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Carbohydrate intake is correlated with biomarkers for coronary heart disease in a population of overweight premenopausal women

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Abstract

The associations between macronutrient intake and plasma parameters associated with increased risk for coronary heart disease (CHD) were evaluated in 80 overweight premenopausal women. We hypothesized that higher carbohydrate intake would be associated with a more detrimental plasma lipid profile. Dietary data were collected using a validated food frequency questionnaire (FFQ). Plasma total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined from two fasting blood samples. In addition, selected apolipoproteins (apo) and LDL peak size were measured. Values for TC, TG and HDL were not in the range of risk classification; however, the mean values of LDL-C, 2.7±0.7 mmol/L, were higher than the current recommendations. Carbohydrate intake was positively associated with TG and apo C-III ($P<.01$) concentrations, and negatively associated with LDL diameter ($P<.01$). Participants were divided into low (<53% of energy) or high (≥53% energy) carbohydrate intake groups. Individuals in the <53% carbohydrate group consumed more cholesterol and total fat, but also had higher intake of polyunsaturated and monounsaturated fatty acids (SFAs). In contrast, subjects in the ≥53% group consumed higher concentrations of glucose and fructose than those in the low-carbohydrate (LC) group. In addition, subjects consuming <53% carbohydrate had lower concentrations of LDL-C and apo B ($P<.01$) and a larger LDL diameter ($P<.05$) than the ≥53% group. These results suggest that the lower LDL-C in the LC group may be related to both the amount of carbohydrate and the type of fatty acids consumed by these subjects.

Keywords: Obesity; Coronary heart disease; Dense LDL; Plasma lipids; Dietary carbohydrates

1. Introduction

Though research does not support the theory that one weight loss program is successful for all populations [1–3], the approach is still disputed, especially with the escalating obesity rates and associated comorbidities. Obesity is a public health challenge that claims approximately 300,000 lives a year [4] and drains an estimated 9.4% of the health care budget [5]. Approximately 65% of the U.S. population has a body mass index (BMI) of ≥25 kg/m^2 [6], which classifies them as overweight. Clinically, overweight and obese (BMI≥30 kg/m^2) persons are at increased risk for coronary heart disease (CHD) and diabetes mellitus type 2 (DM2) [7,8]. Coronary hear disease, the number one killer in the US, affects an estimated 13 million people [9] and accrues direct and indirect costs of $112 billion each year [10]. In addition, from 1990 to 1998, in adults between 30 and 40 years of age, DM2 prevalence rose 70% [6]. Expenditures for treatment and management of DM2 is approximately $100 billion in direct and indirect costs per year [11]. Practical solutions for the management and prevention of obesity, CHD and DM2 are needed for the immediate future. However, experts disagree on dietary modifications that will result in weight loss, decreased plasma concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TGs) that would still maintain or increase high-density lipoprotein cholesterol (HDL-C) [12–14].

History has shown that when a single food or nutrient is depicted as a "bad" dietary component, the public has difficulty substituting a healthy choice for the bad food that gets eliminated from the diet to sometimes detrimental effects [15,16]. For example, when the public was advised to severely decrease dietary fat, total caloric intake actually

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increased along with the incidence of overweight, obesity and CHD [14] partly due to the availability of low fat, increased sugar foods. Although current research focuses on the macro- or micronutrient composition of the diet, ecologic data suggest that overall diet quantity and quality is more pertinent for achieving weight loss and for maintaining a healthy plasma lipid profile [17,18]. The Lyon Diet Heart Study demonstrated that the Mediterranean diet with its increased intake of vegetables, fruits, fish, unsaturated fatty acids and whole grains afforded some protection against nonfatal heart attacks and cardiac death [17,18]. In contrast to refined grains, whole grains contribute a greater amount of fiber, phytochemicals and essential fatty acids [19–21], and may increase satiety [7] because of decreased intestinal transit time. Cohort studies have also shown an inverse association between complex carbohydrates and CHD [22].

Overweight and obese premenopausal women are an important population to study because of their increased risk to develop CHD and DM2 [7,8]. From 1971 to 2000, females have significantly increased their caloric intake by 335 kcal (21.5%) compared to the significant increase of 168 kcal (6.9%) seen in males [23]. We have reported risk factors for CHD and DM2 in this population of overweight/obese premenopausal women [8]. We found that 24 of the 80 subjects were classified with insulin resistance and 9 with the metabolic syndrome [8]. Based on these data, our next goal was to evaluate the food choices and if they contributed to the presence of biomarkers for chronic disease in these individuals. Therefore, the purpose of this study was to evaluate the self-selected diet in 80 overweight, obese premenopausal women by utilizing a semiqualitative food frequency questionnaire (FFQ). This allowed for an exploration of the associations between macronutrient intake, mainly carbohydrates, and biomarkers for CHD.

2. Methods and materials

2.1. Study population

Eighty overweight and obese premenopausal women were recruited from the University of Connecticut and surrounding communities. Study protocol was approved by the University of Connecticut Institutional Review Board and informed consents were obtained from all subjects. Participants (74% Caucasians) were between the age of 20 and 45 years and BMIs ranged from 25 to 37 kg/m^2. Exclusionary criteria included pregnancy, lactation and history of kidney or liver disease and diabetes. Nine subjects were identified as current smokers and 61 reported some alcohol consumption. Nine participants were taking a multivitamin, six were taking calcium and one participant was taking calcium, vitamins A, E and folic acid. Twenty-six participants were on some form of birth control, eight were on thyroid medication (stable for at least 2 years) and one participant had been taking a cholesterol-lowering medication for over 5 years. Three participants reported anemia, but did not report taking any prescription or nonprescription drugs for the condition. Using the International Physical Activity Questionnaire [24], the majority of participants considered themselves to be sedentary to moderately active.

2.2. Dietary assessment

Dietary information was collected using a 120-food item FFQ developed by the Fred Hutchinson Cancer Research Center (Seattle, WA). One participant was excluded because of an incomplete FFQ. For each food, participants selected their serving size in comparison to the medium size listed. Pictures of small, medium and large food items were provided to increase reporting accuracy. Participants recorded how many times, on average, in the past 3 months they had consumed each food listed in the FFQ and the serving size.

2.3. Classification of participants into carbohydrate-consuming groups

After the results from the FFQ were calculated, subjects were divided into two groups based on carbohydrate intake. In previous studies exploring different carbohydrate levels on various biochemical parameters, numerous levels have been chosen as cutoff points. In a study by Landry et al. [25], high-carbohydrate and low-carbohydrate groups consumed on average 60% and 46%, respectively, of calories from carbohydrates. In Archer et al.'s [26] study, high- and low carbohydrate average intakes were 58.3% and 44.7%, respectively. In order to compare this study's population with groups from previous studies, similar carbohydrate intakes were needed. To meet this end, the cut-off of 53% of total calories from carbohydrates was used to group this study population. The low-carbohydrate (LC) group was classified as those participants who consumed $<53\%$ of calories from carbohydrates and the high-carbohydrate (HC) group consumed $\geq 53\%$ of calories from carbohydrates.

2.4. Anthropometric measurements

Blood pressure was measured on the right arm using a Welch Allyn, Tycos blood pressure cuff (Welch Allyn, Arden, NC) with the participant seated, following a 5-min rest. Waist circumference (WC) was measured at the midway point between the lowest rib and iliac crest to the nearest 0.1 cm [27,28], and hip circumference was measured at the widest point of the hips to the nearest 0.1 cm. Weight was measured to the closest 0.5 lb and height was measured to the closest 0.5 in. on a portable stadiometer [27]. The weight and height were converted into metric measures to calculate the BMI (kg/m^2). All anthropometric measurements were done at the same time that blood was collected.

2.5. Biochemical measurements

Enzymatic TC and TG kits were obtained from Roche-Diagnostics (Indianapolis, IN). EDTA, aprotonin, sodium azide and phenyl methyl sulfonyl fluoride (PMSF) were

Table 1
Descriptive characteristics of the study population reported as mean± standard deviation and range of values in parentheses

Parameter	$N=80$
Age (years)	29.2±8.4
BMI (kg/m^2)	29.6±3.2
WC (cm)	90.4±8.4
WHR	0.82±0.07
Systolic blood pressure	118.6±7.5
Diastolic blood pressure	76.2±6.9
TC (mmol/L)	5.1±0.8
LDL-C (mmol/L)	2.7±0.7
HDL-C (mmol/L)	1.7±0.3
TG (mmol/L)	1.33±1.7
Apo B (mg/L)	745±150
Apo C-III (mg/L)	186±50
Apo E (mg/L)	34.7±14.7
LDL peak diameter (nm)	26.75±0.04

obtained from Sigma (St. Louis, MO). Two fasting (12 h) blood samples were collected on different days in a single week, from each subject, into tubes containing 0.15 g/100 g EDTA. Plasma was separated by centrifugation at 1500×*g* for 20 min at 4°C, placed into vials containing PMSF (0.05 g/100g), sodium azide (0.01 g/100 g) and aprotonin (0.01 g/100 g) and stored at −80°C until analysis. Plasma samples were used to determine plasma lipids, LDL size and apolipoprotein (apo) concentrations.

2.6. Plasma lipids and apolipoproteins

Our laboratory is a participant in the Centers for Disease Control — National Heart, Lung and Blood Institute Lipid Standardization Program, since 1989 for quality control and standardization for plasma TC, HDL-C and TG assays. Coefficients of variance assessed by the program during the study period were 0.76–1.42 for TC, 1.71–2.72 for HDL-C and 1.64–2.47 for TG. Total cholesterol was determined enzymatically utilizing Roche-Diagnostics standards and kits [29]. High-density lipoprotein cholesterol was measured in the supernatant after precipitation of apo B-containing lipoproteins [30], and LDL-C was determined using the Friedewald equation [31]. Triglycerides were determined using Roche-Diagnostics kits, which adjust for free glycerol [32]. Apo B concentrations were measured by an immunoturbidimetric method using kits from Wako (Richmond, VA), and turbidity was measured at 340 nm [33]. Apo C-III [34] and apo E [35] were measured with a Hitachi Autoanalyzer 740 utilizing kits from Wako.

2.7. Low-density lipoprotein size determination

The Lipoprint LDL system (Quantimetrix, Redondo Beach, CA) was used to determine LDL particle size. Briefly, 25 μl of plasma was added to precast polyacrylamide gel tubes and overlayered with 200 μl of loading gel. Tubes were then photopolymerized for 30 min and placed into the electrophoresis chamber. Electrophoresis buffer (Tris–hydroxymethyl aminomethane 66.1 g/100 g, boric acid 33.9 g/100 g, pH 8.2–8.6) was added to the top and bottom portion of the chamber. The gel was run until the HDL fraction was approximately 1 cm from the end of the gel tube, approximately 90 min at 72 mV. Gels were allowed to sit for 30 min and then scanned with a densitometer.

2.8. Statistical analysis

Values are reported as mean±standard deviation. Pearson correlations determined significant relationships of carbohydrate intake with measured parameters. Unpaired Student's *t* test was used to compare subjects with lower and higher intakes of carbohydrates. A *P* value of <.05 was considered significant. After significant correlations were found between intake of carbohydrates and some of measured parameters, a one-tail *t* test was used to compare the means of total TGs, apo C-III concentrations and LDL size between the high and LC groups.

3. Results

The mean age of the study population was 29.2 years and the mean BMI was 29.6 kg/m^2 (Table 1). Waist circumference and waist to hip ratio (WHR), 90.4 cm and 0.82, respectively, were above optimal values [27]. The mean systolic and diastolic blood pressures were within recommended levels. Plasma concentrations of TC (5.1 mmol/L), HDL-C (1.7 mmol/L) and TG (1.33 mmol/L) were within recommended levels. However, this population's mean LDL-C concentration (2.7 mmol/L) was above the suggested level according to the newest recommendations from the National Cholesterol Education Program's Third Adult Treatment Panel, therefore classifying these subjects at higher risk [36,37]. Apo B and C-III concentrations were on the high end of the recommended ranges. A high prevalence of small, dense LDL particles was also noted in this population.

Table 2 shows Pearson correlations between carbohydrate intake and dietary cholesterol, and selected plasma lipid parameters. Carbohydrate intake was positively associated with dietary cholesterol ($r=.334$, $P<.01$). Carbohydrate intake was also positively associated with plasma TG ($r=.303$, $P<.01$) and apo C-III ($r=.301$, $P<.01$) concentrations, and negatively associated with LDL diameter ($r=-.344$, $P<.01$).

Participants were separated into two groups based on FFQ data: participants who consumed <53% of calories from carbohydrates (LC) and participants who consumed ≥53%

Table 2
Pearson correlation values between dietary items and plasma lipids and total carbohydrate intake

Variable	Total carbohydrate intake
Dietary carbohydrate	–
Dietary cholesterol	0.334*
Plasma TG	0.303*
Plasma apo C-III	0.301*
Plasma LDL diameter	0.344*

* $P<.01$.

Table 3
Dietary intake and plasma lipids of subjects consuming <53% or ≥53% total carbohydrates[a]

Variable	CHO<53%	CHO≥53%	*P* value
	N=44	*N*=35	
Energy (calories)	2052.9±793.0	1931.3±697.2	NS
CHO (% energy)	44.9±20.4	58.5±19.1	.023
PRO (% energy)	17.3±6.0	15.7±5.6	NS
FAT (% energy)	37.6±15.2	27.5±14.3	.001
MUFA (% energy)	14.3±6.0	10.2±5.9	.001
PUFA (% energy)	7.9±3.8	5.7±2.9	.001
SFA (% energy)	12.5±5.0	9.3±5.0	.001
Dietary cholesterol (mg/day)	345.1±196.4	233.4±144.4	.006
Animal protein (%)	12.4±4.6	10.3±4.5	.008
Glucose (g/day)	21.4±12.3	38.0±25.5	.000
Fructose (g/day)	20.6±13.2	40.3±28.4	.000
TC (mmol/L)	4.61±0.71	4.92±1.12	NS
LDL-C (mmol/L)	2.42±0.58	2.84±0.65	.004
HDL-C (mmol/L)	1.61±0.30	1.59±0.27	NS
TG (mmol/L)	1.31±0.73	1.38±0.67	NS
Apo B (mg/dl)	71.9±15.1	78.5±13.6	.023
LDL diameter (nm)	26.81±0.29	26.62±0.44	.031[b]

[a] Values are expressed as mean±SD for 79 subjects.
[b] Evaluated with a one-tail *t* test.

of calories as carbohydrates (CHD) (Table 3). This resulted in mean carbohydrate intakes of 44.9% (LC) and 58.5% (HC), which are similar to previous studies after separating groups into low and HC intake [25,26]. There were no significant differences between the two groups with relation to BMI, WC, blood pressure or energy intake. Although the protein intake was similar across groups, fat intake was significantly different between the two groups, 37.6% in the LC group and 27.5% in the HC group. Low-carbohydrate participants consumed significantly higher intakes of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) than the HC participants. In addition to consuming significantly more fat, the LC group also had higher intakes of dietary cholesterol and animal protein; however, vegetable protein intake was similar among groups. The participants in the LC group consumed less glucose and fructose but more artificial sweetener (e.g., aspartame) than those in the HC group.

Participants in the LC group had significantly lower levels of LDL-C. Participants in the HC group had a mean LDL-C concentration of 2.84 mmol/L, which is above the new recommendation of 2.6 mmol/L. In addition, apo B was significantly higher and LDL diameter smaller ($P<.05$) in the participants in the HC group than those in the LC group. In contrast, plasma levels of TC, TG, HDL-C, and apo C-III and E were not different between the LC and HC groups (Table 3).

4. Discussion

Recently, weight loss and its effect on plasma lipids and other CHD biomarkers have received much attention. An area of intense research is to identify optimal weight loss diets that concurrently improve lipoprotein profiles. Research has shown that caloric restriction in conjunction with a decrease in dietary SFA can result in beneficial changes in lipoprotein profiles that go beyond what can be achieved by weight loss without changes in SFA intake [38]. Research has also shown that increasing dietary cholesterol does not consistently result in increased plasma concentrations of TC [14,39,40]. In addition, plasma TC does not account for total increased risk of CHD [41]. Recently, research has focused attention on the effect of carbohydrate quality on weight loss and CHD risk.

Increased carbohydrate consumption, especially simple carbohydrates, has been associated with elevated plasma TG [9,38,42], which in turn is associated with obesity, increased concentrations of very low density lipoproteins (VLDLs) [42], increased concentrations of apo C-III [43,44] and a decrease of the mean diameter of the LDL particle, all of which are associated with increased CHD risk [38]. The decrease in LDL diameter results in smaller, denser LDL particles that are richer in TG when compared to the larger, more buoyant LDL [42]. A predominance of small, dense LDL particles is indicative of the pattern B phenotype, which has been shown to be associated with a threefold increase in CHD risk [1,45]. In addition, this pattern B LDL is typically seen in those individuals with insulin resistance [41], metabolic syndrome, and DM2 [46].

When studying LDL particle atherogenicity, the delipidation cascade commencing with VLDL synthesis needs to be explored. In healthy individuals, the delipidation cascade converts 80–90% of VLDL into LDL via lipoprotein lipase (LPL) [43,47]. Apo C-III down-regulates LPL activity; therefore, elevated levels of apo C-III indicate decreased TG hydrolysis from VLDL [44,47,48]. Apo C-III has also been shown to inhibit the hepatic uptake of VLDL and its remnants via disrupting the binding domain of the apo B resulting in decreased affinity for the LDL receptor that extends circulation time for both VLDL and LDL [43]. Pattern B LDL is thought to result from increased TG-rich VLDL production and increased residence time in circulation [41,49]. Genetic factors account for some influence on the delipidation cascade but cannot explain the whole process; therefore, this study considered dietary influences.

In this study population, carbohydrate intake was found to be positively associated with plasma TG and apo C-III concentrations and negatively associated with LDL diameter. After separating the participants into the LC and HC groups, the mean carbohydrate intake for the LC and HC groups were similar to previous studies in which the effect of carbohydrate intake on plasma parameters was explored [25,26]. The size of the groups for this study, 44 participants (56%) in the LC and 35 participants (44%) in the HC, reflects the national trend of approximately two thirds of Americans are trying to reduce their carbohydrate intake [3,50]. As would be expected in this study, the LC group consumed significantly less carbohydrate and significantly more total fat, SFA, MUFA and PUFA in addition to dietary cholesterol.

However, the LC group had lower concentrations of LDL-C and apo B and larger LDL particles.

Surprisingly, plasma TG concentrations were not significantly different between the LC and HC groups. However, the elevated apo B concentrations in the HC group would suggest that hepatic VLDL secretion is elevated. The trend of smaller LDL particles in this group also suggests the presence of a dysregulated delipidation cascade. Although the LC group had significantly higher intakes of SFA (12.2%), they also had significantly higher intakes of MUFA and PUFA. It has been shown that diets high in MUFA or PUFA are very effective in lowering plasma LDL-C [51], thus, high intake of these unsaturated fatty acids could have counterbalanced the effects induced by saturated fat.

As expected, the HC group consumed more glucose and fructose than the LC group. This implies that the HC group chose processed carbohydrates (simple carbohydrates) made with high fructose corn syrup instead of high fiber, whole grain carbohydrates (complex carbohydrates). Research has shown that negative associations exist between CHD risk and whole grain foods [9]. Conversely, there is a positive association between CHD and sugar and syrup intake [9]. Fructose is absorbed and metabolized differently from glucose [52]. In the cell, fructose is phosphorylated into fructose-1-phosphate, which can then be easily synthesized into long chain fatty acids, phospholipids and TG [52]. This results in an unregulated supply of substrates for hepatic lipogenesis [52]. The increase in obesity prevalence reflects the increased utilization of high-fructose corn syrup in the American food supply [52] and emphasizes the public's need to distinguish between simple and complex carbohydrates just as they are learning to distinguish between saturated and unsaturated fats [9]. Another explanation for the increased intake of foods containing high fructose corn syrup is that consumers have not identified complex carbohydrate products they prefer. The difference in texture and taste between simple and complex carbohydrate products may act as a barrier. However, the presence of a barrier does not change the fact that elevated consumption of fructose has a deleterious effect on carbohydrate and lipid metabolism.

The data from the present study of overweight and obese premenopausal women support further research on total diet quality as well as the importance of individualizing nutrition care plans for clients to be sure that concepts such as complex and simple carbohydrates are understood. Differences in carbohydrate type (complex vs. simple) are not clear to most people. This lack of understanding is not unexpected since the difference between good (monounsaturated) and bad (saturated) fats took over 20 years for the public to begin to understand. In this study, the HC group increased intake of simple carbohydrates high in fructose and increased their risk of CHD as suggested by their current LDL-C levels (2.7 mmol/L). In contrast, the LC group, which consumed less carbohydrate but more dietary fat and cholesterol, had significantly lower concentrations of LDL-C compared to those participants who consumed more carbohydrates. Therefore, utilizing the lipoprotein profile rather than diet information, the LC group is considered to be at lower risk for CHD than the HC group.

References

[1] Krauss RM. Atherogenic lipoprotein phenotype and diet–gene interactions. J Nutr 2001;131:340S–3S.

[2] Mensink RP, et al. Effects of dietary fatty acids and carbohydrates on the ration of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 2003;77:1146–55.

[3] Volek JS, Sharman MJ, Gomez AL, Scheett TP, Kraemer WJ. An isoenergenic very low carbohydrate diet improves serum HDL cholesterol and triacylglycerol concentrations, the total cholesterol to HDL cholesterol ratio and postprandial lipemic responses compared with a low fat diet in normal weight, normolipidemic women. J Nutr 2003;133:2756–61.

[4] Must A, et al. The disease burden associated with overweight and obesity. JAMA 1999;282:1523–9.

[5] Colditz G. Economic costs of obesity and inactivity. Med Sci Sports Exerc 1999;31:S663–7.

[6] Virgin SE, Schmitke JA. Metabolic syndrome. AAOHN J 2003;51: 37–8.

[7] Liu S, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. Am J Clin Nutr 2003;78:920–7.

[8] Lofgren I, et al. Waist circumference is a better predictor than body mass index of coronary heart disease risk in overweight premenopausal women. J Nutr 2004;134:1071–6.

[9] Schaefer EJ. Lipoproteins, nutrition, and heart disease. Am J Clin Nutr 2002;75(2):191–212.

[10] Association, A.H. Statistical Fact Sheet. 2004.

[11] Ford ES, et al. Serum total cholesterol concentrations and awareness, treatment, and control of hypercholesterolemia among US adults. Findings from the National Health and Nutrition Examination Survey, 1999 to 2000. Circulation 2003;107:2185–9.

[12] Lichtenstein AH, Schwab US. Relationship of dietary fat to glucose metabolism. Atherosclerosis 2000;150:227–43.

[13] Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. Arterioscler Thromb 1992;12:911–9.

[14] Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: a critical review. J Am Coll Nutr 2001;20(1): 5–19.

[15] Schneeman BO. Evolution of dietary guidelines. J Am Diet Assoc 2003;103:S5–9.

[16] Gifford KD. Dietary fats, eating guides, and public policy: history, critique, and recommendations. Am J Med 2002;113(9B):89S–106S.

[17] Kris-Etherton P, et al. Lyon heart study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association Step 1 dietary pattern on cardiovascular disease. Circulation 2001;103:1823–5.

[18] de Lorgeril M, et al. Effect of a Mediterranean type of diet on the rate of cardiovascular complications in patients with coronary artery disease. Insights into the cardioprotective effect of certain nutriments. J Am Coll Cardiol 1996;28:1103–8.

[19] Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. JAMA 2002;288(20):2569–78.

[20] Liu S, et al. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. Am J Clin Nutr 1999;70: 412–9.

[21] Fernandez ML. Soluble fiber and nondigestible carbohydrate effects on plasma lipids and cardiovascular risk. Curr Opin Lipidol 2001;12: 35–40.

[22] Rajala M, Scherer PE. Minireview: the adipocyte — at the crossroads of energy homeostasis, inflammation and atherosclerosis. Endocrinology 2003;144(9):3765–73.
[23] Control CfD. Trends in intake of energy and macronutrients — United States, 1971–2000. Morb Mort Wkly Rep 2004;53(4):80–2.
[24] Craig CL, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003;35: 1381–95.
[25] Landry N, et al. Whole-body fat oxidation rate and plasma triacylglycerol concentrations in men consuming an ad libitum high-carbohydrate or low-carbohydrate diet. Am J Clin Nutr 2003;77(3): 580–6.
[26] Archer WR, et al. High carbohydrate and high monounsaturated fatty acid diets similarly affect LDL electrophoretic characteristics in men who are losing weight. J Nutr 2003;133(10):3124–9.
[27] Lean MEJ, Han TS, Morrison CE. Waist circumference as a measure for indicating need for weight management. BMJ 1995;311:158–61.
[28] Janssen I, Katzmarzyk PT, Ross R. Body mass index, waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines. Arch Intern Med 2002;162:2074–9.
[29] Allain CC, et al. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20(4):470–5.
[30] Warnick GR, Benderson J, Albers JJ. Dextran–sulphate–Mg^{+2} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28:1379–88.
[31] Friedewald WT, Levy RI, Frerickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative centrifuge. Clin Chem 1972;18(6): 499–502.
[32] Carr T, Anderssen CJ, Rudel LL. Enzymatic determination of triglycerides, free cholesterol, and total cholesterol in tissue lipid extracts. Clin Biochem 1993;26:39–42.
[33] Rifai N, King ME. Immunoturbidimetric assays of apolipoproteins A-I, A-II, and B in serum. Clin Chem 1986;32:957–60.
[34] Frendenrich A, et al. Plasma lipoprotein distribution of apo C-III in normolipidemic and triglyceridemic subjects: comparison of the apo C-III to apo E ration in different lipoprotein fractions. J Lipid Res 1997;38:1421–32.
[35] Cohn JS, et al. Plasma concentration of apolipoprotein E in intermediate-size remnant-like lipoproteins in normolipidemic and hyperlipidemic subjects. Arterioscler Thromb Vasc Dis 1996;16: 149–59.
[36] Expert Panel on Detection, E.a.T.o.H.B.C.i.A. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001;285(19):2486–97.
[37] Jones PH. Low-density lipoprotein cholesterol reduction and cardiovascular disease prevention: the search for superior treatment. Am J Med 2004;116(6A):17S–25S.
[38] Grundy SM, Denke MA. Dietary influences on serum lipids and lipoproteins. J Lipid Res 1990;31:1149–72.
[39] McNamara DJ. The impact of egg limitations on coronary heart disease risk: do the numbers add up? J Nutr 2000;19:540S–8S.
[40] Herron KL, Fernandez ML. Are the current dietary guidelines regarding egg consumption appropriate? J Nutr 2004;134(1):187–90.
[41] Griffin BA. Lipoprotein atherogenicity: an overview of current mechanisms. Proc Nutr Soc 1999;58(1):163–9.
[42] Dreon DM, et al. LDL subclass patterns and lipoprotein response to a low-fat, high-carbohydrate diet in women. Arterioscler Thromb Vasc Biol 1997;17:707–14.
[43] Jong MC, Hofker MH, Havekes LM. Role of ApoCs in lipoprotein metabolism. Functional differences between apoC1, apoC2, and apoC3. Artherioscler Thromb Vasc Biol 1999;19:472–84.
[44] Sacks FM, et al. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the cholesterol and recurrent events (CARE) trial. Circulation 2000;102:1886–92.
[45] Ichikawa T, et al. Overexpression of lipoprotein lipase in transgenic rabbits leads to increased small dense LDL in plasma and promotes atherosclerosis. Lab Invest 2004;84:715–26.
[46] Packard CJ. Triglyceride-rich lipoproteins and the generation of small, dense low-density lipoproteins. Biochem Soc Trans 2003;31(5): 1066–9.
[47] Breyer ED, et al. Apolipoprotein C-III displacement of apolipoprotein E from VLDL: effect of particle size. J Lipid Res 1999;40:1875–82.
[48] Blackett PR, et al. Apolipoprotein C-III bound to apolipoprotein B-containing lipoproteins in obese girls. Clin Chem 2003;49(2):303–6.
[49] Kwiterovich POJ. Clinical relevance of the biochemical, metabolic, and genetic factors that influence low-density lipoprotein heterogeneity. Am J Cardiol 2002;90(8A):30i–47i.
[50] Sloan AE. The top 10 functional food trends 2004. Food Technol 2004;58(4):28–51.
[51] Committee AS. AHA scientific statement: summary of the scientific conference on dietary fatty acids and cardiovascular health. J Nutr 2001;131:1322–6.
[52] Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr 2004;79:537–43.