



Cholesterol and Unsaturated Fat Diets Influence Lipid and Glucose Concentrations in Rats

Panayiotis N. Adamopoulos,* Christakis M. Papamichael,*
Antonios Zampelas† and Spyros D. Mouloupoulos‡

*DEPARTMENT OF PREVENTIVE MEDICINE, ALEXANDRA UNIVERSITY HOSPITAL,
ATHENS, GREECE; †NUTRITION AND FOOD SAFETY RESEARCH CENTRE, SCHOOL OF
BIOLOGICAL SCIENCES, UNIVERSITY OF SURREY, GUILDFORD, SURREY, U.K. GU2 5XH; AND
‡DEPARTMENT OF CLINICAL THERAPEUTICS, SCHOOL OF MEDICINE, UNIVERSITY OF ATHENS, ATHENS, GREECE

ABSTRACT. The present study investigated the effects of dietary cholesterol and monounsaturated and polyunsaturated fatty acids on plasma lipids and glucose concentrations. Four groups of ten male Wistar albino rats were fed diets of different fatty acid composition for 40 days. The control group consumed nonpurified diet (containing fat 3.7 g/100 g diet), and cholesterol, olive oil, and safflower oil groups consumed the nonpurified diet enriched with 14 g fat/100 g diet with egg yolk, olive oil, or safflower oil, respectively. Compared with the control, the diet enriched with cholesterol significantly increased fasting plasma cholesterol ($P < 0.01$), triacylglycerol ($P < 0.01$), total lipid ($P < 0.01$) and glucose ($P < 0.05$) concentrations; in the olive oil group, cholesterol and triacylglycerol levels were significantly increased compared with control group ($P < 0.01$ in both instances). In safflower oil group, triacylglycerol levels were also significantly increased ($P < 0.05$) compared with the controls. After comparing diets providing the same amount of fat (cholesterol, olive oil, and safflower oil groups), higher cholesterol, triacylglycerol and total lipid levels were observed in the cholesterol group than in the olive oil group ($P < 0.01$, $P < 0.05$ and $P < 0.01$, respectively), and safflower oil group ($P < 0.01$ in all instances). High-density lipoprotein-cholesterol concentrations were significantly lower in the cholesterol group than in the olive oil and safflower oil groups ($P < 0.05$ in both instances) and fasting plasma glucose levels were higher in the cholesterol than in the olive oil ($P < 0.05$) and safflower oil groups ($P < 0.01$). Finally, after comparing lipid and glucose levels in the unsaturated fatty acids-enriched diets, higher plasma cholesterol concentrations were observed in the olive oil than in the safflower oil group ($P < 0.05$). These data suggest that not only the amount but also the type of dietary fat can influence serum lipid levels. *COMP BIOCHEM PHYSIOL* 113B, 659–663, 1996.

KEY WORDS. Rats, diet, plasma lipids, cholesterol, triacylglycerol, glucose, monounsaturated fatty acids, polyunsaturated fatty acids.

INTRODUCTION

Research on the influence of the amount and type of dietary fat on plasma lipid levels has shown that diets rich in saturated fatty acids (SFA) and cholesterol have a hypercholesterolaemic effect (8,9,11), while diets rich in polyunsaturated fatty acids (PUFA), of the n-6 series (commonly found in vegetable oils), decrease fasting cholesterol (TC) concentrations (1,11,19). Recent attention has focused on the Mediterranean diet (low in polyunsaturated and high in monounsaturated fatty acids) and studies have suggested a potential protective role of monounsaturated fatty acids (MUFA) of the n-9 series (commonly found in olive oil) against the development of coronary heart disease (CHD), probably through a lowering

effect of MUFA on TC concentrations (8,10,13). However, the extent by which MUFA decrease TC levels compared with PUFA is not yet clearly defined. TC concentrations have been found to decrease more with a PUFA diet than a MUFA diet (3) but, on the other hand, low-density lipoprotein-cholesterol (LDL-C) is shown to be decreased to a larger extent following a MUFA diet than a PUFA diet (15). High-density lipoprotein-cholesterol (HDL-C) concentrations have been found to decrease (15), remain stable (18) or even to increase in volunteers on MUFA diets (5,12) compared with volunteers on PUFA diets.

Fasting triacylglycerol (TAG) concentrations are not considered as an independent factor for assessing the risk of developing coronary heart disease because in multivariate analysis is any effect of TAG tends to be eliminated once HDL-C levels are taken into account (2). However, the recent guidelines from the European Atherosclerosis Society (1992) put TAG levels, together with LDL-C and HDL-C, as a risk factor

Correspondence to: A. Zampelas, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH, Surrey, U.K. Tel. 01483-300800 ext. 3379; Fax 01483-576978.

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for developing CHD (16). The impact of dietary fat on fasting and post-meal TAG is still under investigation. Current suggestions are that long-chain SFAs increase TAG concentrations but short- and medium-chain SFAs don't. MUFA do not seem to have an increasing effect on TAG concentrations (7,14), and sometimes can decrease TAG concentrations compared to diets rich in SFA (20). On the other hand, PUFA of the n-6 series may have some lowering effect, TAG levels but the latter suggestion is still to be confirmed (8).

Recent studies have also indicated possible adverse effects of PUFA on the development of CHD, due to their susceptibility to peroxidation and production of free radicals, and current dietary recommendations do not encourage an increase of these fatty acids in the habitual dietary fat intake in the United Kingdom (6). On the other hand, even though effects of dietary MUFA on plasma lipid concentrations have been extensively investigated, controversial suggestions have arisen. In addition, the effects of MUFA diets on TAG concentrations have not been extensively investigated. Since MUFA are not as susceptible as PUFA to peroxidation, interest in their potential role in the diet has been increasing recently.

As a result, since the hypolipidaemic effects of olive oil are not yet clearly defined, the aim of the present study is to compare the effects of cholesterol and unsaturated fat diets on plasma lipid and glucose concentrations.

MATERIALS AND METHODS

Animals and Diets

Forty male Wistar albino rats, each weighing between 200 and 250 g, were divided into four test groups. Each group was fed a particular diet for 10 weeks. The diets were as follows: a nonpurified diet, providing 3.7% fat (w/w) (control group); a nonpurified diet enriched with 14% cholesterol, coming from egg yolk (cholesterol group); a nonpurified diet enriched with 14% olive oil; or a nonpurified diet enriched with 14% safflower oil. All groups were housed under the same conditions in wire cages at 25°C and they had free access to food and water. The nutrient composition of the nonpurified diet and the fatty acid composition of the supplemented egg yolk, olive oil and safflower oil are given in Table 1 and Table 2, respectively. On the day of sacrifice, 24-h fasted rats were anaesthetized under ether and blood was collected by cardiac puncture. Blood samples were centrifuged at 2,500 rpm for 15 minutes to separate the plasma, which was subsequently

TABLE 1. Nutrient composition of the nonpurified diet (control diet)

Component	Percentage (g/100 g)
Vitamins and minerals	4.9
Crude fat	3.7
Crude protein	20.6
Carbohydrate	70.8

TABLE 2. Fatty acid composition of the dietary fat supplemented in the nonpurified diet (g/100 g)

Fatty acid	Egg yolk	Olive oil	Safflower oil
C 12:0	0.0	0.0	0.0
C 14:0	0.0	Trace	Trace
C 16:0	7.2	12.0	8.0
C 18:0	2.3	2.3	2.5
C 20:0	0.0	0.4	0.2
C 22:0	0.0	0.0	0.3
C 24:0	0.0	0.0	0.0
C 14:1	0.0	0.0	0.0
C 16:1	1.1	1.0	0.1
C 18:1	10.9	72.0	13.0
C 20:1	0.0	0.0	0.1
C 22:1	0.0	0.0	0.0
C 18:2	2.9	11.0	75.0
C 18:3	0.0	0.7	0.5
C 20:4	0.2	0.0	0.0
C 22:6	0.3	0.0	0.0
Cholesterol	1260 mg/100 g	Trace	Trace

aliquoted and stored at -20°C until analysis of lipids and glucose concentrations.

Measurement of Lipids and Glucose

The analysis of the blood samples was carried out in the Preventive Medicine Research Laboratory in Alexandra University Hospital, Athens. The Research Laboratory is associated with the system of Quality Control of the World Health Organisation (WHO) European Center. TC concentrations were measured by an enzymatic colorimetric method using a test kit supplied by Abbott Laboratories, Athens, Greece (17). TAG and glucose concentrations were measured by using enzymatic colorimetric methods and the test-kit agent (Abbott Laboratories, Athens, Greece). Measurements of TC, TAG and glucose concentrations were carried out using an automated analyser (Abbott VP Super System, Irving, Texas, U.S.A.). The Helena Lipoprotein Electrophoresis Procedure was used to determine HDL-C concentrations.

Statistical Analysis

Data in Table 3 are given as mean values with their standard deviations (mean \pm SD). Comparisons of lipids and glucose concentrations among the four dietary groups were carried out and differences between groups were identified using one-way analysis of variance. The Duncan's range test was applied to locate and assess the level of significance of any differences found. A *P* value less than 0.05 ($P < 0.05$) was taken as the lowest level of statistical significance.

RESULTS

Lipids and glucose concentrations of the four dietary groups after the 40 days dietary period are given in Table 3.

TABLE 3. Effect of dietary cholesterol and fatty acid composition on plasma lipid and glucose concentrations from rats fed diets enriched with cholesterol, olive oil and safflower oil

Parameter	CO diet	CH diet	OO diet	SO diet
TC (mg/dl)	63.5 ± 9.0	100.7 ± 16.5 ⁺	79.7 ± 7.1 ⁺	66.2 ± 10.4 [*]
TAG (mg/dl)	153.0 ± 49.8	384.3 ± 127.7 ^{*+}	295.4 ± 118.1 ⁺	263.3 ± 52.0 [*]
HDL-C (mg/dl)	43.0 ± 9.8	37.9 ± 7.0 [*]	49.0 ± 8.6	46.2 ± 6.9
Total lipids (mg/dl)	469.0 ± 12.9	572.2 ± 15.6 ⁺	483.3 ± 21.2	472.0 ± 16.2
Glucose (mg/dl)	151.1 ± 20.9	190.8 ± 27.9 ^{*+}	156.4 ± 34.2	132.7 ± 41.9

CO: control diet; CH: cholesterol-enriched diet; OO: olive oil-enriched diet; SO: safflower oil-enriched diet; TC: total cholesterol; TAG: triacylglycerol; HDL-C: high density lipoprotein-cholesterol. CH diet: TC concentrations: ⁺significant ($P < 0.01$) from other dietary groups. TAG concentrations: ^{*}significant ($P < 0.05$) from OO and ⁺significant ($P < 0.01$) from CO and SO. HDL-C concentrations: ^{*}significant ($P < 0.05$) from OO and SO. Total lipid concentrations: ⁺significant ($P < 0.01$) from other dietary groups. Glucose concentrations: ^{*}significant ($P < 0.05$) from CO and OO, and ⁺significant ($P < 0.01$) from SO. OO diet: TC concentrations: ^{*}significant ($P < 0.01$) from CO. TAG concentrations: ^{*}significant ($P < 0.01$) from CO. SO diet: TC concentrations: ^{*}significant ($P < 0.05$) from OO and TAG concentrations: ^{*}significant ($P < 0.05$) from CO.

The cholesterol diet significantly increased TC concentrations compared with the control diet from 63.5 ± 9.0 mg/dl to 100.7 ± 16.5 mg/dl ($P < 0.01$). TAG and total lipid concentrations were also significantly increased following the cholesterol diet from 153.0 ± 49.8 mg/dl to 384.3 ± 127.7 mg/dl ($P < 0.01$) and from 469.0 ± 12.9 mg/dl to 572.2 ± 15.6 mg/dl ($P < 0.01$) respectively. Glucose concentrations were significantly higher in the cholesterol dietary group (151.1 ± 20.9 mg/dl) than in the control group (190.8 ± 27.9 mg/dl) ($P < 0.05$). However, HDL-C concentrations were not significantly altered even though there was a small tendency toward lower HDL-C levels following the cholesterol diet.

The rats on the safflower oil diet had higher TAG concentrations than rats on the control diet (153.0 ± 49.8 mg/dl, and 263.3 ± 52.0 mg/dl, respectively, $P < 0.05$), but TC, total lipid, HDL-C and glucose concentrations were not significantly altered.

The diet high in n-9 fatty acids (olive oil diet) resulted to higher TC and TAG concentrations than the control diet. In particular, TC concentrations rose from 63.5 ± 9.0 mg/dl to 79.7 ± 7.1 mg/dl ($P < 0.01$) and from 153.0 ± 49.8 mg/dl to 295.4 ± 118.1 mg/dl ($P < 0.01$), respectively. Total lipid, HDL-C and glucose concentrations were not significantly altered.

Statistically significant differences were also observed after comparing the diets, which provided the same amount of fat (cholesterol, olive oil and safflower oil diets). In particular, the cholesterol diet resulted to higher TC concentrations than the olive oil and safflower oil diets (100.66 ± 16.5 mg/dl, 79.66 ± 7.1 mg/dl and 66.2 ± 10.4 mg/dl respectively) ($P < 0.01$ in both instances). TAG concentrations were significantly lower in the rats on the olive oil diet (295.4 ± 118.1 mg/dl) than on the cholesterol diet (384.3 ± 127.7 mg/dl) ($P < 0.05$) and they were also lower in the rats on the safflower oil diet (263.3 ± 52.0 mg/dl) than on the cholesterol diet ($P < 0.01$). The cholesterol diet led to significantly higher total lipid concentrations than the olive oil and safflower oil diets. In particular, total lipid levels were 483.3 ± 21.2 mg/dl (olive oil diet) and 572.2 ± 15.6 mg/dl (choles-

terol diet) ($P < 0.01$) and 472.0 ± 16.2 mg/dl (safflower diet) and 572.2 ± 15.6 mg/dl (cholesterol diet) ($P < 0.01$). HDL-C levels were higher on the olive oil diet (49.0 ± 8.6 mg/dl) and safflower oil diet (46.2 ± 6.9 mg/dl) than on the cholesterol diet (37.9 ± 7.0 mg/dl) reaching a level of statistical significance of $P < 0.05$ at both instances. Furthermore, glucose levels significantly increased following the cholesterol diet, from 156.4 ± 34.2 mg/dl (olive oil diet) and from 132.7 ± 42.0 mg/dl (safflower oil diet) to 190.8 ± 27.9 mg/dl ($P < 0.05$ and $P < 0.01$, respectively).

Finally, after comparing serum lipid and glucose levels following the diets rich in unsaturated fatty acids, it was observed that TC concentrations were significantly lower following the safflower oil diet (66.2 ± 10.4 mg/dl) than following the olive oil diet (79.7 ± 7.1 mg/dl) ($P < 0.05$).

DISCUSSION

The initial process of atherogenesis is attributed to the loss of endothelial cell barrier integrity. It is related to chemical and physical agents as well as to the intrinsic properties of the barrier itself. Among the chemical agents with direct effects on the endothelium of the cell is cholesterol. Its ester appears to be toxic to the endothelium, particularly the oxidised form of the fatty acid moiety. Increased TC concentrations reflect a condition in the circulation that may be caused partly by diet and increases the risk for developing CHD.

In the present study, TC, TAG, total lipids and glucose levels were affected by changes not only in the amount of fat in the diet but also in its fatty acid composition. In the cholesterol dietary group, TC and TAG concentrations were significantly higher than in the other dietary groups. In addition, there was also a trend toward lower HDL-C concentrations, which reached statistical significance only when compared with the level of the olive oil and safflower oil dietary groups, an observation that strengthens the current suggestion that diets rich in cholesterol can increase the risk for development of coronary heart disease through increases in fasting TC and TAG concentrations and decreases in HDL-C. Furthermore, glucose levels were also significantly higher, an observation

that suggests a possible onset of insulin resistance following diets rich in cholesterol. However, more research is necessary in this area.

On the other hand, diets rich in either MUFA or PUFA seem to play a protective role against raising fasting serum lipid levels. It is noteworthy that the most profound effect was observed following the diet enriched with n-6 fatty acids (safflower oil diet). In particular, TC, TAG and total lipid levels were significantly lower compared to the cholesterol dietary group, and TC concentrations were significantly lower than those in the olive oil dietary group. The results, therefore, of the present study agree with the suggestion from other studies (3,4) that n-6 PUFA have a more pronounced effect on lowering TC concentrations than n-9 MUFA. On the other hand, the increase in the total amount of dietary fat, although of vegetable oil origin, had a raising effect on TAG concentrations (safflower oil versus control diet), which suggests that an increase in total fat intake, even n-6-PUFA originated, has a hypertriglyceridaemic effect. However, glucose levels were unaffected by the safflower oil diet.

As has already been mentioned, the olive oil diet had a less pronounced effect on lipid levels compared with the safflower oil diet. In particular, even though TC, TAG and total lipid concentrations were lower following the olive oil diet than the cholesterol diet, TC concentrations were higher in the olive oil dietary group than in the safflower oil dietary group. Furthermore, the increase in the total amount of dietary fat, although MUFA-enriched, had a raising effect on TC and TAG concentrations, compared with the control diet. However, we did notice a small trend toward higher HDL-C concentrations in the olive oil dietary group compared with the other groups and this reached statistical significance when compared with the cholesterol group. This could suggest that in the long term one of the possible mechanisms through which olive oil could protect against the development of atherosclerosis is an increase in HDL-C concentrations (12), which are involved in the reverse-cholesterol transport. The actual level of olive oil in the habitual diet that would secure this HDL-C-raising effect is not yet known. In fact, there are studies that have found that olive oil diets have no effect on HDL-C concentrations (3,18,14,21) or just a small lowering effect (15).

In conclusion, both the amount and the type of dietary fat can influence serum lipid levels in the rat. High-cholesterol diets can be atherogenic by causing increases in TC, TAG and total lipid concentrations, through a possible development of insulin resistance, and through possible decreases in HDL-C concentrations. Diets rich in n-6 fatty acids could protect against increases in TC and TAG concentrations but could have a smaller effect on HDL-C concentrations than the high n-9 MUFA diets. On the other hand, diets rich in n-9 fatty acids could also protect against increases in lipid levels but in a less pronounced way than n-6 fatty acids. However, interpretation of the present results in human context needs some consideration since lipid metabolism in the rat is not identical

with the human. The animal model though, would give the opportunity to compare four different diets, something that would not be feasible using human subjects. Further work needs to be done on the effects of olive oil on lipid and especially HDL-C concentrations both in fasting and in the postprandial state in humans, taking into account that n-9 fatty acids are less susceptible to peroxidation than n-6. The overall effects of olive oil on lipid metabolism could prove to be beneficial, offering a greater protective role against the development of CHD than n-6 fatty acids.

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