One-Year Effects of Increasingly Fat-Restricted, Carbohydrate-Enriched Diets on Lipoprotein Levels in Free-Living Subjects (44564E)

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Abstract. Restriction of all dietary fat is a popular strategy for restricting saturated fat intake to lower LDL cholesterol. Some authorities advise the restriction of fat intake to the extreme of less than 10% of daily energy on the assumption that more fat restriction is better. The two studies described herein address questions relating to whether increasing fat restriction produces proportionally increasing benefit on cardiovascular risk factors in hyperlipidemic subjects.The first study is the Dietary Alternatives Study (DAS). The DAS was conducted in 531 male Boeing employees over a 2-year period. Subjects were defined as hypercholesterolemic (HC) or combined hyperlipidemic (CHL) based on age-specific 75th percentiles for plasma LDL-C and triglyceride levels. Hypothesis test analyses were performed at 1 year. HC subjects were randomized to diets taught to attain fat intakes of 30, 26, 22, and 18% (Diets levels 1–4, respectively). CHL subjects (slightly fewer in number) were randomized to Diets 1–3. After 1 year, subjects' total fat intakes were 27, 26, 25, and 22% of energy (en%), resulting in saturated fat intakes of 8, 7, 7, and 6%, respectively. In HC subjects the greatest LDL-C decrease was with Diet 2 (mean of 13.4%) and in CHL subjects with Diet 1 (7.0%). Surprisingly, plasma triglyceride concentrations rose in HC subjects 20% and 40% above baseline on Diets 3 and 4, respectively, with reciprocal reductions in HDL cholesterol of 2.5% and 3%, respectively. Furthermore, apo B reductions were attenuated below Diet 2 in HC subjects and Diet 1 in CHL subjects, and no further reductions were seen in plasma glucose and insulin concentrations, blood pressure, or body weight. Measurements of plasma total fatty acid composition showed a slight increase in plasma palmitate, whereas stearate decreased slightly, supporting the idea that *de novo* synthesis of palmitic acid was increased in the chronic high-carbohydrate feeding condition. The second study asked if the most effective diet in HC subjects, Diet 2, has an equivalent effect in women and men. To answer this question, men and women Boeing employees were taught the closely similar National Cholesterol Education Program (NCEP) Step II diet. After 6 and 12 months, equivalent reductions in LDL cholesterol were observed in women compared with men. HDL cholesterol levels in men were unchanged from baseline at 6 and 12 months, but were reduced 8% in HC women, with accompanying decreases of 18% in HDL2-cholesterol and 5% in apoprotein A-I (all $P < 0.01$).

These data indicate that intakes of fat below about 25 en% and carbohydrate intake above ≈ 60 en% yield no further LDL-C lowering in HC and CHL male subjects and can be counterproductive to triglyceride, HDL-C, and apo B levels. This lack of benefit appears to be explained by an enhanced endogenous synthesis of palmitic

This work was supported by the National Institutes of Health (NIH) grants HL28891 and HL-44878 from the National Heart, Lung, and Blood Institute, the Clinical Nutrition Research Unit #DK-35816, the Diabetes and Endocrinology Research Center #DK-17047, and a gift from the Robert B. McMillen Family Trust.

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0037-9727/00/2253-0191$15.00/0

acid, which negates the benefit of further saturated fat restriction. The HDL-C decrease in HC women may have a similar cause and points to an underlying male-female difference. Alternative dietary approaches to limit saturated fat intake deserve intensive study.
[P.S.E.B.M. 2000, Vol 225:191–199]

Restriction of dietary saturated fatty acids to reduce LDL cholesterol is central to the dietary management of hyperlipidemia (1, 2). A simple approach to this dietary goal is to restrict total fat intake. Thus the National Cholesterol Education Program (NCEP) advises restriction of total fat intake to less than 30% of calories as does the national advisory, Healthy People 2000, and Nutrition Goals for 2010 (2–4). However, these diets have been criticized because of their relative lack of efficacy (5).

In an effort to render low-fat diets more effective, some propose restricting total fat intake to less than 10% of calories on the theory that more fat restriction is inherently better (6–8). Little has been done to test the efficacy of these diets objectively in their own right apart from concomitant interventions. For instance, Ornish *et al.* (7, 8) reported a reduction in coronary artery disease in individuals ingesting a 10% or less fat diet and undergoing marked weight loss and a meditation program compared with Step II Diet alone. However, it is unclear if the reduction in heart disease is due to the diet, the marked weight loss, the meditation, or some combination thereof. Limited previous studies of the dietary component alone find no further LDL lowering with extreme versus moderate fat restriction (9, 10).

An established problem with low-fat, high-carbohydrate diets is that plasma triglyceride levels rise, as originally observed by Ahrens *et al.* (11) in the early 1960s. This effect, known as carbohydrate induction might be negligible if the effect were not sustained over time (12). However, the literature is divided on this point (13). More recently it has been learned that hypertriglyceridemia alone or associated with low HDL is a risk factor for arteriosclerosis (14, 15). This risk complex appears to be more strongly associated with coronary artery disease in women compared with men (16, 17). The hypertriglyceridemia may be due to enhanced endogenous fatty acid synthesis, which could in turn negate the reduction of dietary saturated fat intake (13, 18–20). These observations raise the possibility that extreme low-fat, high-carbohydrate feeding may even reverse the antiatherosclerotic effect of the low-fat diet.

Herein we review two studies reported from the Northwest Lipid Research Clinic addressing these questions. The first was performed in a cohort of 531 male Boeing employees to test the effect of four different levels of increasing fat restriction and carbohydrate augmentation. The second study tested the effect of the National Cholesterol Education Program (NCEP) Step II Diet for the equivalency of its effect on lowering LDL cholesterol in women versus men and conjoint effects on other lipoproteins (21–25). The first study is known as the Dietary Alternatives Study (DAS), and the second study is referred to as the Boeing employees Fat Intervention Trial (BeFIT). In both studies subjects were screened at two visits for consistent LDL cholesterol elevations at or above the age and gender-specific 75th percentile. In addition, subjects were further divided into those with at least one triglyceride level at or above the age and gender-specific 75th percentile (about 175 mg/dl in men and 150 mg/dl in women). Thus two hypercholesterolemic groups were recruited, those with normal triglyceride levels (i.e., simple hypercholesterolemia (HC)), and those with elevated triglyceride levels (i.e., combined hyperlipidemia (CHL)).

Dietary Alternatives Study (DAS)

The design of the DAS is presented in Figure 1. The 531 subjects were screened at two visits for LDL and triglyceride as described above. It would be expected in stratifying subjects in this manner that there would be one-fourth as many individuals with CHL as HC ($0.25 \times 0.25 = 0.0625$). We were surprised to discover that for every four individuals identified with HC there were 2.6 individuals with CHL (320 HC subjects, 211 CHL subjects) where one would be expected (21). This observation means that in this hypercholesterolemic cohort, hypertriglyceridemia is over-represented nearly three-fold. This observation underscores the importance of CHL as a common and very important lipid disorder and is in keeping with the original observations of Goldstein and others (26) that familial combined hyperlipidemia (defined by 95th percentile cutpoints) was more common among myocardial infarction survivors than any other sporadic or familial form of hyperlipidemia in the Seattle Myocardial Infarction Study.

HC subjects were randomized to four diets targeted to contain 30, 26, 22, and 18% of fat as calories. Because of their somewhat fewer number, CHL subjects were random-

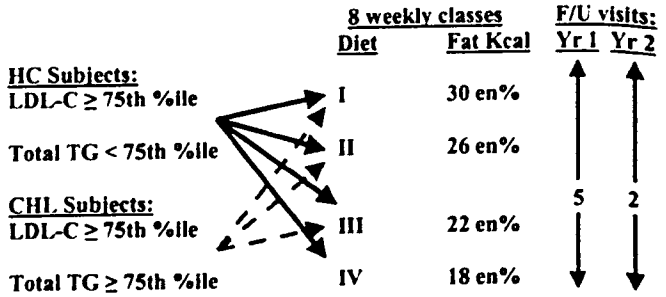

Figure 1. Design of the Dietary Alternatives Study in which 531 HC or CHL subjects were randomized into one of four (HC) or three (CHL) diets of increasing fat restriction (21). Hypothesis test analyses were performed at one year (22).

ized to only the first three diets. All subjects were seen for five visits during the first year at Months 1, 3, 6, 9, and 12. In addition, they were contacted for two reinforcement visits by a dietitian and a behavioral psychologist especially if dietary adherence declined. In the second year a less aggressive follow-up procedure consisted of return visits at 18 and 24 months without behavioral strategies or interventions. The number of subjects originally screened (10,000), the number of dropouts, and the reasons have been recorded (21). Of 531 subjects randomized into the study, 444 comprise the analysis at Year 1 with all data complete (22).

Subject Characteristics. Characteristics of the subjects are represented in Table I. The two cohorts of HC and CHL subjects analyzed after 1 year of the diet, consisted of 270 and 174 subjects, respectively. Their ages and heights were equivalent. The CHL subjects were slightly heavier, and their body mass index was slightly but statistically significantly greater. There were equivalent numbers of smokers (which were few) and alcohol users (which were average), and the majority of subjects were college graduates. More than 85% of both groups reported moderate exercise.

Baseline lipids differed by definition in plasma triglyceride levels, averaging 101 mg/dl in the HC group and 186 mg/dl in the CHL group, the result of dichotomizing subjects below and above the 75th percentile for triglyceride (Table I). The mean LDL cholesterol concentrations were almost identical at 176 and 177 mg/dl, respectively, in the two groups. HDL cholesterol levels were slightly above the male average at 49.9 in the HC group and slightly below the male average at 42.9 mg/dl in the CHL group (27). Plasma glucose concentrations were normal in both groups but were 4 mg/dl higher in the CHL group, a significant difference. Insulin concentrations likewise were higher in the CHL groups, as was systolic blood pressure at 127 vs 121 mmHg, respectively.

Dietary Regimen Adherence. Dietary attainment at 1 year is described in Table II for the HC and CHL groups. Baseline total fat intake ranged from 34%–37% across the four categories in the HC subjects and the three categories in the CHL subjects. Saturated fat intake averaged 12%–15% at baseline in both groups. Total fat was restricted to 27, 26, 25, and 22% in Diets 1–4, as estimated by 4-day food records. The restriction of saturated fat intake was proportionally successful, with ingestion of 8, 7, 7, and 6% of calories as saturated fat in the four HC dietary groups and with similar attainment in the three CHL dietary groups. Correspondingly, dietary cholesterol intake was reduced from a range of 301–347 mg/day to 238, 164, 137, and 136 mg/day in the four HC diet groups. Similar reductions were seen in the three CHL diet groups (Table II). Subjects were encouraged to augment their fiber and, in particular, their soluble fiber intake by augmenting vegetable and fruit intake (21). These intakes were progressively greater in the four dietary categories increasing from 20–23g/day at baseline to 27, 29, 32, and 33g/day in the four HC diet groups. Body weight reductions were 2–3 kg in all groups (Table II) (see correction in footnote).

Lipoprotein Results in HC Subjects. The percentage changes in lipoprotein levels and body weight in HC subjects are shown in Figure 2. Mean total and LDL cholesterol levels were reduced a maximum of 10 and 13.4% with Diet 2. No further reductions in total or LDL cholesterol were seen with Diets 3 or 4. The median cholesterol decreases with Diet 2 were slightly higher, 12% for total cholesterol and 15.4% for LDL cholesterol (data not shown). Mean percentage changes in triglyceride concentrations are also shown in Figure 2. Diets 1 and 2 were

Table I. Characteristics of Subjects with Simple Hypercholesterolemia (HC) Versus Combined Hyperlipidemia (CHL)

	Mean ± SD		*P*
	HC	CHL	
(*n*)	(270)	(174)	
Age (yrs)	47 ± 10[a]	47 ± 10	ns
Height (cm)	177 ± 7	177 ± 7	ns
Weight (kg)	82 ± 12	87 ± 15	<0.001
BMI (kg/m^2)	26.1 ± 3.0	27.8 ± 3.9	<0.001
Smokers (%)	5.6	6.9	ns
Alcohol users (%)	43.3	51.7	ns
College grads (%)	74.1	71.8	ns
Reported moderate exercise (%)	86.5	89.1	ns
Triglyceride (mg/dl)	101 ± 46	186 ± 85	<0.001
LDL-C (mg/dl)	176 ± 27	177 ± 27	ns
HDL-C (mg/dl)	49.9 ± 9.7	42.9 ± 8.5	<0.001
Glucose (mg/dl)	89.3 ± 9.7	93.1 ± 9.9	<0.001
Immunoreactive insulin (μU/ml)	15.6 ± 10.7	20.8 ± 10.5	<0.001
Systolic blood pressure (mm Hg)	121 ± 12	127 ± 15	<0.001
Diastolic blood pressure (mm Hg)	77 ± 9	81 ± 9	<0.001

Note. Total cholesterol in HC and CHL is defined as ≥75th age-specific percentile. Plasma triglyceride in HC is <75th age-specific percentile and in CHL is ≥75th age-specific percentile. Data from Ref. 22.

Table II. Changes in Dietary Intake 0–12 Months: DAS

Diet (*n*)	Mean ± SD						
	HC Subjects				CHL Subjects		
	I (78)	II (62)	III (71)	IV (59)	I (57)	II (55)	III (62)
Total fat (en%)							
Month 0	36 ± 6	36 ± 6	35 ± 6	35 ± 6	36 ± 6	36 ± 7	34 ± 7
Month 12	27 ± 5[a]	26 ± 5[a]	25 ± 6[a]	22 ± 6[a]	28 ± 6[a]	26 ± 6[a]	25 ± 6[a]
Saturated fat (en%)							
Month 0	12 ± 3	13 ± 7	12 ± 3	12 ± 3	12 ± 3	12 ± 3	12 ± 3
Month 12	8 ± 2[a]	7 ± 2[a]	7 ± 2[a]	6 ± 2[a]	8 ± 2[a]	7 ± 2[a]	7 ± 2[a]
Cholesterol (mg/day)							
Month 0	347 ± 156	325 ± 149	301 ± 132	314 ± 134	321 ± 129	319 ± 129	323 ± 162
Month 12	238 ± 82[a]	164 ± 51[a]	137 ± 50[a]	136 ± 78[a]	244 ± 95[a]	170 ± 108[a]	136 ± 50[a]
Fiber (grams/day)							
Month 0	22 ± 8	20 ± 6	23 ± 9	20 ± 6	19 ± 7	20 ± 7	21 ± 7
Month 12	27 ± 9[a]	29 ± 10[a]	32 ± 12[a]	33 ± 15[a]	24 ± 8[a]	27 ± 10[a]	32 ± 11[a]
Body weight (kg)							
Month 0	82 ± 13	82 ± 10	82 ± 12	81 ± 13	89 ± 14	88 ± 17	85 ± 14
Month 12	79 ± 12[a]	79 ± 11[a]	80 ± 12[a]	79 ± 14[a]	87 ± 14[b]	85 ± 16[b]	82 ± 14[a]

Note. Data from Ref. 22 with errors in mean body weights in CHL Diet 2 and 3 subjects in that publication corrected.
[a] $P < 0.01$ vs Month 0, [b] $P < 0.05$ vs Month 0.

without any effect on plasma triglyceride concentrations, but Diet 3 increased plasma triglyceride 22% and Diet 4 by 39%, both statistically significant increases. Statistically significant reductions in HDL cholesterol were also observed at the more extreme Diets 3 and 4, 2.3% and 2.7%, respectively. In contrast, a slight increase in HDL cholesterol was seen with Diet 1.

Plasma apo B levels reflect changes in plasma triglyceride-rich lipoproteins (VLDL) as well as LDL. Reductions of 8% and 10% were observed in Diets 1 and 2, but reductions were only 4% and 5%, respectively, in Diets 3 and 4, consistent with the increase in triglyceride-rich lipoprotein concentrations and the lesser decreases in LDL cholesterol. No significant differences or trends were seen in body weight reduction from Diets 1 through 4, with the reductions ranging from 2%–3.5%.

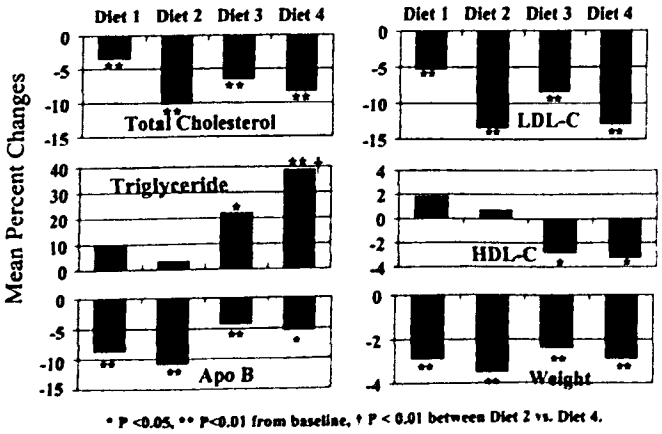

Figure 2. Effects of four levels of progressive dietary fat restriction in HC subjects on plasma lipoprotein lipids, apoprotein B, and body weight after 1 year compared to baseline (22).

Lipoprotein Results in CHL Subjects. The effects of fat-restricted diets in the three CHL diet groups are shown in Figure 3. No benefit of further fat restriction was seen in either total or LDL cholesterol levels below Diet 1. In fact, LDL cholesterol reductions tended to be less in Diets 2 and 3 than in the less fat-restricted Diet 1. However, no induction of hypertriglyceridemia was observed in the three CHL dietary groups, with the greatest triglyceride rise of 24% being seen in Diet 1. Also in contrast to the HC diet groups, HDL cholesterol levels tended to rise with increasing fat restriction, paralleling a trend of increasing weight loss in Diets 1–3, though there were no significant differences among the three groups. Despite the absence of a carbohydrate-induced hypertriglyceridemia in these subjects, apo B reductions were less in Diets 2 and 3 compared with Diet 1 as a trend, consistent with the lesser LDL-C decreases in Diets 2 and 3 versus Diet 1.

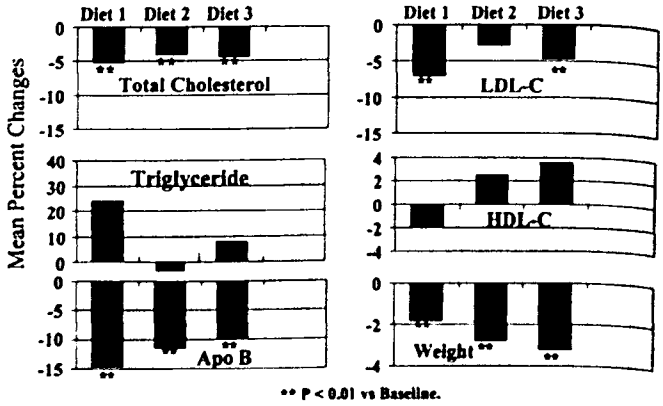

Figure 3. Effects of three levels of progressive dietary fat restriction in CHL subjects on plasma lipoprotein lipids, apoprotein B, and body weight after 1 year compared to baseline (22).

Effects on Carbohydrate Metabolism and Blood Pressure. The effects of study diets on carbohydrate metabolism and blood pressure are shown in Table III. A trend toward lower plasma glucose concentrations was observed in HC subjects with a significant decrease in the Diet 1 group. Reductions in plasma glucose concentrations were less consistent in CHL subjects, with a nonsignificant reduction in the Diet 2 group. Consistent with the significant reduction in glucose in the HC Diet 1 subjects, the greatest plasma immunoreactive insulin reduction (3.6 μU/ml) was also seen in this group ($P < 0.05$). Plasma insulin levels were progressively less reduced with increasing dietary fat restriction (Diets 2 and 3), and with Diet 4 a slightly greater immunoreactive insulin level was observed. None of these between-diet differences is statistically significant, but the insulin/glucose ratio in HC Diet 1 subjects is significantly lower than HC Diet 4 ($P = 0.012$). In contrast to the trend in HC subjects, significant reductions in plasma insulin levels were observed in the CHL subjects ingesting Diets 2 and 3. Thus, the carbohydrate metabolism response to diet differed in HC and CHL subjects, as did lipid metabolism.

Reductions in systolic and diastolic blood pressure were observed in all dietary groups of HC and CHL subjects. The reductions were all approximately equivalent from baseline to 1 year in both HC and CHL subjects. No trend of increasing blood pressure reduction was observed with increasing fat restriction.

Carbohydrate Induction Is Sustained Over 2 Years. To determine if ingestion of a consistently high level of carbohydrate intake is associated with a sustained elevation of plasma triglyceride concentrations over time, DAS subjects were selected for a consistent carbohydrate intake over 3, 12, and 24-month periods within four calorie ranges: <45%, 45%–54.9%, 55%–59.9%, and ≥60% (23). As shown in Figure 4, significantly elevated plasma triglyceride concentrations were observed at 3, 12, and 24 months among HC subjects ingesting a carbohydrate intake greater than 60% of calories, which corresponds to a fat intake less than 25% of calories. A mirror image of this effect was seen in CHL subjects (Fig. 4). At 3 months, the least reduction in plasma triglyceride concentrations was seen in the CHL group eating an excess of 60% of calories as carbohydrate. Similar trends were seen at 12 and 24 months (Fig. 4), but at 12 months the CHL group now had regained their hypertriglyceridemia and at 24 months had exceeded the baseline level, with the exception of the group with the lowest carbohydrate intake (<45% carbohydrate, >40% fat intake). Thus, evidence of carbohydrate induction was seen in both CHL and HC subjects at 24 months, but with the induction threshold set at a much lower level of carbohydrate intake in CHL subjects (45% of calories as carbohydrate). These data suggest that the CHL subjects are already inherently carbohydrate induced, even at average levels of carbohydrate intake and may help explain anecdotal observations of improved lipid profiles with very high-fat diets in such subjects. The data show that carbohydrate induction is sustained for as long as the diet is sustained.

Plasma Fatty Acid Composition. Changes in percentage total plasma fatty acid composition are shown in Table IV. Some of these data have been previously published (22). The plasma fatty acid concentrations as palmitic acid were 20% of all fatty acids in the HC group and 21% of all fatty acids in the CHL group. This difference is statistically significant, meaning that a greater endogenous reservoir of palmitate, the primary fatty acid synthesized by the body, is present in CHL subjects. After 12 months of dietary intervention, no significant change was seen in percentage palmitic acid content in HC or CHL subjects. The stable percentage palmitic acid content with low-fat diet contrasts

Table III. Effects of Four Levels of Fat Restriction on Glucose, Insulin, and Blood Pressure

Diet	Mean ± SD						
	HC Subjects				CHL Subjects		
	I	II	III	IV	I	II	III
Glucose (mg/dl)							
Month 0	90 ± 8	90 ± 9	90 ± 9	88 ± 13	90 ± 9	95 ± 11	92 ± 10
Month 12	88 ± 8[a]	90 ± 8	88 ± 8	86 ± 7	90 ± 8	94 ± 13	92 ± 10
Insulin (μU/ml)							
Month 0	17.7 ± 12.3	15.6 ± 14.4	15.1 ± 7.2	13.1 ± 6.3	20.2 ± 11.6	21.3 ± 9.5	20.6 ± 10.6
Month 12	14.1 ± 7.2[a]	13.9 ± 5.6	14.9 ± 6.0	14.6 ± 7.0	19.0 ± 8.1	17.3 ± 7.2[b]	17.7 ± 8.5[a]
Systolic blood pressure (mm Hg)							
Month 0	120 ± 13	120 ± 12	123 ± 11	122 ± 11	129 ± 18	125 ± 11	126 ± 15
Month 12	116 ± 11[b]	116 ± 11[a]	119 ± 11[b]	119 ± 12[a]	123 ± 15[a]	122 ± 16	121 ± 13[a]
Diastolic blood pressure (mm Hg)							
Month 0	77 ± 8	77 ± 9	79 ± 9	77 ± 8	83 ± 10	79 ± 9	81 ± 8
Month 12	75 ± 9[b]	75 ± 9[b]	76 ± 9[b]	75 ± 7	80 ± 10[a]	75 ± 10[b]	79 ± 9[a]

Note. Data from Ref. 22.
[a] $P < 0.05$; [b] $P < 0.01$. Differences adjusted for baseline inequality.

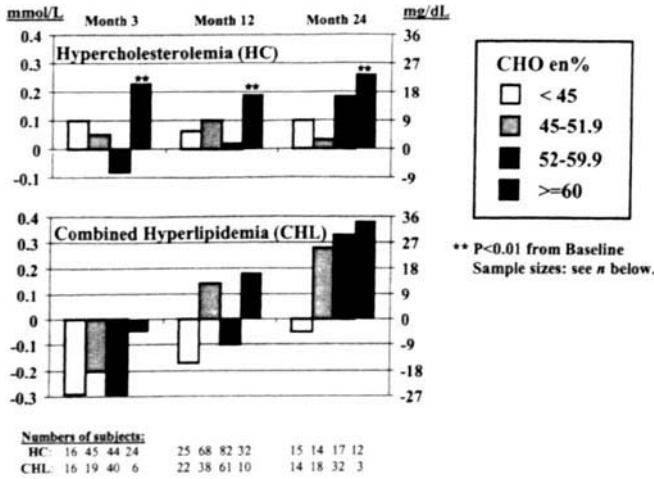

Figure 4. Effects of four levels of consistent carbohydrate intake on plasma triglyceride levels in HC and CHL subjects after 3, 12, and 24 months. (Redrawn from Ref. 23).

Table IV. Effects of Fat Restriction on Plasma Fatty Acid Composition

	Percentage of Total Plasma Fatty Acids (Mean ± SD)									
	HC				CHL			All		
Diet	I	II	III	IV	I	II	III	HC	CHL	*P*
(*n*)	(75)	(59)	(71)	(58)	(53)	(58)	(61)	(263)	(172)	
Palmitic										
Baseline	20.2 ± 1.8	20.1 ± 1.7	20.2 ± 1.5	19.8 ± 1.5	21.0 ± 1.9	21.0 ± 1.7	21.3 ± 1.9	20.1 ± 1.6	21.1 ± 1.8	<0.001
12 month	20.0 ± 2.1	19.7 ± 1.7	20.6 ± 2.0	20.4 ± 2.9	21.4 ± 2.3	21.9 ± 2.5	21.6 ± 2.4	20.2 ± 2.2	21.3 ± 2.4	<0.001
Stearic										
Baseline	7.3 ± 0.8	7.0 ± 0.8	7.0 ± 0.8	7.1 ± 0.8	6.8 ± 0.7	6.8 ± 0.7	6.8 ± 0.8	7.1 ± 0.8	6.8 ± 0.7	<0.001
12 month	6.6 ± 0.7[a]	6.4 ± 0.6[a]	6.3 ± 0.8[a]	6.5 ± 1.3[b]	6.3 ± 0.7[b]	6.2 ± 0.7[a]	6.3 ± 0.9[a]	6.5 ± 0.9[a]	6.3 ± 0.8[a]	<0.05
Oleic										
Baseline	19.1 ± 2.7	19.6 ± 2.5	19.5 ± 2.4	19.0 ± 2.6	21.4 ± 2.8	21.4 ± 3.2	21.8 ± 3.0	19.3 ± 2.6	21.5 ± 3.0	<0.001
12 month	18.0 ± 3.1[b]	17.5 ± 2.6[a]	18.5 ± 2.0[b]	18.7 ± 3.6	20.2 ± 3.0[c]	20.8 ± 5.0	20.7 ± 2.5[c]	18.2 ± 2.9[a]	20.6 ± 3.7[b]	<0.001
Linoleic										
Baseline	33.4 ± 3.8	33.6 ± 3.6	34.0 ± 3.7	33.9 ± 3.2	31.3 ± 3.5	31.0 ± 4.0	29.9 ± 4.2	33.7 ± 3.6	30.7 ± 4.0	<0.001
12 month	34.8 ± 4.7[b]	35.8 ± 3.8[a]	34.3 ± 4.0	32.7 ± 6.1	32.8 ± 3.7[c]	31.7 ± 4.0	30.9 ± 4.7	34.4 ± 4.8[b]	31.7 ± 4.9[b]	<0.001

Note. From text of ref. 22 with additional tabular detail.
[a] $P < 0.001$ versus baseline; [b] $P < 0.01$; [c] $P < 0.05$; ( ) = *n*.

with the statistically significant reductions in plasma percentage stearate content across all diets in HC and CHL subjects. Similar reductions in oleic acid content were also observed (Table IV). Stable plasma palmitic acid despite increasing dietary saturated fat restriction and carbohydrate augmentation are consistent with the observations of Hudgins *et al.* (20) reported in this symposium and elsewhere that high carbohydrate feeding induces increased palmitate synthesis at fat intakes of 10% compared with 30% when fat calories were substituted with a carbohydrate mixture of starch and sugar.

The Boeing Employee Fat Intervention Trial (BeFIT): Dietary Response in Men and Women

Having seen that Diet 2 in male subjects achieved the greatest LDL reduction without long-term triglyceride elevations or HDL reduction, a second study in Boeing employees was conducted to determine if the NCEP Step II diet, which Diet 2 closely resembles, was equally efficacious in men and women. Again subjects were divided into HC and CHL subject groups. Totals of 409 and 383 men and women were analyzed at 6 and 12 months, respectively (24, 25).

To make sure that the dietary intervention was due to the diet ingested rather than attention afforded from participating in a clinical trial, subjects were randomized to an immediate dietary instruction or a delayed dietary instruction after 6 months. As shown in Figure 5, LDL cholesterol reductions, respectively, were similar in HC and CHL female subjects and HC and CHL male subjects in the immediate dietary instruction groups. No statistically significant LDL reductions were observed in the delayed diet instruction groups. In subsequent analyses, the immediate and delayed dietary instruction groups were pooled, there being no difference in the response of both groups to the dietary intervention (24).

Dietary Adherence. The dietary adherence of the BeFIT subjects is shown in Table V. In this cohort, the average baseline fat intake was 33.4%–34.1%, the percentage of caloric intake across HC and CHL subjects and both

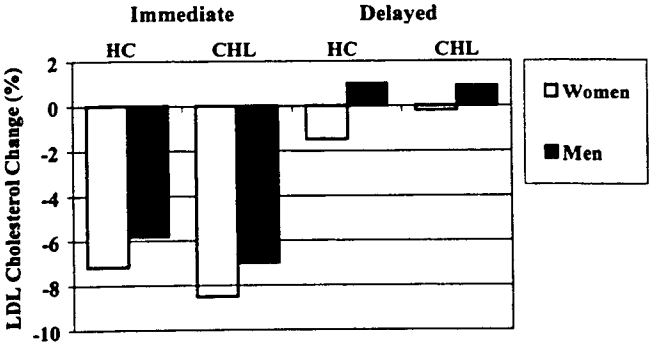

Figure 5. Reductions in LDL-C levels in the BeFIT study of HC and CHL women and men taught an NCEP Step II Diet immediately or after a delay of 6 months. Results showed that the LDL-C reductions are due to the dietary intervention (24).

genders. After 6 months, total fat intake was reduced to 24% and 25.5% of calories in the HC and CHL male groups and 25.2% and 24.8% in the HC and CHL female groups, respectively. The baseline saturated fat intake of these subjects was also slightly lower than in the DAS subjects, ranging from 11.5% to 12.0% of ingested calories. Saturated fat intake after 6 months was reduced to 7.1, 7.7, 7.6, and 7.5% of calories in the male and female HC and CHL groups, respectively. No statistically significant increase in plasma triglyceride was observed in either male or female HC or CHL subjects. As seen in DAS, the mean baseline triglyceride levels in men approximated 112 mg/dl in the HC group and 186 mg/dl in the CHL group. The corresponding triglyceride levels in women were marginally lower at baseline, 100 mg/dl in the HC and 176 mg/dl in the CHL subjects.

With respect to LDL cholesterol, the pooled reductions of immediate and delayed diet groups were similar again in male (10% and 9%) and female (both 9%) HC and CHL subjects, respectively (Table V and Fig. 6). Regarding the HDL cholesterol response, reductions of 0.8–1.1 mg/dl were seen in HC and CHL men at 6 months, but the reductions in women were on the order of 4.2 mg/dl in HC and 2.7 mg/dl in CHL groups (Table V and Fig. 6) (24).

To determine if the selective reductions in HDL cholesterol in female subjects persisted at 12 months, the data were analyzed, including measurements of LDL, HDL2, and HDL3 cholesterol and apoprotein A-I levels (25). LDL cholesterol reductions were again similar in male and female subjects, though not as marked as at 6 months (Fig. 7). Plasma triglyceride concentrations were again not significantly elevated, on the order of 10% or less (unpublished data). However, an HDL cholesterol reduction of 7.6% from baseline persisted in the female HC subjects compared with 1.3% in male HC subjects (Fig. 8). With respect to HDL2 cholesterol levels, the reduction from baseline was 16.5% (3 mg/dl) in HC women and 0.5% in HC men. In contrast, CHL subjects, both male and female, had approximately equivalent reductions in HDL cholesterol. The apo A-I concentration was statistically significantly reduced by 5.3% in female HC and 0.4% in female CHL subjects, not at all in male HC and 1.8% in male CHL subjects (Fig. 8).

These data show that reductions in HDL cholesterol and its constituents persist for 1 year in female HC subjects ingesting a Step II Diet and involve both the lipid and apoprotein moieties of the particle. The HDL_2 cholesterol reduction of 3 mg/dl is particularly striking as it is half of the 6 mg/dl baseline difference in HDL_2 between men and women, which comprises the majority of the difference in HDL cholesterol between men and women.

The reason for the greater HDL and HDL_2 cholesterol reductions in women compared with men is not clear. The HDL-C decrease in women cannot be attributed to a greater increase in plasma triglyceride since plasma triglyceride increases on the diet were minor and statistically insignificant in all four groups. The HDL-C reductions in women were also disproportionately greater than one would expect from the 10 mg/dl greater HDL cholesterol levels in women versus men (27). That is, the reductions are not explained by the higher initial value in women. A mechanistic possibility is that hepatic HDL formation or removal may be altered by the diet in a manner that does not directly involve measurable increases in plasma triglyceride concentrations but is linked to a gender or hormone-specific alteration in hepatic palmitate formation (19). Further research is required to understand the underlying mechanism of the HDL-C decrease in women as well as its significance for coronary artery disease susceptibility.

Conclusions

In male hypercholesterolemic subjects, restriction of dietary fat below 25% of calories and augmentation of car-

Table V. Dietary and Lipoprotein Changes in the BeFIT Study After 6 Months*

	Mean ± SE							
	Men				Women			
	HC		CHL		HC		CHL	
Month:	0	6	0	6	0	6	0	6
Total fat (en%)	34.1 ± 7.4	24.0 ± 7.2[a]	33.5 ± 7.1	25.5 ± 7.0[a]	33.4 ± 6.9	25.2 ± 7.4[a]	33.9 ± 7.2	24.8 ± 7.0[a]
Saturated fat (en%)	11.6 ± 3.1	7.1 ± 2.7[a]	11.5 ± 3.0	7.7 ± 2.8[a]	11.5 ± 3.2	7.6 ± 2.6[a]	12.0 ± 3.1	7.5 ± 3.0[a]
Triglyceride (mg/dl)	112 ± 60	112 ± 48	186 ± 91	181 ± 86	100 ± 40	97 ± 35	176 ± 70	179 ± 85
LDL-C (mg/dl)	176 ± 28	158 ± 26[a]	176 ± 28	161 ± 36[a]	171 ± 36	156 ± 34[a]	173 ± 31	157 ± 31[a]
HDL-C (mg/dl)	46.0 ± 7.7	45.2 ± 7.7	41.3 ± 7.0	40.2 ± 7.3	57.6 ± 12.0	53.4 ± 10.0[a,b]	49.9 ± 10.8	47.2 ± 11.2[a,b]
Weight (kg)	84.8 ± 13.0	81.4 ± 12.6[a]	87.6 ± 12.5	84.7 ± 12.0[a]	70.3 ± 14.8	68.7 ± 14.9[a]	79.4 ± 17.0	77.2 ± 17.0[a]

P versus baseline: [a] $P < 0.01$; [b] $P < 0.01$, change from baseline significantly different in women versus men. From Ref. 24.

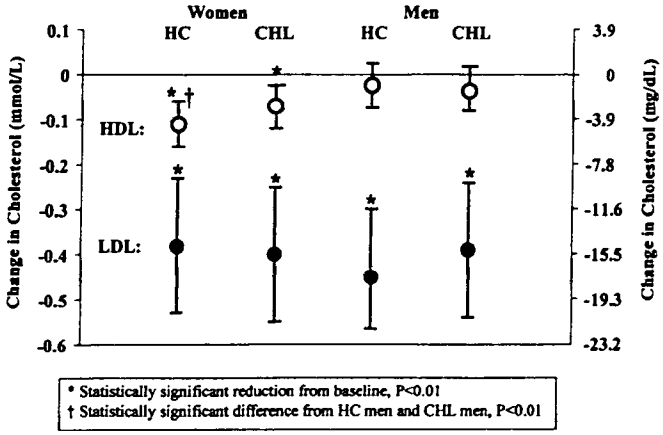

Figure 6. LDL-C and HDL-C reductions in HC and CHL men and women after 6 months of Step II Diet (24).

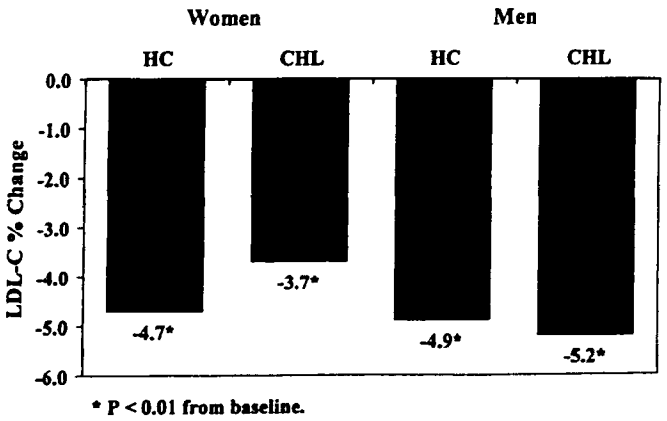

Figure 7. Percentage LDL-C reductions from baseline to 12 months in subjects taught an NCEP Step II Diet (25).

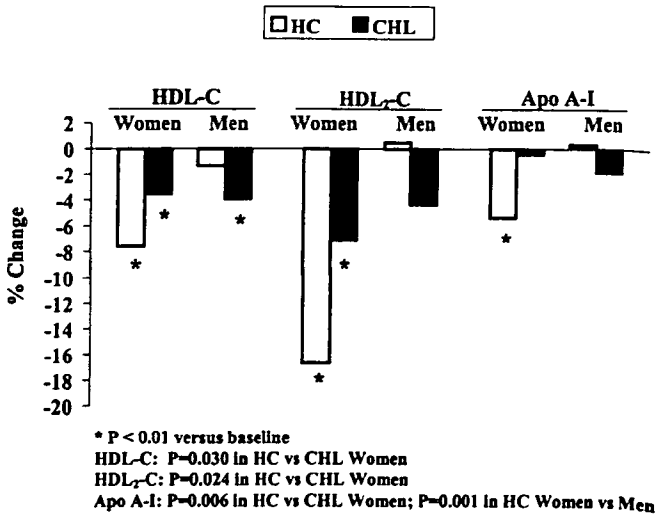

Figure 8. Reductions in HDL-C, HDL2-C, and apoprotein A-I in female and male HC and CHL subjects 1 year following an NCEP Step II Diet. (Illustration redrawn from Ref. 25).

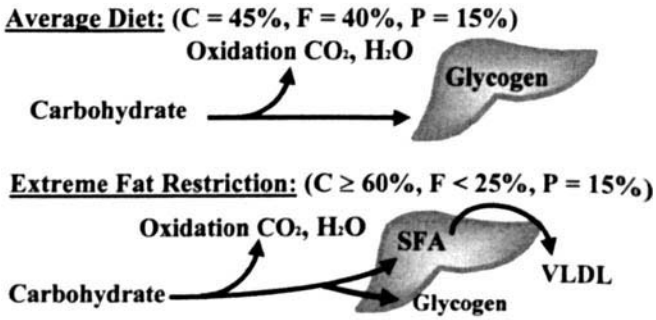

Figure 9. Model of the effect of high-carbohydrate feeding on glucose storage as glycogen (a limited reservoir) and the conversion of the remainder of the ingested carbohydrate into saturated fat and its export from the liver in the form of VLDL. This palmitate-driven increase in VLDL triglyceride concentrations is one mechanism of carbohydrate-induced hypertriglyceridemia (i.e., "carbohydrate induction", first described by Ahrens *et al.* (11) and recently elucidated by Hudgins *et al.* (20)). Impaired triglyceride removal may also contribute to "carbohydrate induction" as proposed by Parks *et al.* (28), but it does not explain the lack of a palmitate drop with low-fat diet and the lack of further LDL reduction seen in DAS.

bohydrate above 60% of calories are associated with an induced hypertriglyceridemia, reduced HDL cholesterol concentrations, and attenuated LDL and apo B reductions. These changes may be due to an increased synthesis of the major endogenous saturated fatty acid made by the body, palmitic acid, formed in the face of an increased dietary carbohydrate intake that exceeds the ability of the body to use it for energy or store it as glycogen as shown in Figure 9. This hypothesis is consistent with the demonstration of carbohydrate-stimulated palmitate synthesis elegantly demonstrated by Hudgins *et al.* (18–20) in previous publications and elsewhere in this symposium. The hypothesis is supported by the lack of a decrease in percentage palmitic acid content despite successful dietary saturated fat restriction and a lack of further LDL reduction. This observation is not explained by impaired triglyceride removal, though it may contribute to "carbohydrate induction" (28).

Our observations demonstrate the value of moderate fat restriction (= 25 en%) as an approach to saturated fat restriction and generally support the recommendations of the American Heart Association and National Cholesterol Education Program Step I and Step II Diet Guidelines. The equivalent reductions in LDL-C levels in women and men with the Step II Diet is encouraging, but the selective and persistent reduction in total and HDL_2 cholesterol and apo A-I in HC women subjects is unexpected, and could diminish the cardiovascular benefit of the LDL-C reduction observed with this diet. The selective HDL reduction in women could be due to increased endogenous palmitate synthesis even though triglyceride did not rise, as the two parameters are reportedly not tightly linked (19). Collectively, these observations suggest that alternative approaches to saturated fat restriction, such as substitution with mono- or polyunsaturated fat may be more effective overall in the dietary prevention of coronary artery disease, a hypothesis that is supported by the observational studies of Hu *et al.* (29) and Willett elsewhere in this symposium.

The authors thank the Boeing Medical Department, especially Dr. George Gey, the employee participants, and the staff of the Northwest Lipid Research Clinic who made these studies possible.

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