EFFECTS OF DIFFERENT DIETARY FATS ON PLASMA LIPID PROFILES IN RABBITS

Ali Sehattei1, Minoo ilkhanipour1, NasAhmadei-asl2, Samad Zare1, Reza Heidari1

1Biology Department, Science Faculty, Urmia University, Urmia, Iran.
2Department of Physiology, Medical Science University, Iran

ABSTRACT

Many factors are associated with decreased risk for coronary heart disease. One of these main factors is the distribution of plasma cholesterol over low density and high density lipoproteins (LDLs & HDLs). This can be modified by changing the source and amount of fats and oils in the diet. Therefore the main purpose of this investigation is to assess the effects of three dietary high fat diets on distribution of plasma lipoproteins in rabbit. Twenty male New Zealand White rabbits aged 4.5 months were randomly divided into four different groups. To each group was assigned a total of 5 rabbits and dietary feeding was commenced 15 days later at the age of four months after the rabbits had been acclimatised to their new environment in the animal experimental unit. The rabbits were fed for a total of two months a 10% (w/w) high fat diet containing the dietary oils. I. Fed basal diet on commercial diet (CO), II. Fed basal diet contains 10% batterfat-ghee-oil (GH), III. Fed basal diet with 10% hydrogenated vegetable fat (HD) & IV. Fed basal diet with 10% unsaturated sunflower oil (SU). At the end of two months feeding the rabbits fasted overnight and had their blood collected by heart puncture. Blood samples were collected for measuring and comparison of the concentrations of HDL and LDL cholesterol, total cholesterol and triglycerides in serum. Results show that Plasma lipids and lipoproteins are affected by type of dietary fats and it seems that the impact of the hydrogenated oil is higher than others, and it increased significantly TG, HDL-chol, VLDL-chol respectively (P<0.05). However there was no difference in total cholesterol and LDL-chol between treatments. HDL-cholesterol increased in the sunflower oil significantly (P<0.05). Total cholesterol increased in ghee-oil-treatment group compared with basal-control diet (P<0.05). LDL/HDL ratio elevated significantly in 2nd group and decreased significantly in 4th group compared with control group respectively (P<0.05). Our findings elucidated that plasma lipids and lipoproteins are affected by the type of the diet, and it seems that the effect of hydrogenated vegetable oil (trans) is more prominent in contrast to the public assumption ghee-oil, as compared with sunflower vegetable oil that had smaller increasing effect on plasma lipids. However, in order to gain confirmation more studies are required.

Key words: Ghee, hydrogenated oil, monounsaturated fatty acids, plasma lipids, cholesterol.

INTRODUCTION

Cardiovascular disease (CVD) is one of the major causes of death in developed countries. The TC/HDL ratio is the best single lipid predictor of CVD. The role of dietary fats as a determinant of plasma lipids and lipoproteins is well documented (Grundy and Denke, 1990; Gurr, 1992). Plasma lipid levels are influenced not only by the amount of fat consumed but by its nature as well. Trans fatty acids are geometrical isomers of the unsaturated fatty acids and are produced as a result of the hydrogenation of edible oils used in margarine and shortening manufacture.

The degree of saturation and unsaturation, stereo isometric differences and fatty acid chain length can all determine the response of plasma cholesterol to dietary fats. Several clinical studies have shown the adverse effects of trans fatty acids (Mauher et al., 2003; Lichtenstein et al., 1999; Matthan et al., 2002). They increase TC, lipoprotein(a) and reduce the protective high density lipoprotein cholesterol (HDL-C). The adverse effects of trans on lipoproteins shown in these clinical studies have additionally been supported by the epidemiological data of Willett and coworkers (1990). A recent study of Sundram et al. (1994) addressed an outstanding question of whether the trans fatty acids are nutritionally better or worse in these regards than the dietary saturated fatty acids they were designed to replace in solid fat products. They concluded that the negative impacts of trans elaidic acid on the lipoprotein profile of humans were worse than the saturates of chain length 12, 14 and 16 carbons. Recent studies suggest that the cholestrolaemic effects of the saturated fats are not uniform (Dreon et al., 1998; Sundram et al., 1994; Pereira et al., 1999) Saturated fatty acids (SFAs) have the potential to increase the blood lipids. Saturated fats are reported to affect serum lipids unfavourably. Saturated fats were found to increase total cholesterol (T-chol), LDL-chol: HDL-chol ratio as compared to unsaturated fats (1994). Butterfat, also known locally as ghee (GH), is a saturated fat of bovine or sheep milk origin. In Iran ghee oil is the more important animal fat produced by most families. It is highly saturated and contains discredited saturated animal fats. The allegation that ghee oil raises total serum cholesterol, thereby increasing the risk of coronary heart disease, however, it was not based on actual experimental studies. The batterfat controversy has been further fuelled by different effects in healthy human subjects and animal model designs. Ghee was found to raise plasma triglycerides (TG) and reduce
plasma LDL and HDL compared to the coconut oil (Ima-Nirwana, 1996). However, Foxall and Schwaery (1990) observed that a ghee-enriched diet was less hypercholesterolaemic compared with a fish oil-enriched diet. Polyunsaturated fatty acids are more prone to lipid peroxidation (LP) as compared to saturated fatty acids (Hallwell and Gutteridge, 1985). The cholesterol found in GF can itself undergo peroxidation (Hallwell and Gutteridge, 1985). Oxidation of cholesterol was found to occur during processing of butter to ghee (Jacobson, 1987). In the present study we postulated that the adverse effect of butter fat (ghee) compared with other dietary fats observed in some previous human or animal studies may lead to different effect when fed to rabbits over a short-term duration. This hypothesis was therefore tested with using the other different dietary oils as used in studies. The rabbit is a frequently used as an experimental model to evaluate dietary fat effects and atherosclerosis. We determined the potential of unfavourable effect of three common dietary fats of different origins, namely hydrogenated soybean oil and sunflower oil of plant origin, and ghee of animal origin, by comparing their effects on serum lipid profile. Therefore, the scientific community needs to conduct controlled studies on the effects of ghee oil and its relation to cardiovascular disease and maintain a responsible perspective. It is necessary to undertake properly controlled studies on the effects of ghee oil on blood lipids and on the risk of CVD.

MATERIALS AND METHODS

Twenty male White New Zealand rabbits aged 4.5 months were randomly assigned to four different dietary groups. To each group was assigned a total of 5 rabbits and dietary feeding was commenced 15 days later at the age of four months after the rabbits had been acclimatised to their new environment in the animal experimental unit. The rabbits were fed ad libitum for a total of two months a 10% (w/w) high fat diet containing the dietary oils whose fatty acid composition is described in Table 1.

Table 1. Fatty acid composition of dietary fats incorporated into diets in this study (g/100 g of dietary oil).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Butterfat(Ghee)oil</th>
<th>Hydrogenated oil</th>
<th>Sunflower oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0</td>
<td>7.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6:0</td>
<td>4</td>
<td>ND</td>
<td>0.6</td>
</tr>
<tr>
<td>8:0</td>
<td>3</td>
<td>ND</td>
<td>0.2</td>
</tr>
<tr>
<td>10:0</td>
<td>5.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12:0</td>
<td>5.1</td>
<td>ND</td>
<td>0.6</td>
</tr>
<tr>
<td>14:0</td>
<td>14.5</td>
<td>ND</td>
<td>0.7</td>
</tr>
<tr>
<td>16:0</td>
<td>27.8</td>
<td>14.2</td>
<td>16.2</td>
</tr>
<tr>
<td>18:0</td>
<td>9.5</td>
<td>9.5</td>
<td>10</td>
</tr>
<tr>
<td>18:1</td>
<td>15.5</td>
<td>26.8</td>
<td>28.8</td>
</tr>
<tr>
<td>18:1t</td>
<td>4.5</td>
<td>33.2</td>
<td>23.1</td>
</tr>
<tr>
<td>18:2t</td>
<td>ND</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>18:2</td>
<td>ND</td>
<td>8.4</td>
<td>13.2</td>
</tr>
<tr>
<td>18:3</td>
<td>0.8</td>
<td>0.4</td>
<td>ND</td>
</tr>
<tr>
<td>SFA</td>
<td>77.2</td>
<td>25</td>
<td>23.5</td>
</tr>
<tr>
<td>MUFA</td>
<td>15.5</td>
<td>36</td>
<td>61</td>
</tr>
<tr>
<td>PUFA</td>
<td>2.9</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>TRAFA</td>
<td>4.3</td>
<td>29</td>
<td>ND</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>0.04</td>
<td>0.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

ND, not detected; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P/S, polyunsaturated saturated fatty acid ratio.

Commercial rabbit diet (in pellet form) was used as the basal diet. The commercial pellets were obtained as a single production batch for the entire study duration to reduce variations in their nutrient content. This contained 2.5% fat, 16.0% protein, 48.8% carbohydrates, 18% fibre, 12.8% moisture and 1.9% vitamins, minerals (including calcium) and trace elements required for optimum maintenance of the rabbits. The protein source in this basal diet was mainly of vegetable (soy) origin. Incorporation of the oil blends into the basal rabbit pellets to increase the fat energy density of the diets was achieved as follows: 100g of each oil blend mixed with 1.0 kg of the rabbit pellet. The oil enriched pellets were further oven dried. All animals were weighed at regular monthly intervals and monitored for their general well being and feed intakes. At the end of two months feeding, the rabbits were fasted overnight, anaesthetised with sodium pentobarbital and had their blood collected by heart puncture.
Measurement of plasma lipids and lipoproteins

Blood collected in tubes containing EDTA (1 mg/ml blood) was used to prepare plasma for the lipid and lipoprotein analyses. Four ml of plasma was pipetted into a Beckmann 50.3 Ti ultraclear centrifuge tube and over layered with 2.0 ml NaCl solution (d20 = 1.006 g/mL). The very low density lipoproteins (VLDL) were isolated by preparative ultracentrifuge in a Beckmann 50.3 Ti rotor in a Beckmann LM8-70 ultracentrifuge. Low density and high density lipoproteins were sequentially isolated at their respective densities (d20) of (1.006<d<1.063) and (1.063<d<1.125) on a Kbr density gradient. These lipoproteins were extensively dialysed to remove their background salt densities. The cholesterol and triglycerides content of the plasma and isolated lipoproteins was analysed enzymatically on an autosampler.

Fatty acid analysis

Fatty acid composition of the dietary oils were analysed by using a Perkin Elmer Autosystem gas chromatogram (Perkin Elmer Corporation, Norwalk, CT, USA) fitted with a 100-metre capillary column (SP2560, Supelco, Belfonte, USA) and temperature programmed from 160°C to 240°C at 4°C/min.

Statistical analysis

The data were analysed with the SPSS/PC+ statistics program (V10, SPSS, Chicago, IL). The differences between the three test groups were assessed with t test (two-tailed). In all cases, statistical significance is P < 0.05 and data are presented in the text and tables as means ±SD. All data were checked for their frequency distribution using the One way-Annova test. Analysis of variance and Student T-test were used to test the differences between dietary treatments.

RESULTS

Rabbits fed the experimental diets demonstrated normal growth throughout the two months feeding duration. The diets fed to these animals had similar total fat energy densities. Effect of dietary oil blends on rabbit lipids and lipoproteins following feeding period are in Table 2.

Table 2. Effect of dietary oil blends on rabbit lipids and lipoproteins following 2-month dietary feeding.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL/HDL-C RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>33.75± 1.5</td>
<td>131 ± 1.9</td>
<td>26 ± 0.4</td>
<td>18.5 ± 1.5</td>
<td>13 ± 0.0</td>
<td>1.42 ± 0.12</td>
</tr>
<tr>
<td>GH</td>
<td>33.5 ± 4.3</td>
<td>196 ± 19 a*</td>
<td>38.8 ± 4</td>
<td>17 ± 4.2</td>
<td>7 ± 0.9 * c</td>
<td>2.5 ± 0.67 d</td>
</tr>
<tr>
<td>HD</td>
<td>46.75 ± 13</td>
<td>174 ± 40 a,b °</td>
<td>34.9 ± 8 b °</td>
<td>17 ± 3.2 °</td>
<td>13.5 ± 3.9 ° c</td>
<td>1.43 ± 0.32</td>
</tr>
<tr>
<td>SU</td>
<td>35.75 ± 1.3</td>
<td>120.75 ± 29 a,b</td>
<td>24 ± 5.8 b</td>
<td>15 ± 1.4</td>
<td>12.2 ± 1.6 ° c</td>
<td>1.3 ± 0.21d *</td>
</tr>
</tbody>
</table>

Values with marker ° are different from values at the end of two months feeding within the same diet group (p<0.05). Values with marker * are different compared with control-diet treatment(P<0.05). Values bearing the same alphabetical superscript are different between treatments groups (p<0.05) Values are given as mean± S.D. Values are means ± SD. (n=5 rabbits per group);

Plasma lipids and lipoproteins were generally modulated by the diet type.Total-chol and LDL-cholesterol was not significantly modulated by these treatment diets, in spite of the pronounced differences in the dietary fatty acid composition.Total cholesterol (TC) increased significantly in the ghee-enriched-oil group, compared to the beginning of feeding. TG value also increased in this group, as compared with the CO diet.Furthermore,LDL-cholesterol:HDL-cholesterol ratio in this group increased significantly, compared to the SU diet group.TG,VLDL-cholesterol:HDL-cholesterol concentrations increased in HD diet group significantly at the end of feeding period and, as compared with the CO diet.However,LDL-cholesterol level increased in this group considerably, but was not significant.HDL-cholesterol increased significantly in SU diet group, in comparison to to the CO diet.Furthermore,in LDL-cholesterol:HDL-cholesterol ratio decreased significantly, as compared to the CO diet.Rabbits fed the HD diet were characterised by higher TG and VLDL-cholesterol concentrations than SU diet group. Furthermore,TG value was higher than GH oil group.HDL-cholesterol value was higher in SU diet group than GH group.

DISCUSSION

In this study rabbits were fed enriched-fat diets continuously for the two-month duration. Changes in blood lipids and lipoproteins resulting from these fatty acid manipulations were evident. The TC/HDL ratio is the single
best lipid predictor of CVD. Although the dietary oils used had very different fatty acid compositions they failed to elicit the expected significant differences in plasma TC and LDL-C concentrations, a finding similar to Ima-Nirwana (1996). Intake of SAFA suppresses the LDL receptor activity and decreases the clearance of LDL from the circulation, resulting in a marked elevation of its level Dreon et al. (1998). SAFA raises the serum TC level thrice as much as PUFA, and MUFA lowers it. Most of this increase is due to an increase in LDL. Although some increase in HDL also occurs, it is not sufficient to offset the marked elevation of LDL.12. Stearic acid (C18:0) is desaturated to oleic acid soon after its absorption, and hence does not raise the TC level (Aro et al., 1997). Ghee oil in this study containing 9.5%, SAFA with chain lengths of 12—16, which have the most cholesterol-raising properties (Khosla and Hyes, 1992). These are lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0). Palmitic acid (16:0) raised plasma TG in hamsters after a 4-week feeding period (Lindsey et al., 1990). Lai et al. (1991) showed that different dietary saturated fats have unique effects on TG metabolism after feeding for 5 weeks. Myristic acid is the most powerful cholesterol-raising SAFA, and increases the TC level 50% more than palmitic acid. Studies in laboratory animals indicate that ghee oil increases both TG and LDL levels (Ima-Nirwana, 1996; Gupta and Prakash, 1997; Vijaya et al., 1999; Foxall and Schwaery, 1990).

Recent studies have shown that caprylic acid (C8:0) and capric acid (C10:0) level (Rudel et al., 1990). Ghee oil in present study contains 8.6% of these two cholesterol-raising SAFA. In our study the ghee-enriched-oil group demonstrated a significant increased TG value compared to the control group. Ghee contained a larger proportion of palmitic acid compared to other dietary fats used in treatments (Table 2). An important point in our study is the short-term feeding period (2 months). We found that short-term feeding had elevated hypertriglyceri-daemic effect in ghee oil group. It is well-documented that rabbits are sensitive to the addition of cholesterol in their diets and will develop cholesterolaemia when exposed to dietary cholesterol for even short durations. Anhydrous ghee contains 25mg cholesterol/100g butterfat (Staprans et al., 2003). Oxidation of cholesterol was found to occur during processing of butter to ghee (Jacobson, 1987). Fats that have been heated for prolonged periods in air contain many dangerous products from oxidation and breakdown of lipids. It is perhaps more harmful than butter due to the added presence of cholesterol oxides, which are generated during its preparation by prolonged heating of butter. Liberal dietary exposure to cholesterol oxides from ghee is a likely contributor to the high frequency of CAD (Gupta and Prakash, 1997). Several clinical studies have shown the adverse effects of trans fatty acids (Willett et al., 1993; Ascherio and Willett, 1995), they increase TC, lipoprotein Lp(a), TG, and reduce the protective high density lipoprotein cholesterol (HDL-C) Sundram et al. (1997) addressed an outstanding question of whether the trans fatty acids are nutritionally better or worse in these regards than the dietary saturated fatty acids when either saturated and trans fatty acids are substituted for cis unsaturated fatty acids, total and LDL cholesterol levels are higher. When trans fatty acids are substituted for saturated fatty acids, HDL cholesterol levels are lower, triglyceride levels are higher and the LDL cho: HDL cholesterol ratio is less favorable. Trans fatty acids have been reported to increase triglyceride levels (Mensink et al., 1992; Nestel et al., 1992; Sundram et al., 1997). Elaidic acid (n-9 trans 18:1) is the principal TRAFA, although several other trans isomers are also formed (Sundram et al., 1997). Consumption of TRAFA has a greater adverse effect on lipoproteins than that of SAFA (Rudel et al., 1990). Whereas both SAFA and TRAFA increase LDL levels considerably, TRAFA also decrease HDL levels, thereby increasing the LDL-cho: HDL-cho ratio, the single best lipid-related risk factor for CAD (Johnson et al., 1993). Important adverse effects of TRAFA consumption include increases in lipoprotein(a), TG, and small-dense LDL levels (Hokanson and Austin 1996). Diets high in MUFA (oleic acid C18:1) like sunflower oils make LDL resistant to oxidation, restore LDL-receptor activity, and markedly lower LDL levels. The reduction in TC is 3-fold higher when MUFA replace SAFA (Zilversmit, 1995). Consumption of MUFA offers the unique advantage of effectively lowering LDL levels without lowering HDL or raising TG levels. Replacing SAFA with PUFA reverses the suppression of LDL-receptor activity by cholesterol-raising SAFA (similar to that of MUFA) (Hokanson and Austin, 1996). PUFA do not raise the TG level, and sometimes lower it (Rudel et al., 1990). The two undesirable effects of PUFA are increased susceptibility for peroxidation, and lowering of the HDL level. HDL inhibits LDL oxidation primarily through its paraoxonase activity; so heated oil for prolonged periods in air (ghee) reduces paraoxonase activity, and thus reduces the ability of HDL to prevent LDL oxidation (Carr et al., 2002). However, we found that addition of ghee increased TC-cholesterol concentration at the end of feeding, increased LDL-cholesterol ratio was comparable to sunflower oil, in fact that ghee contained more palmitic acid and overall saturation fatty acid compared to control diet. An important observation to note is that an upward trend is seen in HDL-cholesterol with both HD and SU fats. In conclusion, ghee increased serum LDL-cholesterol ratio to a greater degree than sunflower oil, making it more atherogenic than HD and SU.
REFERENCES


lipid and Lp(a) changes with substitution of elaidic acid for oleic acid in the diet. *J. Lipid Res.*, 33: 1029-1036.


(Accepted for publication June 2006)