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The effect of a lipid-lowering diet on plasma lipids and lipoproteins in mildly hypercholesterolaemic subjects: A potential role for occasional treats

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The aim of the study was to compare the effect of a lipid-lowering diet containing chocolate confectionery with an equivalent diet that is chocolate-free. In a parallel design trial, 42 free-living subjects (19 men and 23 women), aged 46.9 yr, mildly hypercholesterolemic (6.9 mmol/L) were allocated to an American Heart Association/ National Cholesterol Education Program Step 1 diet that included chocolate confectionery or an identical regimen containing no chocolate. Blood samples for the analysis of plasma lipids were obtained initially, then at 6 and 12 weeks after dietary therapy. Both groups of subjects showed a trend toward a reduction in saturated fat, with those allowed chocolate reaching borderline significance ($P < 0.057$*). Plasma cholesterol and low density lipoprotein cholesterol concentrations were significantly lower (*$P < 0.03$*) whereas plasma triacylglycerol was significantly higher (*$P < 0.02$*) in the control group compared with the chocolate group. High density lipoprotein cholesterol was reduced in both groups. Subgroup analysis on patients with the apo E3/E3 phenotype showed that the response was identical between the control and chocolate groups. The inclusion of a modest amount of chocolate confectionery did not detract from the response of a lipid-lowering diet.* (J. Nutr. Biochem. 11:250–254, 2000) © *Elsevier Science Inc. 2000. All rights reserved.*

Keywords: lipids; cholesterol; diet; chocolate; humans

Introduction

The first step in the treatment of hyperlipidemic patients is diet therapy. Recommendations are to limit the intake of fat, but in particular to reduce the intake of saturated fat and trans fatty acids.[1–3] For a typical patient this is a radical change in eating behavior that is often not adhered to for any extended period (i.e., long-term compliance is poor). Subsequently, patients are placed on lipid-lowering drugs.

An alternative approach in the dietary treatment of patients is to allow a more permissive diet. This can be done by allowing patients a "day off"; however, clinical experience has shown that this often results in bingeing and can be counterproductive. A more positive approach is to allow occasional treats throughout the period of dietary treatment. Chocolate is a common snack food/dessert that many people choose.[4] Although chocolate is relatively high in saturated fat, it is mainly stearic acid, which is considered neutral in its cholesterolemic effect.[5] We proposed to allow a limited amount of chocolate confectionery in the context of a controlled diet for the treatment of hyperlipidemic patients. The aim of this trial was to compare the effect of a lipid-lowering diet containing a modest amount of chocolate confectionery with an equivalent diet that was chocolate-free.

Materials and methods

Recruitment

Male and female hypercholesterolemic subjects (plasma cholesterol > 5.5 mmol/L) were recruited through the Royal Prince Alfred Hospital Lipid Clinic and general practitioner referrals from the Central Sydney Area Health Service (CSAHS). Advertising in

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This study was supported partly by funding from EFFEM Foods, Wodonga, Vic, Australia.
Received September 1, 1999; accepted February 7, 2000.

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0955-2863/00/$–see front matter
PII S0955-2863(00)00072-3

Table 1 Nutrient composition of the lipid-lowering diet

Nutrient	Target
Total fat	<30% of energy
Saturated fatty acids	<10% of energy
Polyunsaturated fatty acids	Up to 10% of energy
Monounsaturated fatty acids	10–15% of energy
Carbohydrates	50–60% of energy
Protein	10–20% of energy
Cholesterol	<300 mg/day
Total energy	To achieve and maintain desirable body weight

Based on the American Heart Association/National Cholesterol Education Program Step 1 Diet.

Table 2 Subjects' baseline characteristics

	Control group	Chocolate group
Number of subjects	22	20
Male/female	10/12	9/11
Age (y)	45.6 ± 10.7	48.2 ± 11.4
Weight (kg)	73.6 ± 14.5	80.9 ± 14.4
BMI (kg/m^2)	26.8 ± 4.7	28.5 ± 4.2
Waist:hip	0.84 ± 0.09	0.87 ± 0.08
Plasma cholesterol (mmol/L)	6.8 ± 0.9	6.9 ± 1.0
apo E phenotype	E3/E3 (n = 12)	E3/E3 (n = 12)
	E3/E4 (n = 7)	E3/E4 (n = 6)
	E4/E4 (n = 3)	E2/E3 (n = 1)
		E2/E4 (n = 1)

Data shown as mean ± SD.
BMI–body mass index.

the local newspapers was carried out on a number of occasions and posters were displayed near the hospital and the University of Sydney campus. Oral presentations were made at information evenings for general practitioners to make them aware of the study. The study protocol was approved by the CSAHS Ethics Committee and all subjects signed informed consent forms prior to their participation in the study.

Study protocol

Patients were allocated to a lipid lowering diet (American Heart Association/National Cholesterol Education Program step 1 diet; *Table 1*) that contained chocolate confectionery or an identical regimen containing no chocolate. The allocation was based on the patient's preference for chocolate as determined by the answer to a casual question by the dietitian. None of the subjects were coerced into entering the study. Those allocated to the chocolate group were advised to consume one item of chocolate confectionery per day as a substitute for a treat or snack but not as a supplement. Along with dietary advice, all participants were asked to participate in any form of exercise 2 to 3 times per week.

Patients assigned to the "chocolate group" were provided with a supply of chocolate confectionery (Milky Way, Maltesers, and Mars, EFFEM Foods, Wodonga, Vic, Australia) and instructed to consume no more than one of the products per day. The weight of the chocolate products was 18.1, 20, and 26 g, which represented 1.9, 3.0 and 2.8 g of saturated fat from the Milky Way, Maltesers, and Mars products, respectively. As a marker of apparent compliance, all subjects were provided with a calendar and asked to document their intake of the chocolate products. Subjects were also asked to forward the wrappers to the Lipid Clinic using the envelopes provided. Occasional phone contact was made with the subjects and rapport with the dietitian was encouraged. Subjects in the control group were treated in an identical manner but the chocolate was excluded. Contact between subjects who were enrolled in the trial was minimal. No adverse side effects of dietary treatment were observed.

On initial interview, the subjects' anthropometric details (height, weight, and waist and hip circumference) were measured followed by a routine physical examination and biochemical screen. This was followed by the assessment of dietary intake and instruction on how to maintain a lipid-lowering diet (with or without the inclusion of chocolate confectionery). Dietary education sessions were conducted in the initial stages of the trial.

Each subject provided a total of five blood samples, twice at baseline before commencing diet therapy, one at week 6 of the trial, and twice on completion of the study (week 12). Thirty milliliters of blood were collected in vacutainers (Becton Dickinson, Franklin Lakes, NJ USA) that contained ethylenediaminetetraacetic acid (EDTA; 1.8 mg/mL). All samples were taken from an antecubital vein. Subjects were in a fasted state (10–12 hr) and in the supine position during the blood collection. Blood samples were centrifuged at 1,500 *g* for 8 min at 4°C and the plasma removed and placed on ice. In order to minimize the recognized biological variation in plasma cholesterol measurements, subjects returned 2 to 4 days later to allow for the collection of a duplicate blood sample[6] for the analysis of plasma lipids.

Plasma cholesterol and triacylglycerol concentrations were analyzed enzymatically using a routine laboratory analyzer (Hitachi 747, Boehringer Mannheim, GmbH, Mannheim, Germany). High density lipoprotein (HDL) cholesterol was determined following the precipitation of apolipoprotein (apo) B containing lipoproteins[7] and low density lipoprotein (LDL) cholesterol was determined using the SI version of the Friedewald equation.[8] The coefficient of variation of a lyophilized standard (Precinorm L, Boehringer Mannheim, Mannheim, Germany) for all cholesterol and triacylglycerol assays was less than 5%.

For the determination of apo E phenotypes, plasma was subjected to ultracentrifugation (106,000 × *g*, 16 hr, 15°C) in a 50Ti rotor (Beckman Instruments, Palo Alto, CA USA). Very low density lipoprotein (VLDL; d < 1.006 g/m) was isolated and delipidated.[9] Analysis of apo E phenotype was determined by isoelectric focussing.[10]

During the second visit (week 6) to the Lipid Clinic, blood samples for the analysis of plasma lipids were obtained and anthropometric measurements were repeated (weight and waist and hip circumference). The dietary protocol given to the patients on their initial review was reinforced by the dietitian followed by a request to complete a second 3-day food record. The dietary records were coded and analyzed (SERVE Nutrition Management System, Williams Pty Ltd, St Ives, NSW, Australia). A similar procedure was followed for the patients' final visit (week 12), in addition to the collection of duplicate blood samples.

The subjects' characteristics on entry into the study are shown in *Table 2*. Subjects allocated to the chocolate group tended to have a higher body mass index relative to those allocated to the control group. Subjects had similar plasma cholesterol concentrations and the distribution of apo E phenotypes showed equal numbers of the apoE3 allele in each group.

Statistical analysis

When comparing the data within a group (i.e., the same subjects before and after intervention), the Student's paired *t*-test was used. However, when groups were compared (control vs. chocolate) an unpaired *t*-test was employed. Statistical significance was taken at a *P*-value of less than 0.05.

Table 3 The effect of diet on the intake of energy (MJ), macronutrients (expressed as % energy), and fiber (g)

	Control group	Chocolate group
Energy (MJ)		
Initial	8.4 ± 2.5	9.7 ± 2.8
Final	7.7 ± 2.2	8.2 ± 2.0
P-value (paired)	NS	0.009
Saturated fat (%en)		
Initial	11.6 ± 4.2	11.2 ± 4.0
Final	9.9 ± 3.9	9.6 ± 2.7
Monounsaturated fat (%en)		
Initial	13.2 ± 3.8	12.2 ± 4.2
Final	14.2 ± 6.4	11.9 ± 4.8
Polyunsaturated fat (%en)		
Initial	6.2 ± 2.8	5.9 ± 1.7
Final	6.7 ± 3.7	6.2 ± 2.0
Carbohydrate (%en)		
Initial	44.5 ± 7.6	47.7 ± 9.4
Final	45.3 ± 13.2	47.5 ± 10.7
Protein (%en)		
Initial	17.7 ± 4.0	18.0 ± 3.9
Final	18.7 ± 4.3	18.7 ± 2.9
Alcohol (%en)		
Initial	2.7 ± 3.8	1.9 ± 2.3
Final	2.3 ± 2.8	2.4 ± 2.8
Fiber (g)		
Initial	24.4 ± 8.9[a]	30.7 ± 8.5[a]
Final	24.5 ± 9.9[b]	33.4 ± 9.8[b]

Data shown as mean ±SD. Values sharing a common superscript are significantly different using the unpaired Student's *t*-test. [a]$P < 0.02$, [b]$P < 0.006$.

Table 4 The effect of diet on plasma triacylglycerol and cholesterol concentrations (mmol/L)

	Control group	Chocolate group
Plasma triacylglycerol		
Initial	1.9 ± 1.1	2.0 ± 0.8
Final	2.1 ± 1.1	2.1 ± 0.7
P-value (paired)	<0.02	NS
Plasma total cholesterol		
Initial	6.8 ± 0.9	6.9 ± 1.0
Final	6.5 ± 0.8	6.7 ± 0.8
P-value (paired)	<0.03	NS
LDL cholesterol		
Initial	4.5 ± 0.9	4.7 ± 1.0
Final	4.3 ± 0.8	4.5 ± 0.9
P-value (paired)	<0.03	NS
HDL cholesterol		
Initial	1.4 ± 0.3	1.3 ± 0.2
Final	1.3 ± 0.3	1.2 ± 0.2
P-value (paired)	<0.002	<0.04

Data shown as mean ±SD. No significant differences between the control and chocolate groups (unpaired).
LDL–low density lipoprotein. HDL–high density lipoprotein.

Results

Effect of diet therapy on the intake of fat and other macronutrients

The effect of diet on the intake of fat is shown in *Table 3*. Analysis of the dietary records showed that dietary therapy resulted in a reduction in the intake of saturated fat. The reduction in the control group was from 25.32 to 18.87 g, a decrease of 6.45 g per day, which reached borderline statistical significance ($P < 0.057$; paired *t*-test). In subjects consuming a small amount of chocolate confectionery as part of their diet, the intake of saturated fat was reduced from 28.73 to 20.42 g, a significant ($P < 0.013$) reduction of 8.31 g. When the change in intake is expressed as a percent of energy intake, the reduction was similar in both groups, reaching borderline statistical significance ($P < 0.057$) in those assigned to the chocolate group.

Subjects in the chocolate group showed a significant reduction ($P < 0.03$) in the consumption of monounsaturated fat. The decrease of 6.4 g had no impact on the intake of monounsaturated fat when expressed a percent of energy. There was little impact of dietary modification on the consumption of polyunsaturated fat.

The intake of dietary fiber was higher in subjects allocated to the chocolate group than in those in the control group. Upon completion of the study, despite a significant reduction in caloric intake in subjects allocated to chocolate, fiber intake remained higher than the control group. Expressed as a percent of total energy, the intake of protein, carbohydrate, and alcohol were not different between groups and were unaffected by the intervention (*Table 3*).

The effect of diet on plasma lipid levels

The effect of diet on the concentrations of plasma triacylglycerol and cholesterol and its distribution among lipoproteins is shown in *Table 4*.

Diet therapy resulted in a significant increase ($P < 0.02$) in plasma triacylglycerol concentrations in the control group. A minor increase in those allocated to the chocolate group was observed. In contrast, plasma cholesterol concentrations fell in both the chocolate and control groups but reached statistical significance ($P < 0.03$) only in the control group. The decrease in plasma cholesterol was paralleled by the LDL fraction. HDL cholesterol concentrations fell in both groups; however, the LDL:HDL and total cholesterol:HDL ratios were not affected.

The distribution of apo E phenotypes varied between the two groups (*Table 2*). There was a greater representation of the apo E4 allele in subjects in the control group compared with those allocated to the chocolate group. As the apo E phenotype may affect the plasma cholesterol response to diet, the data were reanalyzed using only those with the apo E3/E3 phenotype ($n = 12$ per group). In patients with the apo E3 phenotype, plasma triacylglycerol, cholesterol, and LDL cholesterol concentrations were similar in the two groups (*Table 5*). HDL remained significantly lower in both groups after dietary treatment.

Discussion

All subjects reported compliance with diet therapy and based on the calendar reports and receipt of chocolate confectionery wrappers, those allocated to the chocolate group showed no apparent excessive consumption of chocolate confectionery. In the dietary management of hyperlipidemia, rapport between the dietitian and patient is one of

Table 5 The effect of diet on plasma triacylglycerol and cholesterol concentrations (mmol/L) in subject with apo E3/E3 phenotype

	Control group	Chocolate group
Plasma triglycerides		
Initial	2.1 ± 1.3	2.2 ± 0.8
Final	2.4 ± 1.3	2.2 ± 0.5
Plasma cholesterol		
Initial	6.7 ± 0.7	7.1 ± 1.1
Final	6.5 ± 0.8	6.7 ± 0.9
LDL		
Initial	4.4 ± 0.8	4.8 ± 1.1
Final	4.2 ± 0.8	4.5 ± 0.9
HDL		
Initial	1.3 ± 0.3	1.3 ± 0.1
Final	1.2 ± 0.3	1.2 ± 0.2
P-value (paired)	0.004	0.040

Data shown as mean ±SD of 12 subjects per group. No significant differences between the control and chocolate groups (unpaired). LDL–low density lipoprotein. HDL–high density lipoprotein.

the key aspects of a successful outcome. The dietitian was able to establish rapport with all patients enrolled in the study. Although the study was not designed to test the level of satisfaction with treatments, it was apparent that the majority of subjects allocated to the chocolate group enjoyed chocolate.

The goals of dietary therapy were similar for subjects in the control group to those in the chocolate group. Both groups reached those goals by showing a reduction in the intake of saturated fat. The reduction (as a percent energy) was marginally better for those allocated chocolate confectionery in their diet compared with controls. The reduction in saturated fat in subjects allocated to the chocolate group was mainly due to reductions in the intake of animal and visible fats, including butter. Concurrent with this change was a reduction in body weight, which was significant for both groups; however, the waist:hip ratio was not affected significantly. The decrease in saturated fat consumption is clearly a benefit in the management of hyperlipidemic patients[1] and represents a significant reduction in the incidence and cost of coronary heart disease.[11] No significant changes were observed in the consumption of monounsaturated or polyunsaturated fat (as a percent of energy) in subjects allocated to either group.

Diet therapy had variable effects on plasma lipid concentrations. Subjects in both the control and chocolate groups showed reductions in plasma cholesterol levels; however, the reduction in the control group reached statistical significance ($P < 0.03$). The trends observed for plasma cholesterol were reflected in the LDL cholesterol concentrations. The magnitude of the decrease in plasma cholesterol and LDL cholesterol concentrations is similar to that expected from other studies[12] and represents a significant reduction in coronary heart disease risk.[13]

In contrast, plasma triacylglycerol and HDL cholesterol concentrations were affected adversely by the control diet. Plasma triacylglycerol concentrations increased significantly in subjects in the control group but were unaffected in subjects allowed a chocolate treat. HDL cholesterol concentrations fell in both groups. This response pattern is similar to that reported previously[14] when comparing a milk chocolate bar with a high carbohydrate snack in normolipidemic subjects.

The distribution of different apo E phenotypes varied between the two groups of subjects. There was a greater representation of the E4 allele in the control group, which may render the group more responsive to dietary intervention.[5] The commonest form of apo E phenotypes is apo E3/E3, which is found in approximately 60% of the population. The representation of this phenotype in the study population was 12 in each group, which provided sufficient degrees of freedom for a subgroup analysis. Under these circumstances, the cholesterolemic response in the chocolate confectionery group was similar to that in the control group; that is, the consumption of a small amount of chocolate confectionery made no difference to the lipid responses.

There are other aspects of the introduction of a chocolate treat in the dietary management of hyperlipidemic patients that were not considered in this study. Diet therapy involves not only changes in eating pattern but requires changes in food related eating behavior.[15] Meal frequency may also affect the plasma lipids and lipoproteins; however, the status of the research precludes any recommendations regarding this issue.[16] In addition, there is a range of biomarkers that needs to be considered including those that are unrelated to cholesterol metabolism. Chocolate has been reported to contain antioxidants and their inclusion in any type of diet may protect from coronary heart disease by protecting LDL from oxidative modofication.[17] In contrast, stearic acid has been suggested to be a thrombogenic fatty acid and may increase platelet aggregation.[18] Neither of these aspects were explored in this study.

The study was conducted in a setting where current clinical guidelines specify that in patients without other cardiovascular risk factors, the threshold for pharmacologic treatment is 7 mmol/L. The effects of diet intervention are synergistic with those of drug therapy[19] and may offer additional beneficial effects, some of which may be unrelated to cholesterol metabolism. The hypothesis was not that chocolate would be highly beneficial to the patients, but rather that the inclusion of a modest amount would not detract from their response to a great extent. The importance of liberalizing their diet in this way has long-term implications for improved compliance. Improved compliance would be expected to benefit all patients, irrespective of whether or not they required drug therapy as an additional measure. In fact, improved compliance in patients who do receive medications may be an important factor in helping them to achieve treatment targets.

Overall, the inclusion of a chocolate confectionery as part of a lipid-lowering diet did not prevent the reduction in the intake of saturated fat. In that respect, the group allowed chocolate confectionery performed marginally better than the control group in their ability to reduce the intake of saturated fat from sources such as animal and visible fats including butter. However, in contrast to the control group, this reduction in saturated fat intake was not reflected in a statistically significant reduction in plasma total or LDL cholesterol. To maximize the plasma cholesterol lowering effect, the composition of the treat will need to be scruti-

nized in future studies and some consideration given to carbohydrate treats or products such as a chocolate and peanut snack, which have a higher polyunsaturated:saturated ratio than the products used in this study.

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