Effects of dietary fat amount and saturation on the regulation of hepatic mRNA and plasma apolipoprotein A-I in rats

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Abstract

The effects of the amount of dietary fat and saturation together with cholesterol both on hepatic apolipoprotein A-I gene mRNA levels and on plasma levels of this apolipoprotein were studied in male rats. To achieve these goals, seven groups of male Wistar rats were established: control group (n = 5) consuming chow diet; cholesterol group (n = 4) fed on a chow diet containing 0.1% (w/w) cholesterol; coco group (n = 5) fed on a chow diet containing 0.1% (w/w) cholesterol and 40% coconut oil; corn group (n = 5) fed on a chow diet containing 0.1% (w/w) cholesterol and 40% corn oil; and three olive groups consuming a chow diet containing 0.1% (w/w) cholesterol and percentages of 5 (n = 5), 10 (n = 4) and 40% (n = 5), respectively, of olive oil. Animals were kept on these diets for 2 months and then sacrificed for lipoprotein, apolipoprotein and hepatic mRNA analysis. Dietary cholesterol by itself was hypercholesterolemic when compared to chow diet, an effect that was mainly due to an increase in LDL-cholesterol. Corn oil had a hypocholesterolemic action, whether compared to chow or to cholesterol diet, due to a reduction in HDL-cholesterol as well as LDL-cholesterol. HDL-cholesterol levels of 40% olive oil diet were lower than those corresponding to coconut oil and higher than those found in corn oil diet. When compared to control or cholesterol diets, plasma apoA-I concentration appeared significantly increased in coconut and 40% olive oil diets. Coconut oil or corn oil diets did not induce any significant change in apoA-I mRNA compared to control or cholesterol diets. Compared to cholesterol diet, 40 and 10% olive oil diets induced a significant increase in the expression of this message. A positive and significant (r = 0.97, P < 0.01) correlation between plasma apolipoprotein A-I concentration and its hepatic mRNA, was observed when the amount of dietary olive oil was 40% (w/w). A significant negative (r = -0.97, P < 0.01) correlation was found in the corn oil group and no significant association was observed in the remaining groups. Based on the increased plasma levels in coconut oil and in high percentage olive oil diets, and the differences between these two diets for mRNA expression, it can be concluded that different fatty acid containing diets regulate apolipoprotein A-I through different mechanisms, and these mechanisms could be modulated by the fat intake. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Apolipoprotein A-I; Dietary fat; Lipoproteins; Monounsaturated fatty acids; Saturated fatty acids; Polyunsaturated fatty acids

1. Introduction

Epidemiological studies have demonstrated that high-density lipoprotein (HDL) cholesterol is a negative risk factor for coronary artery disease (CAD) independent of other prognostic factors [1–4]. Clinical trials have also proved that increases in HDL-cholesterol are associated with decreases in CAD events, and with atherosclerotic regression [5]. While low HDL-cholesterol levels may be the result of genetic disorders, other environmental factors, such as physical inactivity, cigarette smoking, dietary composition, hormonal status, body mass index and alcohol consumption, are the primary responsible factors for the majority of changes observed in the general population. Pharmacological agents such as niacin, fibrates and statins are also involved in HDL control [6].

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Dietary composition as an important environmental component influencing HDL-cholesterol levels is rather complex. Thus carbohydrate-rich diets are associated with lower HDL levels [6]. On the other hand, the type of supplied fat also modulates the latter parameter, in the sense that saturated, as compared to polyunsaturated, fat consumption induces an increase in HDL-cholesterol levels in humans and in other animal models [7,8]. The effect of monounsaturated diets is still controversial. While classical studies described a neutral role of monounsaturated fatty acids in determining plasma lipids [9], recent studies have shown the effect of monounsaturated fatty acids (oleic acid) in lowering serum levels of low-density lipoprotein cholesterol without modification of HDL-cholesterol levels when substituted for saturated fatty acids in human and non-human primates [10–14].

Apolipoprotein A-I (apoA-I) is the main protein component of high density lipoproteins, and is involved in their structure and metabolism. Therefore, the synthesis and secretion of apoA-I as well as its catabolism may be mechanisms regulating plasma HDL levels (reviewed in Refs. [15] and [16]). The mechanisms whereby different fatty acid and (or) cholesterol regulate plasma apoA-I protein are the focus of current research in the field. Thus, studies carried out in primates have shown that, in the presence of cholesterol, dietary saturated and polyunsaturated fatty acids modulate apoA-I production by transcriptional regulation involving mainly liver apoA-I mRNA, and that the catabolic rate of HDL apoA-I is also involved [17–20]. In rodents, the increase in apoA-I by saturated fat is produced by posttranscriptional mechanisms, not shared by dietary cholesterol [21]. Once again, few studies have addressed the influence of monounsaturated fatty acid containing diets on apoA-I regulation. In non-human primates, Fox et al. [22] described an increase in hepatic apoA-I mRNA with diets enriched in the latter type of fatty acids. Likewise, we have previously shown that rats fed on a diet containing 40% (w/w) monounsaturated fat and 0.1% cholesterol showed induced hepatic expression of apoA-I mRNA [23]. The aim of the present study was to investigate whether the latter effect is influenced by the amount of fat provided and the contribution of hepatic mRNA expression on plasma apolipoprotein concentration.

2. Material and methods

2.1. Materials

Standard rat chow was provided by Pascual (Barcelona, Spain). Cholesterol was obtained from Sigma (Madrid, Spain). Starch was provided by Cofares (Madrid). Olive oil was obtained from Mora (Barcelona, Spain). Molecular biology reagents were purchased from Boehringer Mannheim (Barcelona, Spain) and Sigma. Restriction enzymes were purchased from Pharmacia and Promega (Barcelona, Spain). [2,32P]dCTP was obtained from New England Nuclear (Madrid, Spain).

2.2. Animals and diets

Inbred male Wistar rats (200–250 g, aged 2 months) were kept under different experimental conditions. A detailed description of diets is shown in Table 1. The groups were as follows. Group 1: control animals (five rats) were fed on a standard commercial diet. Group 2: cholesterol group (four rats), commercial diet was supplemented by 0.1% (w/w) cholesterol. Groups 3–5 (five rats): commercial diet was supplemented by 0.1% (w/w) cholesterol and 40% (w/w) fat. All diets contained 1% starch as agglutinant. Three different types of fat were used: coconut oil, corn oil and olive oil. A wide range of polyunsaturated to saturated fatty acid ratio (P/S) was covered (0.03–3.8) and a wide range of monounsaturated fatty acids was also utilized (8.2–74.6%). Groups 6 and 7: commercial diet was supplemented by 0.1% (w/w) cholesterol and 5% (five rats) or 10% (w/w) (five rats) olive oil, respectively. Total fat from diets was extracted (Soxhlet) and fatty acids analyzed using gas-liquid chromatography [24]. All groups were fed ad libitum for 2 months. Before killing, rats were kept on a fasting diet with free access to water for 18 h. Animals used in this study were handled and killed, strictly observing criteria from the European Union for care and use of animal laboratory in research.

2.3. Plasma chemistry

After the experimental period, animals were sacrificed by Avertin (2,2,2-tribromoethanol) injection (Aldrich, Madrid, Spain). The abdominal cavity was opened and intestinal mass removed to visualize the abdominal aorta. Blood samples drawn from the abdominal aorta were collected in 0.1% EDTA tubes. Plasma was separated at 2500 rpm for 20 min at 4°C. Phenylmethyl sulfonyl fluoride (PMSF, 2 × 10⁻⁴ M), and N-ethylmaleimide (NEM, 1.25 mg/ml, Sigma) were added to plasma as proteolytic and lecithin:cholesterol acyltransferase inhibitors. Cholesterol and triglycerides were measured by enzymatic methods [25,26]. Assays were standardized by using Cardiolipid (Sigma) as certified quality control.

2.4. Antibody generation and apolipoprotein A-I assay

Purified rat apolipoprotein A-I was obtained by electroforesis of isolated HDL in a 4-22.5% polyacry-
lamide gel. Gel fragment corresponding to this protein was electroeluted in 0.2 M Tris-acetate (pH 7.4), 1% SDS and 100 mM dithiotreitol. Purified protein concentration was assayed using Biorad Protein Assay. A total of 4 µg of protein diluted in 200 µl of saline phosphate buffer (PBS) (pH 7.4) was mixed with 300 µl of complete Freund adjuvant. This mixture was subcutaneously injected into rabbits. A second boost was given a month later and animals bled after another 20 days. Purified rabbit anti-rat apolipoprotein A-I immunoglobulins G were used to estimate apolipoprotein concentration in a chemiluminescent ELISPOT assay normalized with pure rat apolipoprotein A-I. This assay showed a 5% of interassay variation coefficient.

2.5. RNA preparation

A liver biopsy was flash frozen in liquid nitrogen and stored at –70°C until use. Liver fragments were homogenized in guanidinium isothiocyanate and applied to a cushion of cesium chloride as described by Kingston [27].

2.6. Preparation of DNA probes

The following probes were used: a 0.7-kb EcoRI fragment of rat apoA-I cDNA [28] (generously provided by Dr Lawrence Chan, Baylor College, Houston) and, as an internal standard, an insert of mouse GPDH (Ambion, Madrid, Spain). DNA inserts were purified by gel electrophoresis. Purified DNA probes were labeled with [α-32P]dCTP by a random primer protocol [29] using Rediprime from Amersham-Pharmacia (Madrid, Spain).

2.7. Analysis of mRNA

2.7.1. Northern analysis

Denatured RNA (5 µg) was run in a formaldehyde agarose gel, blotted into nylon membranes, hybridized to a radio labeled probe, washed and exposed to a film following current procedures [30].

2.7.2. Slot-blot analysis

Several amounts of denatured total RNA (1–10 µg) were applied to nylon filters using a slot-blot apparatus (Biorad, Madrid, Spain). Films were scanned by laser densitometry using an LKB 2202 densitometer and an LKB 2400 gel scan XL software. mRNA abundance was measured in absorbance units and normalized to pg of apoA-I mRNA/µg of total RNA using an in vitro transcript of apoA-I generated by using Riboprim (Promega). Blots were stripped and reprobed with GPDH, exposed to film and scanned as described. The relative abundance of apoA-I mRNA message was normalized to values of GPDH as an internal standard.

Table 1
Composition of the different experimental diets

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cholesterol + coconut</th>
<th>Cholesterol + corn oil</th>
<th>Cholesterol +40% olive</th>
<th>Cholesterol +10% olive</th>
<th>Cholesterol +5% olive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy supply (kJ/g)</td>
<td>10.7</td>
<td>10.7</td>
<td>21.4</td>
<td>21.4</td>
<td>21.4</td>
<td>13.4</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>41</td>
<td>41</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Protein</td>
<td>19</td>
<td>19</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Fat</td>
<td>3.2</td>
<td>3.2</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.02</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Main fatty acids

<table>
<thead>
<tr>
<th></th>
<th>&lt;C12:0</th>
<th>Lauric (12:0)</th>
<th>Myristic (14:0)</th>
<th>Palmitic (16:0)</th>
<th>Stearic (18:0)</th>
<th>Oleic (18:1)</th>
<th>Linoleic (18:2n-6)</th>
<th>Linolenic (18:3n-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.3</td>
<td>0.1</td>
<td>1.5</td>
<td>21.6</td>
<td>7.0</td>
<td>33</td>
<td>30</td>
<td>0.3</td>
</tr>
<tr>
<td>Total saturated</td>
<td>33.8</td>
<td>33.8</td>
<td>88.5</td>
<td>13.9</td>
<td>14.9</td>
<td>16.9</td>
<td>18.7</td>
<td>65.3</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>35.2</td>
<td>35.2</td>
<td>8.2</td>
<td>33.6</td>
<td>74.6</td>
<td>70</td>
<td>65.3</td>
<td>80.3</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>0.9</td>
<td>0.9</td>
<td>0.03</td>
<td>3.8</td>
<td>0.7</td>
<td>0.8</td>
<td>0.83</td>
<td></td>
</tr>
</tbody>
</table>

* Components are expressed as g% (w/w). Other components of chow diet are crude fiber, 5%, and minerals, 7%. A total dry matter of 75%. P/S ratio, polyunsaturated to saturated fatty acid ratio.
2.8. Statistical analysis

Results are shown as means and their standard deviations. Analysis of data was done using Instat 2.0 for Macintosh software (GraphPad, San Diego, CA, USA). Some of the analyzed parameters in this study did not show normal distribution according to Shapiro–Wilk test, or failed in homology of variance. Therefore, analysis of statistically significant differences was carried out using Mann–Whitney U-test for unpaired data, and one-way ANOVA was performed according to Kruskal–Wallis test. Alternatively, one-way ANOVA, according to Tukey, was carried out and Tukey–Kramer multiple comparison test as post hoc analysis was used. Differences were considered non-significant when $P > 0.05$. Association between variables was assessed by Spearman’s rank-order correlation coefficient ($r_s$) [31].

3. Results

3.1. Dietary characteristics

Table 1 summarizes the composition of different diets. As shown, distinctive general features were lower carbohydrate and protein, higher percentage of fat and an increase in cholesterol in the different diets, chow being the control. Diets containing different amounts of fat (5, 10 and 40%) were not isocaloric as were diets providing different types of fat at 40% (w/w) content. Chow and cholesterol diets presented a monounsaturated fatty acid content of 33% and almost equal amounts of polynsaturated and saturated fatty acids (P/S ratio 0.9). Coconut oil diet provided a high content of saturated short chain fatty acids (88.5) and a low content of monounsaturated fatty acids. Corn oil diet was particularly enriched in linoleic acid and levels of monounsaturation were similar to the control diet. Olive oil diets were high in monounsaturated fatty acids, differing in their percentages in inverse proportion to the fat content.

3.2. Nutritional evaluation

Table 2 shows that increasing food fat content to percentages higher than 5% produced significant decreases ($P < 0.001$) in the food intake, and these decreases were the highest with 40% (w/w) rates without any difference among type of oil. However, weight gain was decreased only in those groups of experimental animals consuming 40% fat containing diets with again no differences among oils. As results of these data, the ratio of energy supplied related to weight gain was significantly ($P < 0.001$) higher in animals consuming diets enriched in 40% fat, independent of its source.

3.3. Plasma analysis

Table 3 shows the total plasma cholesterol for each of the seven groups at the end of the experimental period. Dietary cholesterol was a hypercholesterolemic agent in these animals since plasma cholesterol significantly ($P < 0.05$ vs. control) increased when rats were fed diets enriched in this compound. When compared to the cholesterol group, simultaneous administration of saturated fat (coconut diet) and cholesterol did not increase plasma cholesterol. Corn oil administration not only prevented hypercholesterolemia caused by cholesterol administration ($P < 0.001$ vs. cholesterol), but reduced plasma cholesterol to levels lower than control group ($P < 0.05$ vs. control). Olive oil administration prevented the hypercholesterolemic effect of cholesterol ($P < 0.05$ vs. cholesterol) maintaining similar values to those found in animals fed on a high carbohydrate diet (control group), irrespective of the dose employed.

The hypercholesterolemic effect of dietary cholesterol (Table 3) is mainly due to an increase in LDL-cholesterol ($P < 0.001$ vs. control), and the hypocholes-
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73

Table 3
Plasma lipid parameters

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Total cholesterol</th>
<th>HDL-cholesterol</th>
<th>LDL-cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td>67 ± 3</td>
<td>44 ± 3</td>
<td>19 ± 4</td>
<td>41 ± 10</td>
</tr>
<tr>
<td>Cholesterol (n = 4)</td>
<td>85 ± 7</td>
<td>45 ± 4</td>
<td>33 ± 3</td>
<td>62 ± 10</td>
</tr>
<tr>
<td>Cholesterol+40% coconut (n = 5)</td>
<td>75 ± 5</td>
<td>50 ± 4</td>
<td>23 ± 3</td>
<td>69 ± 14</td>
</tr>
<tr>
<td>Cholesterol+40% corn (n = 5)</td>
<td>48 ± 7</td>
<td>29 ± 4</td>
<td>15 ± 3</td>
<td>44 ± 9</td>
</tr>
<tr>
<td>Cholesterol+40% olive (n = 5)</td>
<td>67 ± 12</td>
<td>38 ± 5</td>
<td>28 ± 6</td>
<td>47 ± 19</td>
</tr>
<tr>
<td>Cholesterol+10% olive (n = 4)</td>
<td>73 ± 4</td>
<td>44 ± 3</td>
<td>24 ± 3</td>
<td>50 ± 7</td>
</tr>
<tr>
<td>Cholesterol+5% olive (n = 5)</td>
<td>69 ± 12</td>
<td>40 ± 7</td>
<td>24 ± 4</td>
<td>43 ± 9</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P&lt;0.003</td>
<td>P&lt;0.002</td>
<td>P&lt;0.0015</td>
<td>P&lt;0.02</td>
</tr>
</tbody>
</table>

* Data, expressed as mg/dl, are means and their standard deviations. Statistical analysis was done using one way ANOVA according to Tukey and Tukey-Kramer Multiple Comparison Test as post hoc analysis. Different superscripts (b versus control; c versus cholesterol; d versus cholesterol+coconut oil; and e versus cholesterol+corn oil), are significantly different from each other at P<0.05.

The hypocholesterolemic effect of olive oil is mainly due to a decrease (P<0.05 vs. cholesterol) in LDL-cholesterol and is more pronounced in 5 and 10% olive oil containing diets. LDL-cholesterol in saturated and monounsaturated diets was similar and lower than in the cholesterol group. The 40% coconut oil diet was better at reducing LDL-cholesterol compared to 40% olive oil (P<0.05), and as effective as other doses of olive oil. LDL-cholesterol levels of monounsaturated fatty acid diets were found among those of saturated and polyunsaturated diets, reaching the maximum value in animals consuming 10% olive oil diets. The HDL:LDL ratio was actually more favorable regarding the chow diet (2.3) than the olive oil diets (mean of 1.6). Also, the ratio of 2.2 for the cholesterol + coconut oil diet was relatively favorable when compared with the olive oil diets.

Plasma triglycerides (Table 3) increased (P<0.05 vs. control) when rats were fed on cholesterol. This effect was not enhanced by simultaneous administration of saturated fat. Rats receiving polyunsaturated (corn oil) and 5% monounsaturated diets showed a decrease in plasma triglycerides when compared to the cholesterol group (P<0.05 vs. cholesterol).

Fig. 1A shows that apoA-I used to generate the polyclonal antibody was homogeneously purified and panel B shows proof that purified rabbit Ig G anti-apoA-I recognized only this apolipoprotein. Plasma apoA-I concentration (Table 4) appeared significantly (P<0.05 vs. control and vs. cholesterol) increased in saturated and monounsaturated fatty acid provided at 40%. A 10% monounsaturated diet induced levels of apoA-I that were significantly (P<0.05) different from the cholesterol and 40% the olive oil group.

![Fig. 1. Quality characteristics of apolipoprotein A-I, antibody, hybridation conditions and probes used in the present study. (A) Denaturing polyacrylamide electrophoresis showing homogeneously purified apolipoprotein A-I stained with Coomassie blue. (B). Western blotting showing the specificity of immunopurified rabbit Ig G anti-rat apoA-I. Molecular markers used in A and B were: bovine albumin, 66 kDa; egg albumin, 45 kDa; glyceraldehyde dehydrogenase, 36 kDa; carbonic anhydrase, 29 kDa; and α-lactoalbumin, 14 kDa. Northern blot analysis reflecting the specificity of hybridation conditions for apoA-I (C) and GPDH (D) probes. A total of 5 μg of total RNA was subjected to electrophoresis in a formaldehyde 1% agarose gel. The RNA was transferred to a nylon membrane and hybridized to radioactive cDNAs. mRNA sizes are indicated on the autoradiogram in relation to the ribosomal RNAs.](image-url)
vided doses (10 or 40%). The 10% olive oil was not without any significant difference between the two pro-
groups when fat percentage was higher than 10% (w

group, a significant increase was observed in olive oil

cholesterol fed animals were taken as the reference

hepatic apolipoprotein apoA-I mRNA obtained in the

trrophoretic mRNA species of the appropriate molecu-

apoA-I and 1D, GPDH) hybridized to a single elec-

Cholesterol

Cholesterol

Cholesterol

Cholesterol

Cholesterol

Cholesterol

Cholesterol

Cholesterol

ANOVA

\( r_s \) are shown in Table 5. Although a general

positive correlation was found between plasma apoA-I

and HDL-cholesterol, the degree of association ap-

peared highly variable depending on the diet. While

highly significant correlations were noted for corn and

10% olive oil groups, weaker associations were ob-

served in the remaining groups.

Table 5 also shows the relationship between plasma

apolipoprotein A-I concentration and hepatic apoA-I

mRNA. According to the observed correlations, the

increase in plasma apoA-I in olive oil containing diets is

strongly related (\( r_s = 0.97, P < 0.01 \)) to changes in he-

patic apoA-I mRNA. However this dramatic effect is

only observed when supplied dietary fat is 40% (w/w).

A significant negative correlation (\( r_s = -0.97, P <

0.01 \)) was found in the corn oil group and no significant

association was observed in the remaining groups.

4. Discussion

In this experiment we used male Wistar rats, an

animal model that we had previously found to undergo

changes in plasma lipids when saturation of fat was

changed in the presence of cholesterol [32]. In that

study, an extremely high fat percentage 40% (w/w) and

0.1% (w/w) cholesterol were used, and a 60% increase

of hepatic apoA-I mRNA was found when olive oil was

the source of fat. The present work is an extension of

the previous study in order to determine whether the

described effect of olive oil could exist at lower fat

contents with the additional presence of 0.1% choles-

terol and if the observed effect at the hepatic mRNA

level is reflected at all by plasma apoA-I concentration.

The energy supply in animal groups consuming 40% fat

was higher than in groups on chow, cholesterol, 5% olive oil

or 10% olive oil as shown in Table 1. Animals

Table 4

Plasma concentration and hepatic expression of rat apolipoprotein A-I in the different experimental diets*

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Plasma concentration (mg/dl)</th>
<th>Hepatic expression (pg of apoA-I mRNA/μg total RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td>66 ± 34</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>Cholesterol (n = 4)</td>
<td>49 ± 35</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>Cholesterol + 40% coconut (n = 5)</td>
<td>101 ± 34^w</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Cholesterol + 40% corn (n = 5)</td>
<td>71 ± 11</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Cholesterol + 40% olive (n = 5)</td>
<td>102 ± 12^wce</td>
<td>28 ± 11^wce</td>
</tr>
<tr>
<td>Cholesterol + 10% olive (n = 4)</td>
<td>89 ± 6^d</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>Cholesterol + 5% olive (n = 5)</td>
<td>89 ± 16</td>
<td>19 ± 13</td>
</tr>
<tr>
<td>ANOVA</td>
<td>( P &lt; 0.04 )</td>
<td>( P &lt; 0.05 )</td>
</tr>
</tbody>
</table>

* Data are means and their standard deviations. Hepatic expressions are normalized to the levels of GPDH expression. Statistical analysis was done using non-parametric one-way ANOVA according to Kruskal–Wallis test and unpaired Mann–Whitney U-test to test pair-wise differences. Different superscripts (\(^* \) versus control; \(^* * \) versus cholesterol; \(^* * * \) versus cholesterol + coconut oil; \(^* * * * \) versus cholesterol + corn oil; and \(^* * * * * \) versus cholesterol + 40% olive) are significantly different from each other at \( P < 0.05 \).

3.4. RNA analysis

As depicted in Fig. 1, the used cDNA probes (1C, apoA-I and 1D, GPDH) hybridized to a single elec-
trophoretic mRNA species of the appropriate molecular size in each case. Table 4 shows the values of hepatic apolipoprotein apoA-I mRNA obtained in the different groups consuming the experimental diets. When compared to control group fed chow diet, there was no significant change in this message in animals consuming coconut, corn or olive oil. However, when cholesterol fed animals were taken as the reference group, a significant increase was observed in olive oil groups when fat percentage was higher than 10% (w/w) without any significant difference between the two pro-
vided doses (10 or 40%). The 10% olive oil was not

analyzed against coconut or corn oils because the conditions are not comparable due to two different parameters involved: source of fat and fat intake.

The degree of relationship among parameters in the different dietary groups was established using an associ-
ation analysis, and the values of Spearman correlation coefficient (\( r_s \)) are shown in Table 5. Although a general positive correlation was found between plasma apoA-I and HDL-cholesterol, the degree of association appeared highly variable depending on the diet. While highly significant correlations were noted for corn and 10% olive oil groups, weaker associations were ob-
served in the remaining groups.

Table 5 also shows the relationship between plasma apolipoprotein A-I concentration and hepatic apoA-I mRNA. According to the observed correlations, the increase in plasma apoA-I in olive oil containing diets is strongly related (\( r_s = 0.97, P < 0.01 \)) to changes in he-
patic apoA-I mRNA. However this dramatic effect is

only observed when supplied dietary fat is 40% (w/w).

A significant negative correlation (\( r_s = -0.97, P <

0.01 \)) was found in the corn oil group and no significant

association was observed in the remaining groups.

Table 5

Relationship between parameters in the different experimental diets*

<table>
<thead>
<tr>
<th>Plasma apoA-I concentration</th>
<th>Control (n = 5)</th>
<th>Cholesterol (n = 4)</th>
<th>Coconut (n = 5)</th>
<th>Corn (n = 5)</th>
<th>40% Olive (n = 5)</th>
<th>10% Olive (n = 4)</th>
<th>5% Olive (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-cholesterol</td>
<td>0.4</td>
<td>0.6</td>
<td>0.4</td>
<td>0.9**</td>
<td>0.4</td>
<td>0.82*</td>
<td>0.7</td>
</tr>
<tr>
<td>ApoA-I mRNA</td>
<td>0.5</td>
<td>0.8</td>
<td>-0.15</td>
<td>-0.97**</td>
<td>0.97**</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Spearman correlation coefficients \( r_s \) are indicated. \( n \), number of animals in each experimental group.

\* \( P < 0.05 \).

\** \( P < 0.01 \).
Dietary cholesterol induced hypercholesterolemia in rats confirming our previous results [32]. The increase of plasma cholesterol is mainly due to an increase in LDL-cholesterol (Table 3). Our dietary cholesterol percentage (0.1%, w/w) was not high enough to elicit changes in LDLr mRNA levels [32]. Woollett et al. [35] and Dietzchy et al. [36] with similar dietary cholesterol amount have described reduction of LDLr activity. In HepG2 cells, LDL receptor activity was reported to have been reduced without any changes in LDL receptor mRNA [37]. In rats and mice, changes in activity were not associated with protein or mRNA changes [38]. Therefore LDL receptor activity may be more important than protein and mRNA levels to explain plasma LDL levels. The lack of hypercholesterolemic effect of lauric acid (main fatty acid of coconut oil) in this study corroborates our previous work [32] and is in agreement with results obtained by Jackson et al. [39] in hamsters and by Denke and Grundy in humans [40]. Coconut oil induced an increase in HDL-cholesterol in agreement with previous work done in other experimental animals and in humans [10,11,17]. In this study, we found that corn oil is very effective in preventing hypercholesterolemia caused by dietary cholesterol. This effect could be due to the high linoleic acid content of corn oil as reported by Grundy and Denke [11]. This oil decreases both LDL- and HDL-cholesterol, the latter effect being of higher intensity. Decrease in HDL-cholesterol has also been described in monkeys and it seems to be due to lesser hepatic secretion of these particles. Animals fed olive oil in the different percentages showed similar values of cholest erolemia as animals fed chow diet and a hypocholesterolemic effect when compared to cholesterol fed animals. The latter is mainly due to a decrease in LDL-cholesterol and not to changes in HDL-cholesterol, in agreement with other studies using monounsaturated fatty acid diets [10,14]. When provided at 40%, the coconut oil diet surprisingly decreased LDL-cholesterol at a higher rate than the same percentage of olive oil. An explanation of this fact could be that the food provided to the coconut group had a lower amount of palmitic acid than the 40% olive oil (Table 1). It is well documented that palmitic acid raises LDL-cholesterol [40]. Furthermore, the amount of linoleic in the coconut blend could be high enough to neutralize the negative impact of myristic acid, as suggested by Khosla and Sundram [41]. Our data support the hypothesis raised by these authors in the sense that the effect of some oils could be modified by the accompanying fats and results could be different from those obtained when the pure fat is added to a semisynthetic diet.

Dietary cholesterol induced hypertriglyceridemia in rats confirming our previous results [32]. Othani et al. [42] reported a similar effect without any change in the synthesis of apoB and we did not find any change in apoB message caused by dietary cholesterol [32]. In rats, most of VLDL remnants are eliminated from circulation by LDL receptor [11]. The hypertriglyceridemia could be due to the lower activity of LDL receptor found when cholesterol is added to food. No additional effect on the elevation of plasma triglyceride concentration induced by dietary cholesterol was observed when the diet was supplemented with saturated coconut oil (Fig. 1), a similar finding to that reported by Van Heek et al. [43] in rabbits. Plasma triglyceride levels of groups consuming corn or olive oil were similar to those found in control animals and lower than rats fed cholesterol, which may be regarded as an abolition of the hypertriglyceridemic effect of dietary cholesterol. These effects are similar to those found in humans and in rabbits [11,43] and corroborate our previous data [32] regarding neutralizing the cholesterol effect. Percentage of provided olive oil was irrelevant regarding this matter. Our results suggest that, in the rat model, unsaturation of diet is a potent modulator of plasma triglycerides.

Saturated diet did increase apoA-I concentration as described in other animal species and in humans [11,44]. Feeding monounsaturated fatty acids also increased plasma apoA-I levels with high percentages of fat. A similar effect has been described in humans and in rabbits [11,43] and corroborate our previous data [32] regarding neutralizing the cholesterol effect. Percentage of provided olive oil was irrelevant regarding this matter. Our results suggest that, in the rat model, unsaturation of diet is a potent modulator of plasma triglycerides.

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correlation is found between HDL-cholesterol and apoA-I levels. Considering that an enhanced catabolism of apoA-I for these types of diets has been found in other studies [46], it is highly possible that cholesterol content of HDL together with apoA-I are equally removed from the HDL particles in these diets. This high apoA-I catabolism would also explain the low plasma levels despite normal apoA-I mRNA and would be consistent with the strong and negative correlation between apoA-I mRNA and plasma apoA-I (Table 5). Although a lower translation of apoA-I mRNA is possible, this mechanism would have to be proved.

The amount of apoA-I mRNA found in the livers from control animals was 21 ± 4 pg/μg total RNA, a figure similar to that published by Srivastava et al. [47]. Hepatic expression was similar among cholesterol and coconut, corn oil groups and chow diet group. Monounsaturated fatty acid diets induced an increased expression of this message depending on the amount of fat provided. When either 10 or 40% fat was provided, a 43% increase was found, a result that corroborates our previous study, although the magnitude of response was of less intensity [32]. These data indicate that at least 10% olive oil is required to induce hepatic expression. Based on the correlation data shown in Table 5, an even higher olive oil percentage is needed to show a high correlation between plasma apoA-I and its hepatic expression. Thus, dependence of apoA-I on its hepatic mRNA as a main factor determining plasma levels would only happen at these high intakes of olive oil. At this percentage of fat, dissociation of behavior of apoA-I and other HDL components may be important as is suggested by the low correlation coefficient between apoA-I and HDL-cholesterol. Using saturated fat, Dreon et al. [48] have shown that fat intake modulated hepatic lipase activity in humans. An inverse correlation between hepatic lipase and HDL concentrations is currently found [49]. These facts suggest that a similar situation could be involved in olive oil diet which could explain the HDL-cholesterol levels observed at 40% olive oil diet. The extent of dependence of all these mechanisms on olive oil intake in other non-HDL animals seems plausible. In cynomolgus monkeys, when fed a 14% monounsaturated fat diet, there is a trend to increase plasma apoA-I although not statistically significant [44], and in humans, a 20% supply of monounsaturated fatty acids induces an increase in plasma apoA-I [13,14]. As posed by the latter authors, there seems to be a threshold for the effect of monounsaturated fatty acids. Whether this dependence on the amount of monounsaturated fat is a general phenomenon in all species or is something specific for rats, due to the fact that it is an HDL animal, requires more experimental work due to the paucity of studies using monounsaturated fatty acid diets.

In saturated fat-fed animals, hepatic mRNA is not responsible for plasma levels, suggesting that a slow catabolism of apoA-I may be the determinant cause, as described for cynomolgus monkeys [46]. In hamsters consuming saturated fat, an increase in HDL particle size was found also suggesting impairment in the catabolic processing of these particles in the presence of this specific kind of fat [50]. Besides these aspects related to HDL dynamics, other molecular mechanisms might be implicated. Arzolan et al. [51], in transgenic mice, and Srivastava et al. [21], in normal mice, showed that saturated fat induced a more efficient translation of apoA-I mRNA. This mechanism acting for saturated fatty acid diets seems to be specific for rodents since in primates fed these types of diets there is a close correlation between hepatic mRNA and plasma apoA-I levels.

In conclusion, it is clear that the plasma apoA-I increase in saturated and monounsaturated fatty acid diets has different mechanisms. Likewise, for the saturated diet there are posttranscriptional or posttranslational mechanisms with different degree of involvement depending on species. In the case of monounsaturated diets, our study indicates that hepatic mRNA may be responsible for the increase observed in plasma. This dependence on apoA-I mRNA levels is clearly observed at a high fat content. Our results, regarding regulation of plasma apoA-I, provide further support for the notion of different mechanisms of action for the different fatty acids, influence of dietary fat content and species-specific mechanisms. Further research is necessary to gain more insight into these mechanisms and to achieve a complete picture of them.

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