A High Carbohydrate versus a High Monounsaturated Fatty Acid Diet Lowers the Atherogenic Potential of Big VLDL Particles in Patients with Type 1 Diabetes[1,2]

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ABSTRACT The objective of the present study was to compare the effects of two diets on the atherogenic potential of two VLDL subfractions harvested from fasting subjects by measuring the number and composition of particles and the amount of esterified cholesterol accumulated in macrophages. A high (25%) monounsaturated fatty acid (Mono) diet and a high (61%) carbohydrate (CHO) diet were provided for 4 wk in a randomized crossover design to 19 normolipidemic, nonobese patients with type 1 diabetes. The two diets were matched for protein, polyunsaturated/saturated fatty acids, cholesterol and fiber content. The number of circulating big VLDL (S_f 100–400) particles was greater during the high Mono than during the high CHO diet based on the levels of apolipoprotein B (means ± SEM): 31.4 ± 7.4 versus 20.0 ± 3.8 mg/L ($P < 0.025$, paired t test). The following variables did not differ during the diet periods: number of small VLDL (S_f 20–100) particles, esterified cholesterol accumulated in THP-1 macrophages incubated with the same number of big and small VLDL particles and particle composition. We conclude that a high CHO diet might be preferable to a high Mono diet, on the basis of the premise that more big VLDL particles could increase the atherosclerotic risk in patients with diabetes. J. Nutr. 130: 2503–2507, 2000.

KEY WORDS: • *monounsaturated* • *carbohydrate* • *big VLDL* • *diabetes* • *humans*

Atherosclerosis is the leading cause of death in patients with type 1 diabetes above the age of 20 y (The Carter Center 1985). This holds in the presence of a normal fasting lipid profile in the majority of the patients (Howard 1987). However, other abnormalities are present in type 1 diabetes; namely, the composition of triglyceride (TG[4])-rich lipoproteins is abnormal, with the diabetic particles being cholesterol enriched and phospholipid poor compared with normal particles (Georgopoulos and Rosengard 1989). It is possible that these changes can increase the atherogenic potential of the diabetic particles (Georgopoulos et al. 1994).

The hallmark of early atherosclerotic lesions is foam cell formation through lipid accumulation in macrophages, after the uptake of apolipoprotein (apo)B-containing lipoproteins (Bierman 1992). In addition to LDL and lipoprotein(a), VLDL were recently isolated from human atherosclerotic lesions (Rapp et al. 1994).

Esterified cholesterol (EC) accumulation in macrophages is characteristic of foam cell formation. We therefore used EC accumulation in THP-1 macrophages incubated with VLDL subfractions as an in vitro model of foam cell formation. THP-1 macrophages, a human monocytic cell line, has been used by us (Georgopoulos et al. 1994) and others (Auwerx et al. 1988, Hara et al. 1987) as an in vitro model. In previous studies, we (Georgopoulos et al. 1994) and others (Klein et al. 1989) have reported that EC accumulation by macrophages is increased in a dose-dependent manner when the cells are incubated with increasing amounts of TG-rich lipoproteins isolated from the plasma of patients in the fasting and postprandial state who have type 1 diabetes versus lipoproteins isolated from age- and sex-matched normal subjects.

Because the particle number and composition and the amount of EC accumulated in macrophages could affect the VLDL-related atherosclerotic risk in type 1 diabetes, we measured all of these variables in the present study to determine whether there is a difference between a high monounsaturated fatty acid (Mono) diet and a high carbohydrate (CHO) diet on the atherogenic potential of VLDL subfractions isolated from fasting patients with type 1 diabetes.

SUBJECTS AND METHODS

Study subjects and experimental protocol. We studied 19 subjects (11 men and 8 women) with type 1 diabetes (based on

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[4] Abbreviations used: apo, apolipoprotein; CHO, carbohydrate; EC, esterified cholesterol; TG, triglycerides.

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accepted criteria of the American Diabetes Association). Their clinical characteristics were as follows. the body mass index was normal (mean ± SD 24.5 ± 2.1 kg/m^2 body surface); similarly, they had a normal lipid profile and normal liver, kidney, thyroid and hematologic variables and proteinuria of <300 mg/24 h. Their mean age was 29 ± 8.6 y (age range 22–47 y), and their duration of diabetes was 13 ± 7.2 y (range 2–28 y). None of the subjects were taking medications that affect lipoprotein metabolism other than insulin and, in 2 women, oral contraceptives. The lipoprotein measurements in all women were made at the same time of their menstrual cycle during both dietary periods. Patients remained on the same dose of medications other than insulin throughout the study. None of the study participants used alcohol on a regular basis. All participating patients signed the consent form, which complied with the revised Helsinki Declaration and was approved by the institutional review board. The patients maintained steady levels of exercise throughout the study and were asked to keep records of their blood glucose and insulin requirements. A randomized crossover design was used, with each dietary period lasting 4 wk. All food was prepared in the metabolic kitchen of the Minneapolis VA Clinical Research Center and provided to the participants. A 3-d rotating menu plan was used. Prepared extra snacks were provided for periods of exercise or episodes of hypoglycemia. The high Mono diet contained 40% total fatty acids (25% Mono, 6% polyunsaturated and 9% saturated), 45% CHO and 15% protein. The high CHO diet contained 24% total fatty acids (9% Mono, 6% polyunsaturated and 9% saturated), 61% CHO and 15% protein. The polyunsaturated/saturated fatty acid ratio of both diets was 0.67. All other nutrients, including cholesterol (300 mg/d) and fiber (28–30 g/d), were the same in both diets. Nutritional analysis of the menus used in the two diets was carried out with the VA nutrition software program and verified by the National Nutrition Coordinating Center (Minneapolis, MN). Glycemic control was evaluated by measurements of fructosamine [RoTAG; Roche Diagnostic Systems (Johnson et al. 1982)] and hemoglobin A_1c measurements (Glyc-Affin; Isolab Inc., Akron, OH). Blood glucose records were reviewed once or twice per week, and adjustments were made in insulin dose or time and energy distribution of meals to avoid frequent hypoglycemia or sustained blood glucose levels of >13.8 mmol/L. Patients visited the center two or threes times per week to be weighed and pick up their meals. Subjects were asked to abstain from alcohol during the last 10 d of each diet period. During the last week of each dietary period, three blood samples for determination of fasting (12 h) blood glucose and plasma lipid (total, LDL and HDL cholesterol, TG) and apoA-I and apoB levels were obtained from each subject. The clinical, lipoprotein and apoB data for 11 of 19 subjects in this study have also been included in another report in which the differences were compared between the high Mono and the high CHO diets on the metabolism of TG-rich lipoproteins isolated from the plasma in the postprandial and fasting state in patients with type 1 diabetes (Georgopoulos et al. 1998).

Handling of blood samples. To avoid lipoprotein degradation and lipid oxidation, blood samples for lipoprotein analysis were collected in tubes containing EDTA (1 g/L) and placed on ice. Plasma was separated by centrifugation at 10°C, and a 1:100 dilution of a solution containing 1 g/L DTPA, 120 g/L ϵ-aminocaproic acid, 50 g/L glutathione, 10 g/L thimerosal and 10 g/L butylated hydroxytoluene was added. DTPA has been shown to be a more potent antioxidant than EDTA (Heinecke et al. 1986). VLDL subfractions were analyzed within 1–2 wk.

Determination of fasting lipid and lipoprotein variables. Plasma TG levels were measured enzymatically with a commercially available kit (catalogue no. 816370; Boehringer-Mannheim Diagnostics, Indianapolis, IN). The coefficient of variation of these assays in our laboratory is 4%. Plasma total and HDL cholesterol levels were measured enzymatically, the latter after heparin-manganese precipitation according to the Lipid Research Clinics protocol. Both apoB and apoA-I were determined nephelometrically with commercially available kits (Catalogue no. 86071 and 86070 for apoB and apoA-I, respectively; Incstar, Stillwater, MN). These measurements were performed in the VA laboratory and were made under quality control testing with the Northwest Lipid Research Laboratory in Seattle, WA.

Isolation of TG-rich lipoproteins. All isolations were performed under aseptic conditions within 48–72 h from harvesting of plasma by salt density gradient ultracentrifugation with an SW 28 rotor according to the method of Lindgren as modified by Redgrave and Carlson (1979) and as described previously (Georgopoulos et al. 1994). Two subfractions were isolated: S_f 100–400 (31.2×10^6 g · min^{-1}) containing big VLDL and S_f 20–100 (152.0×10^6 g · min^{-1}) containing mostly small VLDL. Each subfraction was collected under aseptic conditions, dialyzed against Tris-EDTA (or Tris-DTPA) buffer at 4°C, concentrated with Aquacide, filtered and stored at 4°C for use in tissue culture experiments within 1–2 wk.

The apoB determinations in the TG-rich lipoprotein subfractions were performed by electroimmunoassay recognizing both apoB-100 and apoB-48 (Koren and Alaupovic 1991) in Dr. Alaupovic's laboratory (Oklahoma Medical Research Foundation, Oklahoma City, OK). The coefficient of variation of the assay was 5%.

Chemical composition of the isolated TG-rich lipoproteins. The total protein concentration of the lipoproteins was determined according to a modification of the Lowry method (Lowry et al. 1951). Free cholesterol and EC were measured by gas-liquid chromatography after lipid extraction as described later (Ishikawa et al. 1974). The EC values were multiplied by 1.68 to account for the fatty acid mass. Phospholipid phosphorous was determined according to the method of Bartlett (1979) and multiplied by 25 to obtain phospholipid mass.

Cell culture. THP-1 monocyte/macrophage cells were obtained from American Type Culture Collection (Rockville, MD). The cells were grown in suspension in RPMI-1640 containing 100 mL/L fetal calf serum, 100 mg/L streptomycin, 10^5 U/L penicillin and 250 mg/L fungizone-containing amphotericin as described previously (Georgopoulos et al. 1994). Cells were maintained at 0.5–1 × 10^9 cells/L by pelleting the cells twice weekly and completely changing the medium. Forty-eight hours before use, the cells were seeded (1–2 × 10^6 cells/35-mm culture dish) and induced to differentiate into macrophages by the addition of 1×10^{-7} mol/L phorbol-12-myristate-13-acetate as described by others (Auwerx et al. 1988, Hara et al. 1987). The cells were washed twice with phosphate-buffered saline before incubation with the lipoproteins for 48 h. The apoB concentration used was 50 mg/L for both VLDL. The incubation medium consisted of lipoproteins in RPMI without fetal calf serum or albumin.

Mass measurement of cellular cholesterol. The masses of free cholesterol and EC were determined by gas-liquid chromatography according to the method of Ishikawa et al. (1974). The lipid extracts were dried under a nitrogen stream, resolubilized and injected into the gas chromatograph at 270°C using an HP-17 cross-linked 50% phenylmethylsilicone capillary column and a helium flow of 30 mL/min. Stigmasterol was used as an internal standard. The areas under the curves were calculated, and the mass of EC was derived from the subtraction of free cholesterol from total cholesterol after saponification.

Statistical analysis. The effects of the diets on lipoproteins and clinical variables, including glycemic control, and on apoB in VLDL subfractions were compared by paired *t* test (Snedecor and Cochran 1989).To compare the effects of lipoprotein subfractions on free cholesterol and EC mass accumulation in macrophages, each lipoprotein subfraction was analyzed in triplicate in every experiment; control cells (without lipoproteins) were also included. The triplicate results were averaged and were analyzed by an analysis of covariance with the BMDP statistical package (1992 BMDP; Dynamic Statistical Software, Los Angeles, CA). EC accumulation in the control wells was used as a covariate to account for the variability of the cells within experiments. Finally, to evaluate differences in the composition of diabetic and normal lipoproteins, a log ratio analysis of compositions was performed as described previously (Georgopoulos et al. 1994).

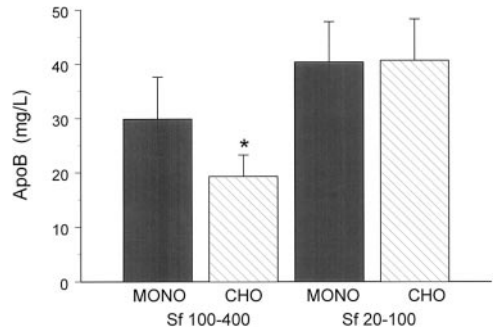

FIGURE 1 Effect of a 4-wk randomized crossover study of a high (25%) monounsaturated fatty acid (Mono)/45% carbohydrate (CHO) diet versus a high (61%) CHO/9% Mono diet on apolipoprotein B concentrations in big VLDL (S_f 100–400) and small VLDL (S_f 20–100) subfractions isolated from plasma of fasting patients with type 1 diabetes. *P = 0.02, paired t test. Values are means ± SEM (n = 19).

RESULTS AND DISCUSSION

EC accumulation in macrophages has been used as an in vitro model of foam cell formation. It has been shown, both by us (Georgopoulos et al. 1994) and by others (Klein et al. 1989, Lindqvist et al. 1983), that the degree of cellular EC accumulation after the uptake and degradation of a TG-rich lipoprotein depends on the concentration of the lipoprotein incubated with the cells. Moreover, at the same concentration, TG-rich lipoproteins from diabetics compared with those from control subjects result in a higher EC accumulation in macrophages (Georgopoulos et al. 1994, Klein et al. 1989, Kraemer et al. 1985). Therefore, the atherogenic potential of a given intervention (i.e., diet) depends on the concentration and corresponding number of the lipoprotein particles and on the degree of cellular EC accumulation that they cause for a given concentration, which is possibly related to particle composition.

In the present study, we assessed these variables by comparing the effect of a high CHO versus a high Mono diet on the atherogenic potential of VLDL subfractions from fasting subjects. Our results show that the particle number, estimated by measuring apoB concentration in the big VLDL particles (S_f 100–400), was higher during the high Mono diet than during the high CHO diet (P = 0.02) (**Fig. 1**). This was not the case for the small VLDL particles (S_f 20–100). We contend that the difference in the big VLDL particle number between the two diet periods is the result of dietary differences, because other confounding factors, like weight, insulin dose, glycemic control and fasting lipid profiles (including LDL, HDL and apoB and apoA-I), that could account for the observed differences were similar at the end of the two dietary periods (**Table 1**). This finding is consistent with our previous study (Georgopoulos et al. 1998), in which compared with the high Mono diet, the high CHO diet was associated with fewer postprandial lipoprotein particles of both hepatic and intestinal origin. The mechanism for the observed difference in big VLDL particle number is unclear. It could be due to decreased production or increased clearance of big VLDL during the high CHO diet versus the high Mono diet. As reviewed by Krauss (1998), in addition to IDL and very small VLDL (S_f 12–60), big VLDL particles are considered to be atherogenic. Animal studies have also shown that the retention of lipoprotein particles is greater for bigger VLDL particles (Nordestgaard 1996). Human turnover studies have supported the hypothesis that accumulation in the circulation of bigger VLDL particles (S_f 100–400) can lead to the generation of a series of atherogenic lipoproteins, including IDL and dense LDL (Packard and Shepherd 1997). Moreover, two recent studies in which nuclear magnetic resonance spectroscopy was used have shown that large VLDL particles are associated with more angiographically verified atherosclerosis (Freedman 1998) and a higher atherosclerotic risk (Framingham Offspring Study; Otvos 1999).

We studied the EC accumulation in macrophages during the two diet periods by using the same apoB concentration to match particle numbers. Because of inherent cellular variability between experiments, ideally the VLDL subfractions isolated from the plasma samples from each patient after the

TABLE 1

Effect of a 4-wk randomized crossover study of a high (25%) monounsaturated fatty acid/45% carbohydrate diet versus a high (61%) carbohydrate/9% monounsaturated fatty acid diet on clinical variables of patients with type 1 diabetes

	High monounsaturated fatty acid diet	High carbohydrate diet
Weight, *kg*	72.4 ± 11.1	72.3 ± 11.1
Insulin dose, *U/24 h*	47 ± 15	48 ± 15
Glycohemoglobin, *%*	9.4 ± 2.2	9.5 ± 2.1
Fructosamine, *μmol/L*	353 ± 64.3	357 ± 64.4
Total cholesterol, *mmol/L*	3.76 ± 0.64	3.81 ± 0.62
Triglycerides, *mmol/L*	0.89 ± 0.39	0.90 ± 0.36
HDL cholesterol, *mmol/L*	1.27 ± 0.29	1.20 ± 0.25
LDL cholesterol, *mmol/L*	2.12 ± 0.53	2.16 ± 0.60
Apolipoprotein A-I, *mg/L*	1504 ± 276	1518 ± 320
Apolipoprotein B, *mg/L*	751 ± 169	777 ± 165

[1] Values are means ± SD (n = 19).
[2] Measurements were performed at the end of each diet period.

TABLE 2

Esterified cholesterol accumulation in THP-1 macrophages incubated with equal number of big VLDL (S_f 100–400) and small VLDL (S_f 20–100) subfractions, isolated from plasma of fasting patients with type 1 diabetes after a 4-wk randomized crossover study of a high (25%) monounsaturated fatty acid/45% carbohydrate diet versus a high (61%) carbohydrate/9% monounsaturated fatty acid diet[1]

Subfraction	High monounsaturated fatty acid diet	High carbohydrate diet
	mmol/mg cell protein	
S_f 100–400	130.3 ± 27.4	187.2 ± 37.8
S_f 20–100	139.6 ± 30.8	147.7 ± 35.4
Control[2]	97.2 ± 35.9	80.4 ± 92.3

[1] Values are means ± SEM (n = 18).
[2] No lipoprotein added.
[3] At the end of each diet period, both VLDL subfractions from a given patient were incubated with cells in triplicate in the same experiment. Triplicates of a no-lipoprotein control were included in every experiment; the results were analyzed by analysis of covariance (BMDP 5V statistical package), with the control used as a covariate to account for the within-experimental variability between the cells.

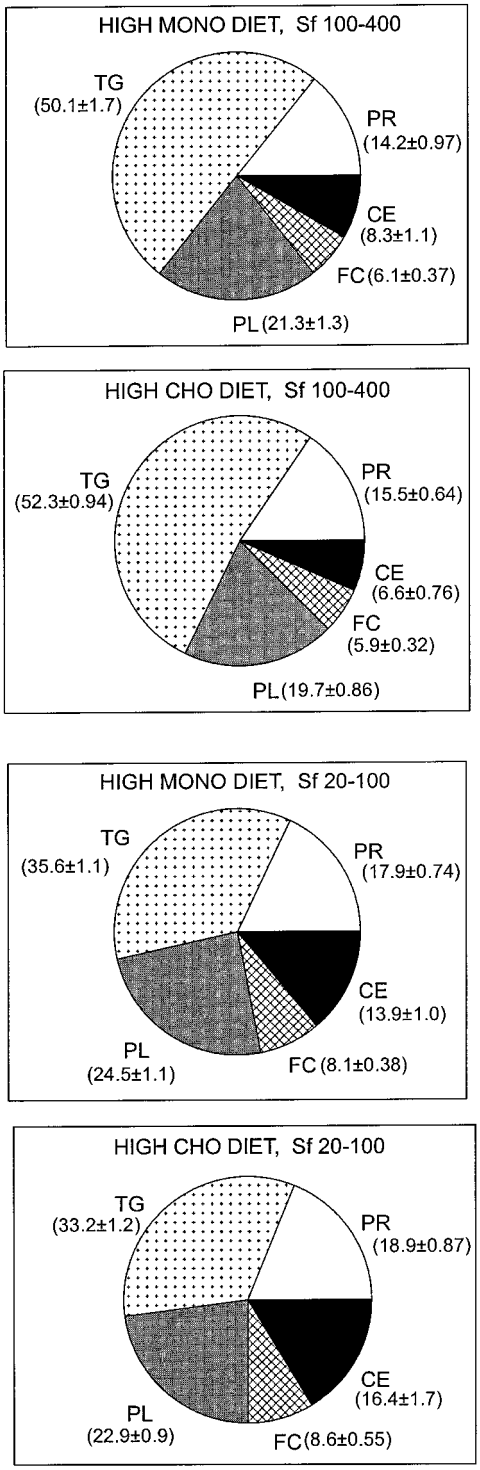

FIGURE 2 Effect of a 4-wk randomized crossover study of a high (25%) monounsaturated fatty acid (Mono)/45% carbohydrate (CHO) diet versus a high (61%) CHO/9% Mono diet on the composition of big VLDL (S_f 100–400) and small VLDL (S_f 20–100) subfractions isolated from plasma of fasting patients with type 1 diabetes. Log ratio analysis of composition showed no significant differences between the diet periods. PR, protein; CE, cholesteryl esters; FC, free cholesterol; PL, phospholipid. Values in parentheses represent mean ± SEM percentages (n = 19).

consumption of both diets should have been incubated simultaneously in the same experiment. However, this could not be done because the TG-rich lipoproteins are unstable and their composition is altered if stored beyond 2 wk (unpublished observations). Therefore, to take into account the cellular variability between experiments, we used the amount of EC accumulated in no-lipoprotein control subjects (**Table 2**) as a covariate in the analysis of the results. We found that this covariate had a significant effect on cellular accumulated EC. After accounting for its effect by analysis of covariance, there was no difference in the effect of the diets on the amount of cellular accumulated EC (Table 2).

To assess possible differences between the diets in particle composition for each isolated subfraction, we calculated the log ratio of the particle components (protein, TG, phospholipid, free cholesterol and cholesteryl esters). There were no differences in the particle composition of either VLDL subfraction between the dietary periods (**Fig. 2**). The lack of difference in the particle composition between the diets could have accounted for the observed lack of difference in the degree of cellular EC accumulation. In previous studies, differences in TG-rich lipoprotein composition were associated with the greater degree of EC accumulation in macrophages incubated with diabetic versus normal TG-rich lipoproteins (Georgopoulos et al. 1994).

We conclude that in normolipidemic, nonobese fasting patients with type 1 diabetes, a high CHO diet versus a high Mono diet results in fewer circulating big VLDL particles and therefore perhaps a lower atherogenic potential.

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