Insulin Resistance, Dietary Cholesterol, and Cholesterol Concentration in Postmenopausal Women

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Questions remain concerning the effect of variations in cholesterol intake on plasma cholesterol concentration, as well as on the role of factors modulating the metabolic impact of this dietary intervention. To define the impact of wide variations in dietary cholesterol intake on plasma total and low-density lipoprotein (LDL) cholesterol concentrations, as well as testing the hypothesis that resistance to insulin-mediated glucose disposal would accentuate the increase in plasma total and LDL cholesterol concentrations in response to a given increment in dietary cholesterol intake, we performed a prospective, randomized study comparing diets varying in cholesterol content in 65 healthy, postmenopausal women, 31 defined as insulin-resistant and 34 as insulin-sensitive. The changes in total and LDL cholesterol in response to increments in dietary cholesterol of up to ~800 mg/day were modest in magnitude, without evidence of a statistically significant diet-induced increase in cholesterol concentration, or of any difference in the responses of insulin-resistant as compared with insulin-sensitive women. These results indicate that relatively large increments in dietary cholesterol intake had little effect on total or LDL cholesterol concentrations in healthy, postmenopausal women, irrespective of whether they were insulin-resistant or insulin-sensitive.

IN CONTRAST TO earlier publications,[1,2] results of multiple recent studies in both healthy men and women have shown that dramatic increases in cholesterol intake are associated with relatively modest increases in plasma cholesterol and low-density lipoprotein (LDL) cholesterol concentrations.[3-9] On the other hand, results of several studies have emphasized that the magnitude of the plasma LDL cholesterol response to an increase in cholesterol intake varies from person to person.[3,4-6] At the present time, there is no obvious explanation to account for the individual differences in the effect of dietary cholesterol on cholesterol concentration. In this context, evidence[10,11] that the increase in plasma cholesterol concentration in response to incremental changes in cholesterol consumption was exaggerated in individuals with combined hyperlipidemia as compared with those who were only hypercholesterolemic was of interest. Given evidence[12] that patients with combined hyperlipidemia are insulin-resistant and hyperinsulinemic, as compared with those with a simple elevation of LDL cholesterol, it seemed reasonable to test the hypothesis that differences in insulin resistance and/or hyperinsulinemia are responsible for the variability in plasma cholesterol response to increases in dietary cholesterol intake. More specifically, we wished to see if the magnitude of the dietary-induced increase in plasma cholesterol concentration would be accentuated in insulin-resistant as compared with insulin-sensitive individuals. To accomplish this goal, we enrolled 65 postmenopausal women, classified as being either insulin-resistant or insulin-sensitive. The decision to limit the study population in this manner resulted from 2 considerations. In the first place, although the results of 1 meta-analyses did not show any gender difference in the plasma cholesterol response to increases in cholesterol intake,[9] the results of studies of somewhat longer duration suggested the presence of increased sensitivity to dietary cholesterol in healthy, young women, as compared with healthy men of the same age.[7,8] Because the risk of coronary heart disease increases substantially as women become postmenopausal,[13] defining a subset of postmenopausal women who might be uniquely sensitive to the untoward effect of increases in cholesterol intake would be of substantial clinical utility. Consequently, we decided to compare various aspects of glucose, insulin, and lipoprotein metabolism before and 3 months after increases in daily dietary cholesterol intake from 113 mg to either 319 mg, 523 mg, or 941 mg in postmenopausal women, stratified into an insulin-sensitive and insulin-resistant group.

MATERIALS AND METHODS

Methods

The study was approved by the Human Subjects Committee at both hospitals, and each research subject gave written informed consent before entering the study. Nondiabetic women recruited for this study were postmenopausal for at least 1 year, with a body mass index (BMI) between 19 and 33 kg/m^2, a fasting plasma total cholesterol concentration less than 280 mg/dL, and a triglyceride concentration less than 400 mg/dL. Subjects were not taking any medication known to affect lipid metabolism, and results of a physical examination, hemogram, and routine biochemical tests were all normal. Subjects using hormone replacement therapy (HRT), other medication, or dietary supplements continued on them throughout the study.

Candidates for the study were admitted to the General Clinical Research Centers at Stanford Hospital or University of Alabama at Birmingham, and insulin-mediated glucose disposal was measured by the insulin-suppression test.[14] After an overnight fast, intravenous catheters were placed in each arm. Blood was sampled from 1 arm for measurement of plasma glucose and insulin concentration and the contralateral arm for administration of test substances. Octreotide was

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Submitted August 2, 2000; accepted November 14, 2000.

Supported by research grants from the Egg Nutrition Center, Washington, DC, and Grants No. RR-00032 and RR-00070 from the National Institutes of Health, Division of Research Resources, Bethesda, MD.

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0026-0495/01/5005-0012$35.00/0

doi:10.1053/meta.2001.22559

administered at a rate of 0.5 μg/min in a solution containing 2.5% (wt/vol) human serum albumin by Harvard infusion pump to suppress endogenous insulin secretion. Simultaneously, insulin and glucose were infused at 25 mU/m^2/min and 240 mg/m^2/min, respectively. Blood was sampled for measurement of plasma glucose[15] and insulin[16] concentrations every 10 minutes until 180 minutes had elapsed. Insulin concentrations typically plateau by 60 minutes, whereas glucose concentrations plateau after 120 minutes. The 4 values obtained from 150 and 180 minutes were averaged and considered to represent the steady-state plasma glucose (SSPG) and steady-state plasma insulin (SSPI) concentrations achieved during the infusion. Because SSPI concentrations are similar in all individuals, qualitatively and quantitatively, and the glucose infusion rate is identical, the magnitude of the resultant SSPG concentration provides an accurate estimate of how effective insulin is in disposal of a glucose load, ie, the higher the SSPG, the more insulin-resistant. After the insulin-suppression test, 32 insulin-sensitive women (SSPG concentrations <100 mg/dL) and 33 insulin-resistant women (SSPG >160 mg/dL) were enrolled. Volunteers not falling into either of these categories were not followed further.

After qualification for enrollment, all subjects were started on a baseline, low-cholesterol diet and randomly assigned to 1 of 3 groups destined to receive an increased amount of cholesterol. The baseline diet contained 113 mg of cholesterol/day, whereas the 3 subsequent diets were calculated to contain either 319 mg, 523 mg, or 941 mg cholesterol per day. Various combinations of egg and egg substitutes were used to attain the desired amount of cholesterol in each of the 4 diets, thus preventing the volunteers from knowing the amount of cholesterol they were consuming on either the baseline or the 3 test diets.

Caloric level for each subject was calculated using the Harris-Benedict equation[17] and adjusted to maintain body weight within 0.5 kg of their baseline weight throughout the study. Calorie intake was distributed with 20% of daily calories consumed at breakfast, 40% at lunch, and 40% at dinner. Subjects were required to visit the research center daily to check body weight, eat 1 meal, and pick up their prepackaged meals. The meal consumed during this visit contained 100% of daily cholesterol content. All 4 of the diets conformed to the macronutrient requirements of the National Cholesterol Education Program (NCEP) Step 1 diet; containing (as percent of total calories) 20% protein, 50% carbohydrate, 30% fat, with 9% saturated fat, 9% polyunsaturated fat, and 12% monounsaturated fat. In addition, the vitamin and mineral content of the diet met recommended dietary guidelines for postmenopausal women. Thus, the only difference between the diets was the cholesterol content.

Volunteers were studied over a 12-week period, 4 weeks on the baseline diet (113 mg of cholesterol/day), a 4-week washout period, followed by a second 4-week diet period consuming 319 mg, 523 mg, or 941 mg of cholesterol/day. At the end of each 4-week diet period, subjects were readmitted to the research center for metabolic measurements. After an overnight fast, blood samples were taken for measurement of cholesterol in all lipoprotein classes, including high-density lipoprotein $(HDL)_2$, HDL_3, lipoprotein (LP)(a), intermediate-density lipoprotein (IDL), and very–low-density lipoprotein (VLDL), by the VAP-II method.[18] VAP-II is a single test direct measurement method, with no estimations involved. It is based on a combination of a rapid (45 minutes) single vertical spin ultracentrifugation and a novel continuous flow enzymatic cholesterol analyzer and is highly sensitive (requiring less than 40 μL of plasma) and reproducible. Furthermore, it separates the commonly measured LDL cholesterol as defined by NCEP, which also includes cholesterol values of Lp(a) and IDL, from r-LDL cholesterol, ie, LDL cholesterol that does not include Lp(a) and IDL. Thus, both the LDL cholesterol-NCEP (as commonly reported by the clinical laboratories) and its individual components (r-LDL cholesterol, LP(a) cholesterol, and IDL cholesterol) are measured by the VAP-II method.

Statistical Analysis

Variables are expressed as mean ± SEM, and the statistical evaluation was performed with the Statistical Analysis System program (SAS Institute Inc, Cary, NC). Student's paired *t* test was performed to evaluate the effect of cholesterol intake on plasma cholesterol concentration.

RESULTS

Baseline characteristics of the 3 groups formed to test the effect of increasing dietary cholesterol intake are shown in Table 1. It can be seen that they were quite similar in terms of demographic variables, as well as lipid and lipoprotein concentrations.

Thirty-one volunteers were defined as being insulin-resistant (SSPG, 206 ± 7 mg/dL) and 34 as insulin-sensitive (75 ± 3 mg/dL). The baseline characteristics of these 2 groups and their lipid and lipoprotein concentration in response to the baseline cholesterol intake of 113 mg/day are shown in Table 2. The 2 groups were similar in the proportion of women on HRT (42% *v* 38%), as well as in age (57 ± 1 *v* 55 ± 1 years) and concentrations of total (173 ± 6 *v* 172 ± 5 mg/dL) and LDL cholesterol (106 ± 5 *v* 109 ± 5 mg/dL) concentrations. BMI was higher in the insulin-resistant women (28.1 ± 0.6 *v* 27.8 ± 0.5 kg/m^2, $P < .001$) as were concentrations of triglyceride, VLDL cholesterol, and apo B. Insulin-resistant women also had lower HDL cholesterol, apo A, and Lp(a) concentrations. However, it should be emphasized that the baseline concentrations

Table 1. Baseline Characteristics of the Three Groups Formed to Assess Effects of Different Daily Cholesterol Intakes (mean ± SE)

Variable	Designated Cholesterol Intake		
	319 mg/d	523 mg/d	941 mg/d
No. of subjects	23	20	22
SSPG (mg/dL)	139 ± 16	148 ± 20	140 ± 15
HRT (+/−)	14/9	11/9	14/8
Sensitive/resistance	12/11	8/12	11/11
Age (yr)	56 ± 1	54 ± 1	57 ± 1
BMI (kg/m^2)	26.1 ± 0.9	26.8 ± 0.8	25.4 ± 0.8
Total cholesterol (mg/dL)	167 ± 6	175 ± 9	176 ± 6
LDL cholesterol (mg/dL)	105 ± 5	111 ± 7	107 ± 5
Real LDL cholesterol (mg/dL)	81 ± 5	87 ± 6	86 ± 5
HDL cholesterol (mg/dL)	46 ± 2	45 ± 2	48 ± 3
HDL_2 cholesterol (mg/dL)	12.5 ± 1.5	11.3 ± 1.1	13.3 ± 1.3
HDL_3 cholesterol (mg/dL)	34 ± 1	33 ± 2	35 ± 2
LDL/HDL cholesterol	2.4 ± 0.2	2.6 ± 0.2	2.4 ± 0.2
IDL cholesterol (mg/dL)	11.9 ± 0.9	16.0 ± 1.9	13.7 ± 1.3
VLDL cholesterol (mg/dL)	16.1 ± 1.5	18.7 ± 2.5	20.7 ± 2.8
$VLDL_3$ cholesterol (mg/dL)	9.7 ± 0.8	10.8 ± 1.6	11.8 ± 1.8
Triglyceride (mg/dL)	67 ± 6	75 ± 9	86 ± 12
LP(a) (mg/dL)	7.5 ± 1.3	8.2 ± 1.7	7.0 ± 1.3
Apo A-1 (mg/dL)	138 ± 5	141 ± 4	141 ± 5
Apo A-2 (mg/dL)	33.0 ± 1.6	33.8 ± 1.2	34.5 ± 1.8
Apo B (mg/dL)	81 ± 4	82 ± 6	82 ± 5
Apo C-3 (mg/dL)	13.2 ± 0.6	13.7 ± 0.9	14.4 ± 1.4
Apo E (mg/dL)	8.7 ± 0.6	8.7 ± 0.6	8.5 ± 0.6

Abbreviations: SSPG, steady-state plasma glucose concentration; HDL, high-density lipoprotein; HRT, hormone replacement therapy; IDL, intermediate-density lipoprotein; BMI, body mass index; VLDL, very–low-density lipoprotein; LDL, low-density lipoprotein; Apo, apoprotein.

Table 2. Baseline Characteristics of the Study Population and Their Lipid and Lipoprotein Concentrations in Response to a Daily Cholesterol Intake of 113 mg (mean ± SE)

Variable	Insulin-Sensitive (n = 34)	Insulin-Resistant (n = 31)	*P* Value
SSPG (mg/dL)	75 ± 3	206 ± 7	<.001
HRT (+/−)	18/31	21/13	
Age (yr)	57 ± 1	55 ± 1	.089
BMI (kg/m^2)	23.8 ± 0.8	28.1 ± 0.6	<.001
Total cholesterol (mg/dL)	173 ± 5	172 ± 5	.968
LDL cholesterol (mg/dL)	106 ± 4	109 ± 4	.603
Real LDL cholesterol (mg/dL)	84 ± 4	85 ± 4	.390
HDL cholesterol (mg/dL)	53 ± 2	40 ± 2	<.001
HDL_2 cholesterol (mg/dL)	16 ± 1	9 ± 1	<.001
HDL_3 cholesterol (mg/dL)	37 ± 1	31 ± 1	.003
LDL/HDL cholesterol	2.1 ± 0.1	2.8 ± 0.2	<.001
IDL cholesterol (mg/dL)	11.9 ± 1.2	15.4 ± 1.1	.024
VLDL cholesterol (mg/dL)	13.8 ± 1.1	22.6 ± 2.1	<.001
$VLDL_3$ cholesterol (mg/dL)	9.9 ± 1.2	11.0 ± 1.0	.436
Triglyceride (mg/dL)	57 ± 4	93 ± 9	.001
Apo A-1 (mg/dL)	144 ± 4	136 ± 11	.090
Apo A-2 (mg/dL)	33 ± 1	34 ± 1	.869
Apo B (mg/dL)	75 ± 4	88 ± 4	.024
Apo C-3 (mg/dL)	12.6 ± 0.7	14.9 ± 0.9	.067
Apo E (mg/dL)	9.0 ± 0.5	8.4 ± 0.5	.403
LP(a) (mg/dL)	10.3 ± 1.4	5.0 ± 0.6	.001

Abbreviations: SSPG, steady-state plasma glucose concentration; HDL, high-density lipoprotein; HRT, hormone replacement therapy; IDL, intermediate density lipoprotein; BMI, body mass index; VLDL, very–low-density lipoprotein; LDL, low-density lipoprotein; Apo, apoprotein.

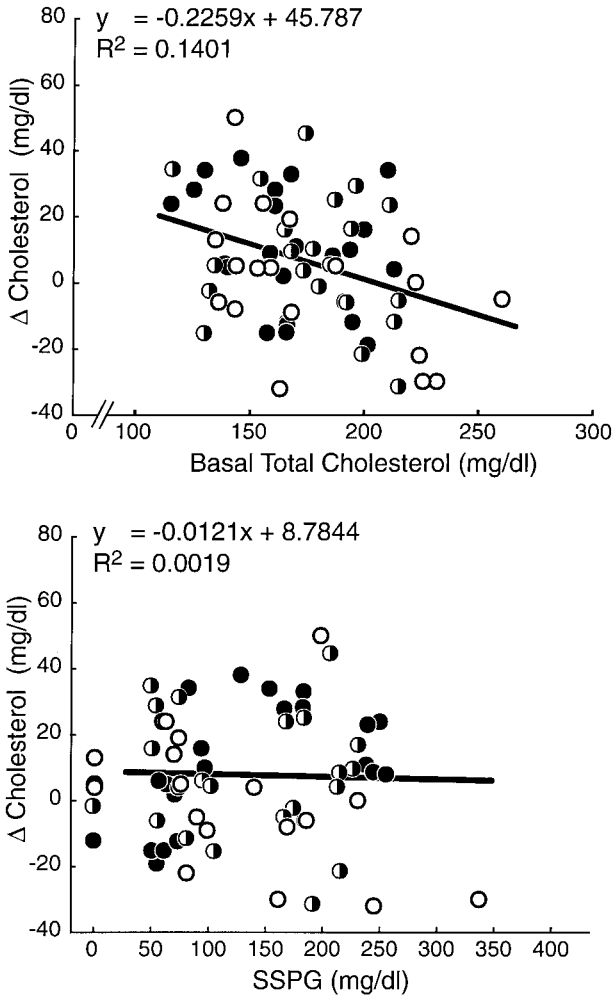

Fig 1. Relationship between total cholesterol (A) and SSPG concentrations (B) at baseline and change (△) in total cholesterol concentration when the cholesterol intake was increased from 113 mg/d to 319 mg/d (●), 523 mg/d (○), or 941 mg/d (◑).

of total and LDL cholesterol were similar in insulin-resistant and insulin-sensitive women.

The absolute changes in total and LDL cholesterol concentrations of the 2 groups in response to the incremental changes in dietary cholesterol intake are shown in Table 3. It can be seen that both total and LDL cholesterol concentrations changed very little in either group when cholesterol intake was increased from 113 mg/day to 319 mg/day, 523 mg/day, or 941 mg/day. It is also apparent that the incremental increases in cholesterol intake did not result in proportional increases in either total or LDL cholesterol concentration in either group. Indeed, if anything, the greatest increase in cholesterol concentration followed the least increment in cholesterol intake. The lack of any discernible effect of increasing dietary cholesterol intake on plasma total and LDL cholesterol was confirmed by 2-way analysis of variance, with neither insulin category (insulin-resistant *v* insulin-sensitive) nor cholesterol intake having

Table 3. Effect of Increasing Dietary Cholesterol Intake on Plasma Cholesterol and LDL Cholesterol Concentration (mean ± SEM)

Group	Cholesterol Concentration		LDL Cholesterol Concentration	
	113 mg/d	319 mg/d	113 mg/d	319 mg/d
Total (n = 23)	167 ± 6	178 ± 6	105 ± 5	112 ± 5+
Sensitive (n = 12)	168 ± 8	172 ± 2	101 ± 6	105 ± 6
Resistant (n = 11)	167 ± 9	184 ± 8	109 ± 7	121 ± 7
	113 mg/d	523 mg/d	113 mg/d	523 mg/d
Total (n = 20)	175 ± 9	181 ± 8	112 ± 7	116 ± 7
Sensitive (n = 8)	190 ± 15	196 ± 12	122 ± 13	127 ± 12
Resistant (n = 12)	166 ± 11	171 ± 10	105 ± 8	108 ± 7
	113 mg/d	941 mg/d	113 mg/d	941 mg/d
Total (n = 22)	176 ± 6	184 ± 6	107 ± 5	113 ± 55
Sensitive (n = 11)	167 ± 9	176 ± 9	100 ± 6	107 ± 7
Resistant (n = 11)	184 ± 8	192 ± 9	114 ± 9	120 ± 8

a statistically significant effect on either total or LDL cholesterol concentrations.

Finally, the results in Fig 1 show that the increment in total cholesterol concentration when dietary cholesterol intake was increased did not vary as a function of either baseline cholesterol or degree of insulin resistance. Essentially identical results were seen when the change in LDL cholesterol concentration (either NCEP or r) was plotted on the vertical axis.

The effects of variations in cholesterol intake on the other variables measured were also quite modest and inconsistent. For example, when the entire population was considered, there was a slight increase in HDL_3 cholesterol in response to a cholesterol intake of 319 mg/day (33.6 ± 1.4 to 35. ± 1.3 mg/day). Similar increases in HDL_2 cholesterol (13.3 ± 1.3 to 15.5 ± 1.7 mg/day), IDL cholesterol (13.7 ± 1.3 to 15.4 ± 1.3), Apo A-1 (141 ± 5 to 147 ± 6), and Apo B (82.0 ± 4.7 to 89.2 ± 4.6) were noted when the cholesterol intake was increased to 941 mg/day. However, even these modest differences disappeared when the insulin-sensitive and insulin-resistant groups were analyzed separately, and the 2 groups behaved in an almost identical manner.

DISCUSSION

The goal of our study was to see if differences in insulin-mediated glucose disposal modulate the impact of cholesterol intake on plasma total or LDL cholesterol concentrations. For this purpose, we screened volunteers to enroll 2 groups, dichotomous as regards their degree of insulin resistance observed during the insulin suppression test. However, despite an approximately 3-fold difference in insulin-mediated glucose disposal between the 2 groups, the results in Table 3 indicate that only a modest increase in total or LDL cholesterol concentration was seen when cholesterol intake was increased from 113 mg/day to as much as 941 mg/day. Furthermore, 2-way analysis of variance did not show any statistically significant change in total or LDL cholesterol as a result of differences in cholesterol intake or of being either insulin-resistant or insulin-sensitive. The lack of any effect of differences in insulin action on the response to wide variations in cholesterol consumption cannot be ascribed to atypical metabolic characteristics of the insulin-resistant women, as they demonstrated the usual changes in lipid metabolism of higher plasma triglyceride and lower HDL cholesterol concentrations.

It is apparent that the results presented are consistent with previous publications showing that differences in cholesterol intake result in relatively modest changes in plasma total or LDL cholesterol concentration.[3-9] However, it should be emphasized that the saturated fat content of the 4 diets in our study was identical. Thus, our results cannot be extended to situations in which significant increases in saturated fat intake accompany a greater cholesterol intake.

Furthermore, our study group consisted of healthy women, whose total plasma cholesterol concentration ranged from 115 to 260 mg/dL. Although the results in Fig 1 suggest that the increment in plasma cholesterol concentration with increases in cholesterol intake is unrelated to baseline cholesterol concentration, our results should not be extrapolated to other population groups with different characteristics. On the other hand, with these 2 caveats in mind, our findings provide additional support for previous findings that plasma cholesterol concentrations are relatively insensitive to major changes in cholesterol consumption[3-9] and do not suggest that cholesterol concentrations in insulin-resistant, postmenopausal women are hyper-responsive to increases in cholesterol consumption.

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