets are administered during 40 days. B group presents increase of

Table 1: Composition of experimental and control diets.

| g/1000g of diet | B F%=40 | B F%=15 | O F%=40 | O F%=15 | HO F%=40 | HO F%=15 | S F%=40 | S F%=15 | C F%=15 |
|-------------------------------|------------|------------|------------|------------|-------------|-------------|------------|------------|------------|
| Casein | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Salts | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 |
| Vitamins | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Butter | 225 | 70 | - | - | - | - | - | - | - |
| olive oil | - | - | 225 | 70 | - | - | - | - | - |
| sunflower high oleic acid oil | - | - | - | - | 225 | 70 | - | - | - |
| Sunflower oil | - | - | - | - | - | - | 225 | 70 | - |
| Soybean oil | - | - | - | - | - | - | - | - | 70 |
| Vit. A | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Choline chloride | 7,1 | 7,1 | 7,1 | 7,1 | 7,1 | 7,1 | 7,1 | 7,1 | 7,1 |
| Dextrine | 521,9 | 676,9 | 521,9 | 676,9 | 521,9 | 676,9 | 521,9 | 676,9 | 676,9 |

Table 2: Diets FA profile (% mean area \pm SD) and ω 6/ ω 3 and PUFA/SFA ratios.

| Diet | B | O | HO | S | C |
|------------------------|-------|-------|-------|-------|-------|
| Palmitic | 26,38 | 8,00 | 3,62 | 6,6 | 10,51 |
| Oleic ω 9 | 20,88 | 69,13 | 85,27 | 28,2 | 22,78 |
| Linoleic ω 6 | 2,69 | 15,89 | 5,99 | 57,5 | 53,31 |
| Linolenic ω 3 | 0,48 | 0,32 | 0,07 | 0,23 | 5,92 |
| ω 6/ ω 3 | 5,6 | 49,6 | 86,0 | 250,0 | 9,0 |
| PUFA/SFA | 0,06 | 1,36 | 0,72 | 5,35 | 3,89 |

Table 3: Diets' intake expressed as mean \pm SD.

| | Diet' intake (g/d) |
|-----------|--------------------|
| C40 | 13,70 \pm 2,08 |
| B GROUPS | |
| F15% | 13,24 \pm 1,68 |
| F40% | 12,94 \pm 1,92 |
| O GROUPS | |
| F15% | 12,71 \pm 1,89 |
| F40% | 10,14 \pm 1,53 |
| HO GROUPS | |
| F15% | 12,91 \pm 1,54 |
| F40% | 12,14 \pm 2,84 |
| S GROUPS | |
| F15% | 13,75 \pm 2,20 |
| F40% | 11,36 \pm 1,43 |

palmitic and oleic acids with a decrease in essential fatty acids respect to C group. The experimental groups O and HO have high oleic acid and low linolenic acid, linolenic acid and DHA levels. S group shows a decrease of linolenic acid and DHA. These results can be seen independent of the F% of the diet. S group shows increase of linolenic acid only when the diet contains F%=40.

Discussion

Various studies on different populations worldwide have demonstrated that average diet exceeds FAO recommendations regarding lipids [21,22]. Our previous findings from food surveys performed on college students in the city of Buenos Aires showed that fat intake was higher than desirable in more than half of the group, and fat provided more than 40% of daily Kilocalories in 10% of the population [23]. For this reason, we try to recreate a similar situation, diets with a high percentage of fat, and the experimental diets had F%=40. Also, FA composition of experimental diets used in the present study was distorted: excessive proportion of saturated FA on B diet, and altered ω 6/ ω 3 ratio on O, HO, and S diet.

Regardless of the F% of diets, the serum profile of B, O and HO groups suggest exacerbation of the route of the family ω 9, with a decrease of linolenic and linolenic acids. This would be a consequence of the distortion in the contribution of FA from dietary sources.

The O and HO diets contribute with large amounts of oleic acid. On the other hand, B diet provides palmitic acid originating oleic acid, by the route of the saturated fatty acids, after denaturation and elongation processes.

Table 4: Serum FA profiles of the experimental groups expressed as %mean area \pm SD.

| GROUPS | F%:15 | F%:40 |
|-----------------------------|-------------------|-------------------|
| PALMITIC ACID | | |
| B | 20,66 \pm 1,86* | 20,63 \pm 2,54* |
| O | 19,44 \pm 2,05 | 18,23 \pm 1,09 |
| HO | 16,81 \pm 4,24 | 12,96 \pm 2,99 |
| S | 18,07 \pm 1,57 | 15,38 \pm 1,48 |
| C | 16,08 \pm 2,15 | |
| OLEIC ACID | | |
| B | 19,26 \pm 1,97* | 20,37 \pm 2,23* |
| O | 21,33 \pm 2,35* | 21,03 \pm 2,41* |
| HO | 24,10 \pm 6,37* | 26,87 \pm 6,47* |
| S | 11,23 \pm 3,78 | 11,46 \pm 3,86 |
| C | 11,29 \pm 2,27 | |
| LINOLEIC ACID | | |
| B | 5,71 \pm 3,35* | 9,79 \pm 1,12* |
| O | 8,40 \pm 0,96* | 14,59 \pm 1,09 |
| HO | 5,26 \pm 1,48* | 7,41 \pm 1,89* |
| S | 18,94 \pm 2,76 | 25,61 \pm 2,42* |
| C | 17,45 \pm 4,11 | |
| LINOLENIC ACID | | |
| B | 0,33 \pm 0,16* | 0,44 \pm 0,14* |
| O | 0,35 \pm 0,15* | 0,28 \pm 0,04* |
| HO | 0,30 \pm 0,16* | 0,32 \pm 0,13* |
| S | 0,38 \pm 0,12* | 0,28 \pm 0,05* |
| C | 0,81 \pm 0,22 | |
| ARACHIDONIC ACID | | |
| B | 8,09 \pm 3,33* | 10,77 \pm 1,14* |
| O | 17,88 \pm 3,22 | 15,14 \pm 3,13 |
| HO | 11,63 \pm 2,28 | 14,47 \pm 2,56 |
| S | 18,47 \pm 3,70 | 20,66 \pm 3,76 |
| C | 15,26 \pm 4,95 | |
| EICOSAPENTAENOIC ACID (EPA) | | |
| B | 0,71 \pm 0,43 | 1,07 \pm 0,23 |
| O | 0,68 \pm 0,24 | 0,7 \pm 0,27 |
| HO | 0,12 \pm 0,02 | 0,75 \pm 0,14 |
| S | 0,68 \pm 0,21 | 0,68 \pm 0,23 |
| C | 0,92 \pm 0,19 | |
| DOCOSAHEXAENOIC ACID (DHA) | | |
| B | 1,69 \pm 0,63 | 2,76 \pm 0,39 |
| O | 1,08 \pm 0,27* | 0,96 \pm 0,28* |
| HO | 0,33 \pm 0,20* | 0,39 \pm 0,07* |
| S | 0,34 \pm 0,05* | 0,40 \pm 0,15* |
| C | 2,22 \pm 0,75 | |

*Differences significant with the control group when $p < 0.01$.

The diets O and HO have a distortion in the relationship ω_6/ω_3 (FAO-WHO recommended for each unit of ω_3 FA supplied by diet, 5-10 units of ω_6 should be consumed). B diet respect O and HO diets, contains higher amount of linolenic acid (ω_3) and least amount of linolenic acid (ω_6). These differences are reflected in serum profile independently of the F%, B group presents a decrease of arachidonic acid (ω_6 family) and the three groups presents a decrease of linolenic acid (ω_3) but B group does not present a decrease in DHA (ω_3 family), as occurs with O and HO groups. In spite of the fact that the diet B has a smaller amount of ω_3 than C diet, this amount is adequate to maintain DHA (ω_3 family) levels within normal values during the 40 days of experience.

In these three diets have more importance the type of lipid source than the F%, because the changes appear when the source of FA is modified and not when the percentage of fat is modified.

In contrast to these three groups, the S group performs differently. When the F% is high (F%=40) it is observed an increase in linolenic acid level. The falling of DHA would be related to the distortion that presents the diet in the relationship ω_6/ω_3 ; the low supply of linolenic acid and high levels of linolenic acid may inhibit the conversion of alpha-linolenic acid in Eicosapentaenoic and Docosahexaenoic acids (EPA and DHA). In S group, the decrease of ω_3 FA and its products and the prevalence of ω_6 FA would lead to a proinflammatory status, taking into account the functions of both series of prostaglandins.

Dietary fat effects on plasma lipid and lipoprotein concentrations are well known. Both quantity and type of lipids intake have a direct effect on serum lipid profile. Diets provided high concentration of saturated fat is associated with a raise in serum LDL cholesterol [24]. On the other hand, polyunsaturated fatty acids from ω_3 and ω_6 families contribute to reduce cardiovascular risk [25]. ω_6 FA are associated with a reduction of serum total and LDL cholesterol [26], even though ω_3 family has a less important impact on cholesterol, it has beneficial effects on coagulation, by diminishing platelet aggregation, reducing fibrinogen levels and improving microcirculation. The results suggest that the type and amount of fatty acids from the diet could be considered by themselves as a risk factor in the development of non-communicable chronic diseases.

Health education, particularly regarding healthy intake habits, is a vital tool in preventing non-communicable chronic diseases. It is important to emphasize not only on the reduction of total fat intake, but also on choosing healthy sources of fat, replacing saturated fatty acids (mainly from animal sources) by mono and polyunsaturated fat from vegetable origin, including oils with higher content of ω_3 , in order to keep a balanced ω_6/ω_3 ratio.

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