Evaluation of the hepatic bioconversion of $\alpha$-linolenic acid (ALA) to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in rats fed with oils from chia (Salvia hispánica) or rosa mosqueta (Rosa rubiginosa)


1School of Nutrition and 2Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago-7, Chile, 3Lipid Center, Institute of Nutrition and Food Technology (INTA), University of Chile and Faculty of Medicine, University of los Andes.

*Corresponding author: gtapia@med.uchile.cl

RESUMEN

Evaluación de la bioconversión hepática de ácido $\alpha$-linolénico (ALA) a ácido eicosapentaenoico (EPA) y ácido docosahexaenoico (DHA) en ratas alimentadas con aceites de chía (Salvia hispánica) o rosa mosqueta (Rosa rubiginosa).

El elevado aporte en la dieta de ácidos grasos omega-6, en relación a los ácidos grasos omega-3, genera alteraciones de la salud cardiovascular, inflamación y otras patologías crónicas no transmisibles. Por otro lado, el pescado rico en ácidos grasos omega-3 es de bajo consumo en Latinoamérica, siendo necesario buscar otras alternativas de aporte de ácidos grasos omega-3, como lo son el aceite de chía (CO) o el de rosa mosqueta (RMO), ricos en ácido $\alpha$-linolénico (ALA), que es el precursor de los ácidos grasos omega-3, eicosapentaenoico (EPA) y docosahexaenoico (DHA). Este trabajo evaluó en forma preliminar la bioconversión hepática del ALA en EPA y DHA y el daño hepático (histología y transaminasa-s) en ratas Sprague-Dawley alimentadas con diferentes aceites vegetales. Se conformaron cuatro grupos experimentales ($n = 9$ animales por grupo) que recibieron durante 21 días: a) aceite de girasol (SFO); b) RMO, c) CO y d) aceite de oliva adicionado de aceite de pescado (EPA + DHA) (OO/FO). RMO y CO aumentaron los niveles hepáticos de ALA, EPA y DHA y disminuyeron la razón n-6/n-3 respecto a SFO ($p < 0.05$), sin cambios en parámetros de daño hepático. Se concluye que CO y RMO pueden ser una alternativa nutricional de aporte de ALA para su bioconversión en EPA y DHA.

PALABRAS CLAVE: Aceite de chía – Aceite de rosa mosqueta – Ácido $\alpha$-linolénico – Ácido docosahexaenoico –Ácido eicosapentaenoico – Bioconversión de ácidos grasos.

SUMMARY

Evaluation of the hepatic bioconversion of $\alpha$-linolenic acid (ALA) to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in rats fed with oils from chia (Salvia hispánica) or rosa mosqueta (rosa Rubiginosa).

The high dietary intake of n-6 fatty acids in relation to n-3 fatty acids generates health disorders, such as cardiovascular diseases, inflammatory diseases and other chronic diseases. The consumption of fish, which is rich in n-3 fatty acids, is low in Latin America and it is necessary to seek other alternatives, such as chia oil (CO) or rosa mosqueta oil (RMO), which are rich in $\alpha$-linolenic acid (ALA), the precursor of the n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This study evaluates the hepatic bioconversion of ALA to EPA and DHA and the damage to the liver (histology and transaminase) in Sprague-Dawley rats fed different vegetable oils. Four experimental groups ($n = 9$ animals each group) were fed the following dietary supplements for 21 days: a) sunflower oil (SFO), b) RMO, c) CO d) olive oil with fish oil added (EPA and DHA) (OO/FO). RMO and CO increased the hepatic levels of ALA, EPA and DHA and decreased the n-6/n-3 ratio compared to SFO ($p < 0.05$) without changes in the parameters of liver damage. It is concluded that CO and RMO may be nutritional alternatives for providing ALA for its bioconversion to EPA and DHA.

KEY-WORDS: $\alpha$-linolenic acid – Chia oil – Docosahexaenoic acid – Eicosapentaenoic acid – Fatty acid bioconversion – Rosa mosqueta oil.

1. INTRODUCTION

Since the discovery in 1929 of the essentiality of some fatty acids in the diet of rodents (Burr and Burr, 1930) and the first references in the early 1980’s to the importance of $\alpha$-linolenic acid (C18: 3 n-3, ALA) as an essential fatty acid and of its metabolic products in human beings (Holman et al., 1982; Holman et al., 1998), this lipid has been intensively studied. ALA is the precursor of the long-chain polyunsaturated fatty acid n-3 (n-3 LCPUFAs) (Schmitz and Ecker, 2008).

ALA is considered an essential nutrient because mammals do not have the enzymatic machinery necessary to insert a double-bond in the n-3 position (Cunnane, 2003; Nakamura and Nara, 2004). This metabolic characteristic (essentiality) is also shared by the precursor of the n-6 family, linoleic acid (C18: 2 n-6, LA). In this context, both ALA and LA are essential for humans and should be incorporated through the diet. ALA is often found...
in low proportions in the majority of vegetable oils, being present in significant amounts in oils such as linseed, rape seed and canola. LA is present in significant proportions (up to 30-60%) in almost all vegetable oils, especially in those oils (or seeds) of high human and animal consumption, such as sunflower, corn and soybean oils (Kamal-Eldin and Andersson, 1997).

Within n-3 LCPUFAs, the most important for human health are eicosapentaenoic acid (C20: 5, n-3, EPA) and docosahexaenoic acid (C22: 6 n-3, DHA). Both EPA and DHA are involved in multiple functions in the human body by playing a central role in the physiology and normal development of individuals from their early embryonic life to the elderly (Li and Hu, 2009; Harris, 2009; Picq et al., 2010). Thus, DHA is crucial during fetal and postnatal neurogenesis (Haggarty, 2010), playing an important role in cognitive and visual development (Hoffman et al., 2009). Today, DHA supplementation is encouraged during pregnancy and lactation (Valenzuela et al., 2006; Valenzuela, 2009; Muhlhauser et al., 2010). Similarly, the anti-inflammatory and anticoagulant properties of EPA, have led to suggest that this fatty acid could be used as part of treatments associated with chronic cardiovascular diseases (Kanai et al., 2011). In this sense, EPA and DHA have been associated with multiple positive health effects (Fetterman and Zdanowicz, 2009), leading to the proposal of its use for the prevention of non transmissible chronic diseases (Kim et al., 2010).

Humans can incorporate n-3 (EPA and DHA) and the n-6 LCPUFA arachidonic acid (C20:4 n-6, AA) into tissues either by direct consumption or through the bioconversion of the precursors ALA and LA, respectively, provided by the diet (Brenner, 2003). As the consumption of preformed n-3 LCPUFAs is usually low in the Latin America population, its contribution tends to come mainly from the bioconversion of ALA (Cunnane, 2003). However, the efficiency of this metabolic pathway varies greatly in humans, and is subject to variables such as physiological status, age and diet, among others (Gormaz et al., 2010). The balance between the n-3 and n-6 fatty acids is one of the most influential factors in the biological response of these fatty acids, since both ALA and LA may compete for the same biosynthetic pathway for the synthesis of their respective LCPUFAs (Cunnane, 2003, Araya et al., 2010).

The western diet usually provides much larger quantities of n-6 than n-3 fatty acids, which leads to n-6/n-3 ratios of up to 20:1 (w/w) in some populations (Simopoulos, 2008). Current nutritional recommendations suggest a maximum ratio of 5:1 (w/w) and an optimal recommended ratio of 1:1 (w/w) (Molendi-Coste, et al., 2011). This imbalance has generated the need to seek new sources of the n-3 precursor ALA from accessible vegetable oils which show no contraindications frequently associated with marine sources of n-3 LCPUFA (availability, price and palatability) (Simopoulos, 2008).

Within the non-traditional sources of ALA are chia (Salvia hispánica) and rosa mosqueta (Rosa rubiginosa) oils which have a high concentration of ALA (close to 60 and 30%, respectively) and n-6/n-3 ratios of 0.7 and 2.5 respectively, which make these oils good sources of the precursor ALA for the formation of n-3 LCPUFAs. Current research on chia (seeds and oil) has demonstrated beneficial effects which improve insulin resistance in dislipidemic obese rats (Chicco et al., 2009), cardiovascular and hepatic protective actions in obese rats (Poudyal et al., 2011) and antitumoral effects in mice (Espada et al., 2007). Rosa mosqueta oil has been characterized for its antioxidant contents (Franco et al., 2007) and its beneficial effects in some dermatological diseases (Valladares, et al., 1986).

Innovative dietary sources of ALA as a precursor for the endogenous biosynthesis of EPA and DHA, require bioavailability studies able to show that a moderate incorporation of ALA into the diet with proper n-6/n-3 ratios does not cause metabolic alterations. Rodents share with humans the capacity to form n-3 LCPUFAs from ALA (Nakamura and Nara, 2004) and therefore are good models for studying ALA – n-3 LCPUFA bioconversion. The aim of this study was to evaluate the biosynthesis of EPA and DHA in rats fed with diets containing chia and rosa mosqueta oils as sources of ALA precursor.

2. MATERIAL AND METHODS

2.1. Materials

The components for the elaboration of the diets were locally purchased. The kits for the determination of transaminases activity: aspartate transaminase (AST) and alanine transaminase (ALT) were purchased from Biosource International (Camarillo, CA, USA). Zolazepam chlorhydrate, tiletamine chloride and Zoletil 50, used for the anesthesia of the animals were obtained from Virbac S.A. (Carros, France). Materials for histology (phosphate-buffered formalin, paraffin, hematoxylin-eosin) and solvents and reagents were obtained from Merck Química Chilena (Santiago, Chile). Fatty acid standards for gas-chromatography and reagents for fatty acid methyl ester derivative preparation were obtained from Sigma Chemical (St. Louis, MO, USA). The mixture of fatty acids used as standard was obtained from Nu-Check Prep. (Elysian, MN, USA).

2.2. Animals and Diets

Thirty-six male Sprague-Dawley rats, weighing 89.5 ± 6.05 g, (Institute of Biomedical Sciences, Faculty of Medicine, University of Chile), were randomly assigned to one of four groups (nine per group) and had free access to the experimental diets. The animals in each group were fed an
isocaloric diet (20% protein, 10% fat and 70% carbohydrates), supplemented with micronutrients according to the nutritional requirements of Sprague-Dawley rats (Molendi-Coste et al., 2011, Orellana, et al., 2002). The fat composition of each diet was: sunflower oil (SFO), rosa mosqueta oil (RMO), chia oil (CO) and a mixture of olive oil and fish oil (90% and 10% w/w, respectively) (OO/FO). The nutritional composition and the fatty acid contents of the experimental diets is shown in Table 1. The dietary intervention was performed for 21 days. Animals were free to eat the diets and drink the water. Food intake and body weight was monitored daily. At the end of the experimental period and after 8 hours of fasting the rats were anaesthetized. Blood samples were obtained by cardiac puncture and serum was separated for the determination of AST and ALT transaminase activity. Liver samples were taken and frozen in liquid nitrogen (–190 °C) for further fatty acid analysis, or fixed in phosphate-buffered formalin, embedded in paraffin and stained with hematoxylin-eosin for morphology assessment. Experimental animal protocols and procedures complied with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 2011).

### 2.3. Assessment of liver injury

Serum AST and ALT activities were measured using specific commercial kits and expressed as U/l. Liver morphology assessment was carried out in tissue samples sliced from paraffin blocks, stained with hematoxylin-eosin and evaluated by a pathologist (double blind assay) in order to determine hepatocellular necrosis and score for liver damage.

### 2.4. Fatty acid analysis

Fatty acid analyses of both diets and liver samples were performed by gas-liquid chromatography (GLC). Samples were assessed for lipid extraction according to Bligh and Dyer (Bligh and Dyer, 1959) and transformed into fatty acid methyl esters (FAME) with methanolic boron trifluoride (12% methanolic solution) according to Firestone (Firestone, 1997), and stored at –20 °C until analysis. The GLC of FAME was performed using Hewlett-Packard equipment (model 6890A), with a capillary column (Agilen HP-88, 60m x 0.25mm; I.D. 0.25 mm) and flame ionization detector for FAME detection. Hydrogen was the carrier gas. The retention times of FAME were compared to a standard mixture (Nu-Check Prep). C23:0 was used as internal standard. Fatty acids were expressed as g/100g liver.

### 2.5. Statistical analysis

The statistical analysis was performed using the GraphPad Prism 5.0 software (GraphPad Software, Inc. San Diego, USA). The Values shown represent the mean ± SEM for the number of separate experiments indicated. Statistical significance of differences between mean values was assessed by a one-way ANOVA and the Newman-Keuls test. A p-value of < 0.05 was considered significant.

### Table 1

<table>
<thead>
<tr>
<th>Nutritional composition of the different diets.</th>
<th>SFO</th>
<th>RMO</th>
<th>CO</th>
<th>OO/FO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>310</td>
<td>310</td>
<td>310</td>
<td>310</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>19.6</td>
<td>19.6</td>
<td>19.6</td>
<td>19.6</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>36.5</td>
<td>36.5</td>
<td>36.5</td>
<td>36.5</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>9.6</td>
<td>9.6</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>SAFA (g)</td>
<td>0.9</td>
<td>0.6</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>1.3</td>
<td>1.6</td>
<td>0.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Oleic acid (g)</td>
<td>1.0</td>
<td>1.5</td>
<td>0.5</td>
<td>6.2</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>7.4</td>
<td>7.8</td>
<td>8.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Total n-6 PUFA (g)</td>
<td>7.3</td>
<td>4.4</td>
<td>2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Linoleic acid (g)</td>
<td>7.2</td>
<td>4.2</td>
<td>2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Total n-3 PUFA (g)</td>
<td>0.1</td>
<td>3.4</td>
<td>6.4</td>
<td>2.1</td>
</tr>
<tr>
<td>ω-3-linolenic acid (g)</td>
<td>0.1</td>
<td>3.3</td>
<td>6.3</td>
<td>0.1</td>
</tr>
<tr>
<td>EPA (g)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>DHA (g)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>n-6/n-3 PUFA ratio</td>
<td>77</td>
<td>1.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

s(SFO), sunflower oil; (RMO), rosa mosqueta oil; (CO), chia oil; (OO/FO) olive oil/ fish oil.
3. RESULTS

3.1. Weight increase and food intake

There were no significant differences in the initial and final weight or in the food intake of all animal groups during the 21 days of intervention (Table 2). No significant differences in average tissue weights (liver, kidney, spleen and brain) among all groups were observed (data not shown). There were no mortalities during the study.

3.2. Assessment of liver damage: serum transaminase levels and histology

All experimental groups showed a normal enzymatic activity for AST and ALT. Only the RMO group showed significantly higher levels for ALT, although no indication of damage was observed (Fig. 1). Liver histology for each experimental group showed normal tissue histo-architecture, reflecting good preservation of the tissue, without signs of inflammation, apoptosis and/or necrosis (Fig. 2). Transaminase analysis and liver histology of the different groups indicated that the different oil-supplemented diets do not create disturbance or damage in liver physiology.

3.3. Hepatic fatty acid content

Table III shows the fatty acid composition of the hepatic samples. No significant differences were observed in the content of total and saturated fatty acid (SAFA) in all groups. The total monounsaturated fatty acid (MUFA) content was significantly higher in the OO/FO group, with oleic acid as the most relevant fatty acid. The total PUFA content was significantly reduced in RMO, CO and OO/FO groups when compared to the SFO group. However, groups RMO and CO showed higher total PUFA values than the OO/FO group. The n-6 PUFA were reduced in the RMO, CO and OO/FO groups when compared to SFO. However, the RMO group showed higher values for n-6 PUFA than CO and OO/FO. Compared to SFO, AL and AA were significantly reduced in the RMO, CO and OO/FO groups. n-3 PUFAs were significantly higher in the RMO, CO and OO/FO groups compared to SFO group, with the CO group showing the highest n-3 PUFA values. The CO group showed the highest

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SFO</th>
<th>RMO</th>
<th>CO</th>
<th>OO/FO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>88.44 ± 2.09</td>
<td>86.44 ± 1.77</td>
<td>88.89 ± 2.34</td>
<td>88.44 ± 1.43</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>222.3 ± 6.39</td>
<td>216.4 ± 5.40</td>
<td>219.7 ± 8.70</td>
<td>218.0 ± 4.58</td>
</tr>
<tr>
<td>Initial intake (g)</td>
<td>4.8 ± 0.3</td>
<td>5.1 ± 0.6</td>
<td>4.9 ± 0.8</td>
<td>4.8 ± 0.7</td>
</tr>
<tr>
<td>Final intake (g)</td>
<td>17.3 ± 0.9</td>
<td>17.1 ± 0.6</td>
<td>16.5 ± 1.1</td>
<td>16.9 ± 0.9</td>
</tr>
</tbody>
</table>

(SFO), sunflower oil; (RMO), rosa mosqueta oil; (CO), chia oil; (OO/FO) olive oil/fish oil. Values represent the mean ± SEM for n = 9 rats/experimental group. No significant differences were observed for the different groups.
values of ALA compared to all other experimental groups. However, the ALA values for the RMO group were also higher than SFO and OO/FO groups. The EPA contents of the RMO, CO and OO/FO groups were significantly higher than SFO values. However, the CO group exhibited the highest EPA concentration obtained from the CO-group are significantly lower than those of the other groups.

4. DISCUSSION

The data presented shows that diets formulated with innovative vegetable oils, such as chia (CO) and rosa mosqueta (RMO), have no adverse effects on animals, allowing similar growth rates and feed intake when compared to the diet elaborated with SFO, the oil most frequently consumed in Latin America. There was no alteration in liver function (normal enzymatic activity and histology) in animals fed the SFO-, RMO-, CO- and OO/FO diets.

The modification in the fatty acid content of the liver from rats fed with the different diets (Table 3) reflects the fatty acid composition of the oils used for the formulation of the diets. In the same context, the group fed the CO-diet showed a significantly higher hepatic content of the n-3 LCPUFAs (ALA, EPA and DHA) when compared to rats fed the other diets (SFO, RMO and OO/FO groups). Less pronounced values, but also important, are the results obtained for the RMO group, with ALA, EPA and DHA being higher than for the SFO. The higher hepatic content of EPA and DHA obtained from the CO- and RMO-diet may suggest a higher level of biotransformation of ALN to EPA.
and DHA. However, this biotransformation appears to be most efficient in the CO-fed group than in the RMO group when EPA formation is considered. This is not the case for DHA, because the fatty acid was not significantly modified in the liver, indicating that RMO and CO oils produced similar hepatic levels of DHA to those achieved from the OO/FO-diet. This observation suggests that regardless of the dose of ALA provided by the diet, liver DHA levels are not significantly modified. Our results are consistent with other studies on rodents, which demonstrated that dietary supplementation with ALA in fact stimulates the elongation and desaturation of this fatty acid to their bioactive products EPA and DHA, mostly favoring the biotransformation to EPA (Cho et al., 1999a, Cho et al., 1999b, Wang et al., 2005). It is important to consider the drastic reduction of the hepatic content of AA produced by the CO-diet, which is not observed for the RMO-diet. This alteration in the n-6/n-3 ratio may produce a homeostatic imbalance. However, we did not observe any adverse effect of this imbalance in the animals during the experimental protocol.

Results presented in Table IV may appear contradictory in terms of the fact that a higher supply of ALA is not translated into a higher hepatic EPA + DHA/ALA ratio (observed in CO- and RMO-groups). However, the hepatic EPA and DHA contents of these groups are higher than the SFO group (Table 3), indicating that not all the ALA provided by the CO- and RMO-diet is transformed into n-3 LCPUFAs metabolic products. The low values for the ratio AA/EPA + DHA obtained for the CO group may suggest a higher bioconversion of the n-3 precursor (ALA) compared to the bioconversion of then-6 precursor (AL). These results may not be compared with those of the OO/FO group, because these animals received preformed EPA and DHA.
Currently, a wide range of clinical studies has conclusively demonstrated the health benefits of n-3 PUFA consumption on the prevention and/or treatment of many diseases (Fetterman and Zdanowicz, 2009). Nevertheless, the low intake of foods considered as good sources of n-3 PUFAs in most western populations, fails to meet the nutritional requirements of these fatty acids, stimulating the consumption of nutritional supplements rich in n-3 PUFAs, mainly from marine origin (mammals, fish or algae oils) (Abete et al., 2009). However, these supplements are also not easily accessible to everybody; they often produce many difficulties, ranging from simple gastrointestinal disturbances in some people, to even the risk of chronic toxicity, due to an increasing expression and molecular mechanisms involved in the metabolism of n-3 LCPUFAs.

Table 4

<table>
<thead>
<tr>
<th>Product/precursor ratio of n-6 (AA/LA) and n-3 fatty acids (EPA + DHA/ALA) and n-6/n-3 LCPUFA ratio (AA/EPA + DHA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA/LA</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>SFO</td>
</tr>
<tr>
<td>RMO</td>
</tr>
<tr>
<td>CO</td>
</tr>
<tr>
<td>OO/FO</td>
</tr>
</tbody>
</table>

(SFO), sunflower oil; (RMO), rosa mosqueta oil; (CO), chia oil; (OO/FO) olive oil/ fish oil. Values represent the mean ratio ± SEM obtained from each experimental group (n = 9). Statistical significance: a: significantly different from SFO; b: significantly different from RMO; c: significantly different from CO; d: significantly different from OO/FO; p < 0.05; one-way ANOVA and Newman-Keuls test.

5. CONCLUSIONS

Results from the present study can establish that the consumption of chia and rosa mosqueta oil is not associated with adverse liver effects in rats. Furthermore, these oils, which are good sources of ALA, can significantly increase hepatic levels of ALA, EPA and DHA, producing much lower n-6/n-3 ratios. These results encourage us to develop future studies on the effects of chia and rosa mosqueta oil consumption in humans, aiming for the possible consideration of these oils, which are traditionally produced in many countries of South America, as a nutritional alternative of the n-3 PUFAs from marine origin. Future works must be focused on the study of desaturase activities, gene expression and molecular mechanisms involved in the metabolism of n-3 LCPUFAs.

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