Original Contributions

Fasting Insulin and Apolipoprotein B Levels and Low-Density Lipoprotein Particle Size as Risk Factors for Ischemic Heart Disease

Benoît Lamarche, PhD; André Tchernof, PhD; Pascale Mauriège, PhD; Bernard Cantin, MD;
Gilles R. Dagenais, MD; Paul J. Lupien, MD; Jean-Pierre Després, PhD

Context.—Epidemiological studies have established a relationship between cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations and the risk of ischemic heart disease (IHD), but up to half of patients with IHD may have cholesterol levels in the normal range.

Objective.—To assess the ability to predict the risk of IHD using a cluster of nontraditional metabolic risk factors that includes elevated fasting insulin and apolipoprotein B levels as well as small, dense LDL particles.

Design.—Nested case-control study.

Setting.—Cases and controls were identified from the population-based cohort of the Québec Cardiovascular Study, a prospective study conducted in men free of IHD in 1985 and followed up for 5 years.

Participants.—Incident IHD cases were matched with controls selected from among the sample of men who remained IHD free during follow-up. Matching variables were age, smoking habits, body mass index, and alcohol consumption. The sample included 85 complete pairs of nondiabetic IHD cases and controls.

Main Outcome Measures.—Ability of fasting insulin level, apolipoprotein B level, and LDL particle diameter to predict IHD events, defined as angina, coronary insufficiency, nonfatal myocardial infarction, and coronary death.

Results.—The risk of IHD was significantly increased in men who had elevated fasting plasma insulin and apolipoprotein B levels and small, dense LDL particles, compared with men who had normal levels for 2 of these 3 risk factors (odds ratio [OR], 5.9; 95% confidence interval [CI], 2.3-15.4). Multivariate adjustment for LDL-C, triglycerides, and high-density lipoprotein cholesterol (HDL-C) did not attenuate the relationship between the cluster of nontraditional risk factors and IHD (OR, 5.2; 95% CI, 1.7-15.7). On the other hand, the risk of IHD in men having a combination of elevated LDL-C and triglyceride levels and reduced HDL-C levels was no longer significant (OR, 1.4; 95% CI, 0.5-3.5) after multivariate adjustment for fasting plasma insulin level, apolipoprotein B level, and LDL particle size.

Conclusion.—Results from this prospective study suggest that the measurement of fasting plasma insulin level, apolipoprotein B level, and LDL particle size may provide further information on the risk of IHD compared with the information provided by conventional lipid variables.

JAMA. 1998;279:1955-1961

OVER THE LAST 30 years, several epidemiological studies have reported a direct relationship between total plasma cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations and the risk of coronary artery disease (CAD),[1,2] and elevated total plasma cholesterol levels are considered by many to be the main cause of coronary atherosclerosis. However, the ability to adequately identify individuals at high risk for the development of CAD solely on the basis of total cholesterol or LDL-C concentration has recently been challenged by evidence suggesting that a considerable proportion of patients with CAD may have cholesterol levels in the normal range (Genest et al[3] reported the proportion to be as high as 50%).[4] There are also data to suggest that a notable proportion of patients undergoing cholesterol-lowering therapy and who achieve significant LDL-C reduction may still develop CAD.[5] These observations have emphasized the need to find additional markers of risk that would allow a more refined identification of individuals at high risk for CAD.

Clinical data have provided evidence that elevated plasma triglyceride levels and reduced high-density lipoprotein cholesterol (HDL-C) concentrations may be associated with a considerable increase in CAD risk. Although the independent contribution of plasma triglycerides to CAD remains controversial,[6] the clinical relevance of elevated triglyceride levels should no longer be overlooked as hypertriglyceridemia may reflect additional metabolic disturbances highly predictive of CAD risk.[7] Results from the Helsinki Heart Study[8] and from the Prospective Cardiovascular Münster

(PROCAM) study[9] have suggested that hypertriglyceridemia should be considered an important risk factor for CAD, particularly when combined with elevated LDL-C and reduced HDL-C concentrations. This cluster of risk factors may represent the metabolic condition most predictive of CAD risk.[10]

With the 5-year prospective data from the Québec Cardiovascular Study, we have recently reported that elevated fasting plasma insulin levels,[11] elevated apolipoprotein B concentrations,[12,13] and the presence of small, dense LDL particles[14] were strongly associated with the development of ischemic heart disease (IHD) in men, independent of established risk factors. Plasma LDL-C, triglyceride, and HDL-C levels were also significant correlates of IHD in the Québec Cardiovascular Study.[7,12,13,15] In the current study, we investigate whether the ability to identify individuals at high risk for the development of IHD could be improved by measuring 3 nontraditional risk factors, namely fasting plasma insulin and apolipoprotein B levels and LDL particle diameter, over and beyond what can be achieved using more traditional lipid risk factors, triglyceride, LDL-C, and HDL-C levels.

METHODS

Study Population and Follow-up

The Québec Cardiovascular Study cohort has been described in detail previously.[13,16] In 1973, a random sample of 4637 men aged 35 to 64 years was recruited from 7 suburbs of the Québec metropolitan area for an evaluation of cardiovascular risk factors using the provincial electoral lists. Subsequent evaluations were performed at regular intervals and data collected in 1985 were used as the baseline characteristics for the present prospective analyses. In 1985, 2443 (61%) of the living cohort came to the lipid clinic in a fasting state for their evaluation. Among the 1557 other potential living subjects, 150 (10%) could not be located, 302 (19%) came to the clinic in a nonfasting state, and 1105 (71%) either refused to participate or were evaluated in a nonfasting state at their home by project nurses. Analyses of data collected in 1973 revealed that the age distribution of the 2443 subjects in 1985 was representative of the original cohort. At the end of follow-up (September 1, 1990), all subjects were contacted by mail and invited to answer a short standardized questionnaire on smoking habits, medication use, history of cardiovascular disease, and diabetes mellitus. For those who reported such diseases and those who died, hospital charts were reviewed. Telephone calls were made to subjects who did not answer a second letter and if the call was unsuccessful, another call was made to a close family member. Mortality and morbidity data were obtained in 99% and 96%, respectively, of the subjects of the initial 1973 screening.

Evaluation of Risk Factors

Data on demographic and lifestyle variables as well as medical history and medication were obtained in 1985 through a standardized questionnaire administered to each subject by trained nurses and further reviewed by a physician. Body weight and height were recorded. Resting blood pressure was measured after a 5-minute rest in a sitting position. The mean of 2 blood pressure measures taken 5 minutes apart was used in the analyses. Information on personal and family history of IHD and diabetes mellitus, smoking habits, alcohol consumption, and medication use was also obtained. Diabetes mellitus was considered in men who self-reported the disease or who were treated with hypoglycemic agents. Only 2% of men were using hypolipidemic drugs in 1985 (mainly clofibrate and cholestyramine), whereas 8% and 4% of men were using β-blockers and diuretics, respectively, on a regular basis at the 1985 screening. Data on drug use at the time of follow-up were not available. Alcohol consumption was computed from the type of beverage (beer, wine, or spirits) consumed in ounces per week and then standardized as an absolute quantity (1 oz of absolute alcohol was equivalent to 22.5 g of alcoholic beverage). Family history of IHD was considered positive if at least 1 parent or 1 sibling had a history of IHD.

Definition of IHD Events

The diagnosis of a first IHD event included typical effort angina, coronary insufficiency, nonfatal myocardial infarction, and coronary death. All myocardial infarction cases met the criteria previously described,[16] namely diagnostic electrocardiographic (ECG) changes alone or 2 of the following criteria: typical chest pain of at least 20 minutes in duration, creatine kinase enzyme level at least twice the upper limit of normal, or characteristic ECG changes. Coronary insufficiency was considered if typical retrosternal chest pain of at least 15 minutes in duration was associated with transient ischemic ECG changes but without significant elevation in levels of creatine kinase. Diagnoses of myocardial infarction and coronary insufficiency were confirmed by hospital charts. All ECG tracings were read by the same cardiologist, who was unaware of the subjects' risk profiles. The diagnosis of effort angina was based on typical symptoms of retrosternal squeezing or pressure-type discomfort occurring on exertion and relieved by rest and/or nitroglycerine. Criteria for the diagnosis of coronary death included confirmation from death certificate or autopsy report confirming the presence of coronary disease without evidence for noncardiac disease that could explain death. Myocardial infarction was considered fatal if death occurred within 4 weeks of the initial event or if it was diagnosed at autopsy. Deaths related to IHD were confirmed from the Provincial Death Registry. Informed consent was obtained to review relevant hospital files. Autopsies were performed in about one third of deaths. The total IHD event frequency during the 5-year follow-up period was similar in men participating in the study (5.4%) and in nonparticipants (6.5%).

Pairing Procedures

Between 1985 and 1990, 114 of the 2103 men who had no clinical evidence of IHD at baseline had a first IHD event: 50 had a myocardial infarction, 40 had effort angina, 9 had coronary insufficiency, and 15 died of IHD-related causes. Each case subject was matched with a control subject selected from among the remaining 1989 men without IHD during follow-up. Subjects were matched on the basis of age, cigarette smoking, body mass index, and weekly alcohol intake. The mean difference within pairs was 0.6 years, 0.2 kg/m², and 0.2 oz/wk for age, body mass index, and alcohol intake, respectively. The mean difference within pairs for cigarette smoking was 0.3 cigarettes per day. Subjects who had an IHD event and who were classified as nonsmokers were systematically matched with nonsmoking control-group subjects.

Laboratory Analyses

Fasting lipoprotein lipid and apolipoprotein levels were measured in plasma in 1985 when subjects came to the clinic for evaluation. Aliquots of fasting plasma were frozen at the time of collection and were later used for the assessment of LDL diameter and fasting insulin concentrations. Total cholesterol and triglyceride levels were determined on a multianalyzer (Technicon RA-500, Bayer Corp, Tarrytown, NY) as previously described.[17] High-density lipoprotein cholesterol was measured in the supernatant fraction after precipitation of apolipoprotein B–containing lipoproteins with heparin–manganese chloride.[18] Low-density lipoprotein cholesterol levels were estimated by the equation of Friedewald et al.[19] Subjects with triglyceride levels higher than 4.5 mmol/L (399 mg/dL) (n = 52) were excluded from the analyses.[13] Plasma apolipoprotein B levels were measured by the rocket immunoelectrophoresis method of Laurell,[20] as described previously.[17] Serum standards for the apolipoprotein assay were prepared in the laboratory and calibrated

Risk Factors in Ischemic Heart Disease—Lamarche et al

against serum samples from the Centers for Disease Control and Prevention. The standards were lyophilized and stored at –85°C until use. The coefficients of variation for cholesterol, HDL-C, triglyceride, and apolipoprotein B measurements were less than 3%.

Low-density lipoprotein particle diameter was assessed using nondenaturing 2% to 16% polyacrylamide gradient gel electrophoresis of whole plasma according to Krauss et al[21] and McNamara et al,[22] as described previously.[23] Plasma samples were applied on gels in a final concentration of 20% sucrose and 0.25% bromophenol blue. Following a 15-minute pre-run, electrophoresis was performed at 200 V for 12 to 16 hours and at 400 V for 2 to 4 hours. Gels were stained with Sudan black B according to standardized procedures and stored in a solution of 9% acetic acid and 20% methanol until analysis using an optical densitometric image analyzer (BioImage Visage 110 1DGEL, Genomic Solutions, Ann Arbor, Mich) coupled with a computer (SPARC Station 2 Sun, Genomic Solutions). Low-density lipoprotein diameter was estimated by comparing the migration distance on the gel of the predominant LDL subspecies for each individual with the migration distance of standards of known diameters. One assay was performed for each subject. Analyses of pooled plasma standards revealed that the assessment of LDL diameter using this method was highly reproducible with a coefficient of variation of less than 3% (A.T., unpublished data, 1996).

Fasting plasma insulin concentrations were measured with a commercial double-antibody radioimmunoassay (human insulin-specific radioimmunoassay method; Linco Research, St Louis, Mo) according to the manufacturer protocol. This assay shows essentially no cross-reactivity with human proinsulin (<0.2%). The coefficient of variation was below 5.5% for both low and high fasting insulin concentrations.[11]

Statistical Analyses

Fasting insulin levels and LDL diameter were measured in 106 and 103 case-control pairs, respectively,[11,14] but data for both variables were available simultaneously in 100 controls and 102 cases. Men who reported having diabetes mellitus or who were receiving hypoglycemic therapy at the baseline evaluation were excluded (15 cases and 1 control). We therefore had data on 87 IHD cases and 99 controls. After excluding all pairs for which 1 of the 2 subjects had missing data, the study sample included 85 complete pairs of IHD cases and matched controls. Baseline characteristics of subjects who developed IHD during the 5-

year follow-up (IHD cases) were compared with the characteristics of those who remained IHD free using paired t tests for means and χ^2 tests for frequency data. Variables with a skewed distribution were log-transformed. Correlation analyses were performed using the Pearson and the Spearman coefficients of correlation for parametric and nonparametric variables, respectively.

The median of the control group was used as the cutoff point to identify men with elevated or low levels of each variable of interest (LDL-C, 3.7 mmol/L [143 mg/dL]; triglycerides, 1.52 mmol/L [135 mg/dL]; apolipoprotein B, 1.1 g/L [110 mg/dL]; fasting insulin, 72 pmol/L [10 μU/mL]; HDL-C, 1.01 mmol/L [39 mg/dL]; LDL particle diameter, 25.82 nm). Thus, by definition, each of these risk factors was found in 50% of the control subjects. The proportion of cases classified as having 1 or more risk factor based on these arbitrary cutoff points was compared with that of control subjects. The proportional hazards regression (PHREG) procedure on SAS (SAS Institute, Cary, NC) for conditional logistic regression analysis was used to estimate the odds ratio (OR) for IHD associated with the presence of each risk factor, as an isolated condition or combined with others. Odds ratios were adjusted for medication use at baseline (β-blockers and/or diuretics), family history, and systolic blood pressure. The potential confounding effects of using β-blockers and diuretics were combined because they both yielded similar risk. Thus, medication use (yes or no) and family history (presence or absence) were

treated as categoric variables whereas systolic blood pressure was treated as continuous.

RESULTS

Table 1 presents the clinical characteristics of the 85 controls and IHD cases. A higher proportion of case patients was using β-blockers and/or diuretics on a regular basis at baseline (17.7% vs 4.7%, P = .007). However, there was no difference between cases and controls in the use of hypolipidemic medication at baseline. As a result of the matching procedure, the frequency of smokers (41%) and the number of cigarettes smoked per day (25 cigarettes per day) were essentially the same in both groups. Systolic blood pressure was also the same in both groups. As expected, there were marked differences in several plasma lipoprotein-lipid parameters as well as in fasting insulin levels at baseline between IHD cases and controls. Triglycerides (18.2%), fasting insulin (18.9%), and apolipoprotein B (15.9%) levels showed the largest case-control differences. Mean plasma HDL-C concentrations and LDL diameter were also significantly different between cases and controls (P = .03). It is important to note that although being tightly matched with IHD cases on the basis of age, body mass index, smoking, and alcohol consumption, the risk profile of control subjects in the current study is very similar to that of the total sample of men who remained free of IHD during follow-up[13] and from which they were selected.

Table 1.—Baseline Characteristics of IHD Cases and Matched Controls*

Characteristics	Controls (N = 85)	IHD Cases (N = 85)	Difference, %†	P Value‡
Age, mean (SD), y	58.5 (7.0)	59.0 (7.7)	. . .	Matched
BMI, mean (SD), kg/m²	26.0 (3.4)	26.2 (3.8)	. . .	Matched
Cigarettes per day, mean (SD)§	24.7 (13.4)	25.3 (12.9)	. . .	Matched
Alcohol intake, mean (SD), oz/wk	5.7 (8.6)	5.3 (8.0)	. . .	Matched
Medication users, No. (%)‖	4 (4.7)	15 (17.7)	13.0	.007
Family history of IHD, No. (%)	45 (52.9)	53 (62.4)	9.5	.21
Systolic BP, mean (SD), mm Hg	133 (19)	135 (17)	1.5	.55
Total cholesterol, mean (SD), mmol/L	5.6 (1.0)	6.1 (1.1)	8.9	<.001
LDL-C, mean (SD), mmol/L	3.8 (0.9)	4.2 (1.0)	10.5	.001
TG, mean (SD), mmol/L	1.70 (0.69)	2.01 (0.74)	18.2	.001
HDL-C, mean (SD), mmol/L	1.03 (0.26)	0.95 (0.23)	–7.8	.03
Fasting insulin, mean (SD), pmol/L	77.6 (27.4)	92.3 (28.3)	18.9	<.001
Apo B, mean (SD), g/L	1.1 (0.27)	1.3 (0.33)	15.9	<.001
LDL diameter, mean (SD), nm	25.75 (0.80)	25.60 (0.51)	–0.6	.03

*IHD indicates ischemic heart disease; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; apo B, apolipoprotein B; and ellipses, data not applicable. IHD cases are men who developed IHD during the 5-year follow-up. To convert total cholesterol, LDL-C, and HDL-C from millimoles per liter to milligrams per deciliter, divide by 0.02586. To convert triglycerides from millimoles per liter to milligrams per deciliter, divide by 0.01129. To convert fasting insulin from picomoles per liter to microunits per milliliter, divide by 7.175. To convert apolipoprotein B from grams per liter to milligrams per deciliter, divide by 0.01.
†Relative difference between cases and controls.
‡Matched indicates variables that were used in the pairing procedure. Differences in triglyceride levels and LDL particle diameters between cases and controls were tested with the Wilcoxon signed rank test for nonparametric variables. Other variables were tested with paired t tests.
§Cigarettes smoked per day by smokers only (n = 35 IHD cases and controls).
‖Medication users includes men taking β-blockers and/or diuretics on a regular basis at the 1985 screening.

Risk Factors in Ischemic Heart Disease—Lamarche et al **1957**

Table 2.—Prevalence of Traditional and Nontraditional Risk Factors Among Cases and Associated IHD Risk*

Risk Factors	Prevalence in Cases, %	OR (95% CI)†	P Value
Nontraditional			
Elevated fasting insulin	81.2	5.5 (2.3-13.6)	<.001
Elevated apo B	69.4	2.7 (1.2-6.0)	.01
Small dense LDL	69.4	2.5 (1.2-5.2)	.01
Traditional			
Elevated LDL-C	68.2	2.4 (1.1-5.1)	.03
Elevated TG	76.5	3.5 (1.6-7.4)	.002
Reduced HDL-C	62.4	1.6 (0.85-3.0)	.15

*IHD indicates ischemic heart disease; OR, odds ratio; CI, confidence interval; apo B, apolipoprotein B; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; and HDL-C, high-density lipoprotein cholesterol. Abnormalities are based on the 50th percentile value of controls for each variable (fasting insulin, 72 pmol/L [10 µU/mL]; apo B, 1.1 g/L [110 mg/dL]; LDL particle diameter, 25.82 nm; LDL-C, 3.7 mmol/L [143 mg/dL]; TG, 1.52 mmol/L [135 mg/dL]; and HDL-C, 1.01 mmol/L [39 mg/dL]). Each risk factor was present in 50% of controls.

†Odds ratios were obtained using conditional logistic regression and represent the odds of developing IHD during the 5-year follow-up in exposed individuals (presence of risk factors) compared with nonexposed participants (absence of risk factors). Odds ratios are adjusted for the potential confounding effects of systolic blood pressure, medication use, and family history of IHD.

Prevalence of cumulative number of risk factors in 85 pairs of ischemic heart disease (IHD) cases and controls. The upper panel shows the proportion of IHD cases and controls with 1 or more of the nontraditional metabolic risk factors (elevated fasting plasma insulin levels, elevated apolipoprotein B levels, and small, dense low-density lipoprotein [LDL] particles) compared with subjects having none of these risk factors. The lower panel presents the frequency of cases and controls with 1 or more of the traditional risk factors (elevated low-density lipoprotein cholesterol [LDL-C] and triglyceride levels and reduced high-density lipoprotein cholesterol [HDL-C] levels) compared with subjects having none of these risk factors. The presence or absence of risk factors is based on the 50th percentile value of controls for each variable (fasting insulin, 72 pmol/L [10 µU/mL]; apolipoprotein B, 1.1 g/L [110 mg/dL]; LDL particle diameter, 25.82 nm; LDL-C, 3.7 mmol/L [143 mg/dL]; triglycerides, 1.52 mmol/L [135 mg/dL]; and HDL-C, 1.01 mmol/L [39 mg/dL]).

Prevalence of Lipoprotein and Insulin Abnormalities

Because there are currently no reference values for apolipoprotein B and insulin levels and for LDL diameter, and in an attempt to compare the contribution to IHD risk of variables having different scales, lipoprotein-lipid and fasting insulin levels were dichotomized using the median (50th percentile) of the control group. Table 2 presents the prevalence of each of the metabolic abnormalities in IHD cases. Based on these prevalences, ORs for developing IHD during the 5-year follow-up were estimated using conditional logistic regression while taking into consideration the potential confounding effects of systolic blood pressure, medication use, and family history of IHD. Eighty-one percent of cases had elevated fasting insulin concentrations based on these criteria, yielding a 5.5-fold increase in the OR for IHD (95% confidence interval [CI], 2.3-13.6, P<.001) compared with men having insulin levels below the 50th percentile of controls. Elevated plasma triglyceride levels were also associated with a marked increase in the risk of IHD (OR, 3.5; 95% CI, 1.6-7.4; P = .002). Elevated apolipoprotein B and LDL-C levels and small, dense LDL particles were observed in a similar proportion of cases (69.4%, 68.2%, and 69.4%, respectively). These 3 abnormalities were associated with a significant 2.4-fold to 2.7-fold increase in the OR for IHD. Finally, 62.4% of IHD cases had HDL-C levels below the 50th percentile of controls. There was a 60% increase in the risk of IHD associated with reduced HDL-C levels (OR, 1.6), which was not significant after adjustment for confounders (95% CI, 0.85-3.0). This analysis did not take into consideration the fact that cases with 1 abnormality may also have had additional metabolic abnormalities in combination. Nevertheless, results presented in Table 2 suggest that among all variab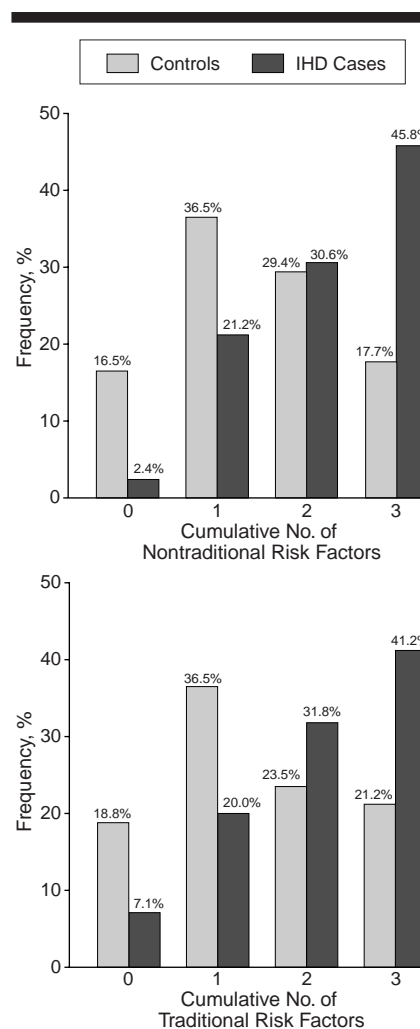les of interest, elevated fasting plasma insulin concentrations, irrespective of the presence or absence of other lipoprotein abnormalities, were associated with the greatest relative increase in the risk of IHD.

Prevalence of Isolated Abnormalities

The prevalence rates of elevated plasma fasting insulin and apolipoprotein B levels as well as of small, dense LDL in their isolated form (ie, associated with none of the other 2 abnormalities) were low in both IHD cases and control subjects. Isolated hyperinsulinemia was observed in only 11 (12.9%) of both IHD cases and controls. However, when considering only subjects with elevated fasting insulin levels (42 controls and 69 cases), 11 (15.9%) of 69 hyperinsulinemic IHD cases did not have elevated apolipoprotein B levels or small, dense LDL in combination compared with 11 (26.6%) of 42 controls. Only 2 (2.4%) of 85 IHD cases had isolated elevations in apolipoprotein B levels compared with 9 (10.6%) of 85 controls. Finally, the small, dense LDL phenotype in its isolated form was found in only 5 (5.9%) of 85 IHD cases. In comparison, twice as many controls (11 [12.9%] of 85) had small, dense LDL in isolation. These results suggest that hyperinsulinemia, elevated apolipoprotein B levels, and small, dense LDL particles may be observed more frequently in combination with each other rather than as isolated conditions, and that a smaller proportion of IHD cases may display these abnormalities in their isolated form compared with controls. We therefore tested whether the cluster of these metabolic risk factors may further increase the risk of IHD.

Prevalence of Nontraditional Risk Factors

The Figure compares the prevalence rates of the cumulative number of abnormalities in IHD cases and controls. To simplify data presentation, fasting plasma insulin levels, apolipoprotein B levels, and small, dense LDL particles are referred to as *nontraditional* risk factors, whereas LDL-C, triglyceride, and HDL-C levels are referred to as *traditional* risk factors. As shown in the Figure (top), only 2 IHD cases (2.4%) had none of the 3 nontraditional metabolic risk factors, compared with 14 controls (16.5%). One of every 5

Risk Factors in Ischemic Heart Disease—Lamarche et al

IHD cases (n = 18, 21.2%) had 1 of the nontraditional risk factors in its isolated form, compared with more than a third of controls (n = 31, 36.5%). The proportion of cases that simultaneously had elevated fasting insulin levels, elevated apolipoprotein B levels, and small, dense LDL particles (cumulative number of risk factors, 3) was 2.6-fold greater than that of controls (45.8% vs 17.7%). Consequently, 98% of IHD cases had at least 1 of the nontraditional risk factors compared with 83% of controls. On the other hand, 82% of controls did not have elevated fasting plasma insulin levels, elevated apolipoprotein B levels, and small, dense LDL simultaneously, compared with 54% of IHD cases.

Prevalence of Traditional Risk Factors

A similar analysis was performed using the traditional risk factors (LDL-C, triglycerides, and HDL-C levels) as discriminating variables for the determination of IHD risk (Figure, bottom). Although differences in the proportion of cumulative number of traditional risk factors between IHD cases and controls were slightly attenuated compared with differences in the proportion of nontraditional risk factors, a similar pattern of distribution was observed. There was a greater proportion of controls that had relatively low LDL-C and triglyceride levels and high HDL-C levels (number of risk factors, 0) compared with IHD cases (18.8% vs 7.1%), whereas the proportion of IHD cases that had elevated LDL-C and triglyceride levels and low HDL-C concentrations simultaneously (cumulative number of risk factors, 3) was 1.9-fold greater than that of controls (41.2% vs 21.2%).

Risk of Developing IHD During Follow-up

Based on the prevalence of the cumulative number of risk factors presented in the Figure, the crude OR for developing IHD during the 5-year follow-up was increased 18.2-fold in subjects who had all 3 nontraditional risk factors simultaneously compared with those who had none of the 3 risk factors (results not shown). By comparison, the OR for IHD in subjects with the 3 traditional risk factors simultaneously was 5.2 (not shown). Multivariate conditional logistic regression analysis was performed to compare the ability to predict IHD using traditional and nontraditional risk factors. The prevalence of IHD cases in subjects with no risk factor (2 and 6 IHD cases for nontraditional and traditional risk factors, respectively) was too small to accurately assess the risk of IHD using this group as a reference. We have therefore performed the multivariate logistic regression analysis by combining subjects with 0 and 1 risk factor only, and by using this group as a reference (OR, 1). As shown in Table 3, subjects that had elevated LDL-C and triglyceride levels and reduced HDL-C concentrations simultaneously (cumulative number of traditional risk factors, 3) showed a 3-fold increase in the risk of IHD (model 1: OR, 3.0; 95% CI, 1.4-6.4; $P = .005$) compared with men having none or only 1 of these risk factors. This increased risk was no longer significant after multivariate adjustment for fasting insulin and apolipoprotein B levels and LDL particle diameter (model 2: OR, 1.4; 95% CI, 0.5-3.5; $P = .50$).

The impact of having elevated fasting insulin and apolipoprotein B levels and small, dense LDL particles in combination with each other on the odds of developing IHD was more prominent. The risk of developing IHD was increased almost 6-fold when subjects simultaneously had elevated fasting insulin and apolipoprotein B levels and small, dense LDL particles (model 3: OR, 5.9; 95% CI, 2.3-15.4; $P<.001$). This increase in risk was essentially unmodified when LDL-C, triglyceride, and HDL-C levels were included as confounders in the multivariate logistic regression model (model 4: OR, 5.2; 95% CI, 1.7-15.7; $P = .003$).

An analysis was carried out to test the 2-way and 3-way interaction terms as predictors of IHD risk. It was found that none of the 2-way or 3-way interaction terms for continuous variables were significant. However, because of the small sample size, the possibility of a significant interaction among the 3 nontraditional or the 3 traditional risk factors cannot be excluded.

Univariate associations between the traditional and nontraditional risk factors and the variables that were used to match IHD cases to controls were investigated. Plasma triglyceride levels ($r = 0.15$, $P = .05$) and HDL-C levels ($r = -0.17$, $P = .02$) showed significant associations with body mass index. Plasma triglyceride levels also showed a significant but inverse correlation with age ($r = -0.23$, $P = .003$) whereas HDL-C levels were positively associated with weekly alcohol consumption ($r = 0.26$, $P<.001$). Low-density lipoprotein particle size was also a significant correlate of age ($r = 0.19$, $P = .01$) but the most significant correlation between risk factors and matching variables was observed between plasma fasting insulin concentrations and body mass index ($r = 0.40$, $P<.001$).

COMMENT

Results of the present prospective study emphasize the potential of plasma fasting insulin and apolipoprotein B levels as well as of small, dense LDL particles as clinically relevant markers of the risk of developing IHD. Our results suggest that this cluster of metabolic abnormalities may even provide more information on IHD risk than the more traditional lipid risk factors, LDL-C, triglycerides, and HDL-C. Indeed, almost 1 (45.8%) of every 2 IHD cases had elevated insulin and apolipoprotein B levels as well as small, dense LDL particles, and this combination of metabolic risk factors resulted in a remarkable 18-fold increase in the risk of IHD. Adjustment for the more traditional cluster of risk factors through multivariate logistic regression did not attenuate this relationship. These observations have consequential clinical implications, particularly in terms of primary prevention of IHD. They imply that identifica-

Table 3.—Risk of IHD According to Cumulative Number of Traditional and Nontraditional Risk Factors*

	Cumulative No. of Risk Factors, OR (95% CI)†		
	0 or 1	2	3
Traditional Risk Factors			
No. of cases (No. of controls)	23 (47)	27 (20)	35 (18)
Model 1‡	1 (. . .)	3.1 (1.1-8.6)	3.0 (1.4-6.4)
Model 2‡§	1 (. . .)	2.6 (0.9-7.8)	1.4 (0.5-3.5)
Nontraditional Risk Factors			
No. of cases (No. of controls)	20 (45)	26 (25)	39 (15)
Model 3‡	1 (. . .)	3.7 (1.2-11.2)	5.9 (2.3-15.4)
Model 4‡‖	1 (. . .)	3.0 (0.9-9.8)	5.2 (1.7-15.7)

*Traditional risk factors are low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglyceride levels. Nontraditional risk factors are fasting insulin and apolipoprotein B levels and small, dense low-density lipoprotein. IHD indicates ischemic heart disease; OR, odds ratio; CI, confidence interval; and ellipses, data not applicable.

†The risk of IHD is expressed as the odds ratio (calculated using conditional logistic regression) of developing IHD during the 5-year follow-up in men having 2 or 3 of the traditional and nontraditional metabolic risk factors compared with men having 0 or 1 risk factor. Presence or absence of risk factors is based on the 50th percentile value of controls for each variable (fasting insulin, 72 pmol/L [10 µU/mL]; apolipoprotein B, 1.1 g/L [110 mg/dL]; low-density lipoprotein particle diameter, 25.82 nm; low-density lipoprotein cholesterol, 3.7 mmol/L [143 mg/dL]; triglycerides, 1.52 mmol/L [135 mg/dL]; and high-density lipoprotein cholesterol, 1.01 mmol/L [39 mg/dL].

‡Odds ratios are adjusted for confounding effects of systolic blood pressure, medication use at baseline, and family history of IHD.

§Model is further adjusted for fasting insulin and apolipoprotein B levels (low or elevated) and low-density lipoprotein particle size (small or large) as dichotomic variables using the 50th percentile value of controls.

‖Model is further adjusted for low-density lipoprotein cholesterol, triglyceride, and high-density lipoprotein cholesterol levels (low or elevated) as dichotomic variables using the 50th percentile value of controls.

tion of individuals at risk could be substantially improved by measuring fasting plasma insulin and apolipoprotein B levels and LDL particle diameter. It should be kept in mind that these findings do not in any way lessen the clinical importance of assessing LDL-C, triglyceride, and HDL-C concentrations. The current study should not be considered an attempt to discredit the well-described and accepted relationship between the so-called lipid triad and the risk of IHD.[8-10] It was apparent that an important proportion of IHD cases was characterized by this dyslipidemia compared with controls.

It may be argued that the paired nature of the study population may have had the adverse effect of overmatching for the traditional risk factors, thereby understating their true impact on a randomly selected population. As expected, there were significant correlations between risk factors and some of the variables used to match IHD cases and controls. Although significant, these correlations were of very low magnitude (with shared variances lower than 7%), with the exception of the relationship between plasma fasting insulin levels and body mass index (with a shared variance of 16%). The paired nature of the study is therefore very unlikely to have biased the estimation of the contribution of the traditional risk factors to IHD risk compared with that of the nontraditional risk factors.

We reported that a very small proportion of IHD cases had no risk factor and that abnormalities in insulin and apolipoprotein B levels and in LDL particle diameter were more frequently observed in combination and not in isolation compared with controls. It is therefore apparent that the risk of developing IHD is largely dependent on the presence of risk factors that, in most cases, emerge as a cluster of metabolic abnormalities. In this context, arguments have been proposed for why plasma insulin and apolipoprotein B levels and LDL particle size may represent better markers of IHD risk than LDL-C, triglyceride, and HDL-C levels.

Small, Dense LDL and the Risk of IHD

Plasma LDL-C levels are merely measurements of the cholesterol content of a lipoprotein particle that has been described as being very heterogeneous in terms of composition, size, and density. Although the cholesterol content of LDL certainly contributes to its heterogeneity, we have failed to find a significant association between LDL density or size and LDL-C levels.[14,23] Recognition of the atherogenic potential of small, dense LDL largely came from cross-sectional case-control studies that reported a higher

prevalence of small, dense LDL in patients with IHD compared with healthy controls.[24-26] Observations from 3 recent prospective reports provided further support for a critical role of small, dense LDL particles in the etiology of atherosclerosis.[14,27,28] The greater susceptibility of these particles to oxidation[29] and their reduced affinity for the hepatic LDL receptor[30] have been proposed as potential mechanisms for the increased atherogenic potential of small, dense LDL.

Apolipoprotein B and the Risk of IHD

Apolipoprotein B is the protein moiety of LDL. The clinical interest of this protein lies in the fact that it provides a relatively accurate estimate of circulating LDL particle numbers. Total plasma apolipoprotein B concentration, as opposed to LDL apolipoprotein B, also accounts for the number of triglyceride-rich lipoproteins (very low-density lipoprotein and intermediate-density lipoproteins), and recent data suggest that these 2 lipoprotein subfractions may also play an important role in the etiology of IHD.[31,32] Plasma apolipoprotein B concentration can therefore be considered a crude marker of the number of atherogenic particles in plasma.[33] Results from the Québec Cardiovascular Study suggest that plasma apolipoprotein B concentration is a strong predictor of IHD risk, independent of traditional risk factors.[12,13] It is therefore suggested that apolipoprotein B, as a measure of the number of atherogenic particles in plasma, may yet provide more information than the amount of cholesterol transported by these particles.

Insulin and the Risk of IHD

The concept of insulin resistance as a central component of a potentially atherogenic dyslipidemic state was first introduced in 1988 when it was suggested that a large proportion of individuals resistant to the action of insulin was also characterized by metabolic disturbances highly predictive of an increased IHD risk.[34] Using fasting or postglucose insulin levels as crude indices of insulin resistance, univariate analyses of large cohorts of nondiabetic populations have shown that hyperinsulinemia in the fasting state or following a glucose load was associated with an increased risk of IHD.[35-37] Results from multivariate analyses have, however, yielded discordant conclusions. We[11] and others[38] have recently reported that elevated plasma insulin levels measured with an antibody showing essentially no cross-reactivity with proinsulin were associated with an increased risk of developing IHD, independent of other risk factors such as triglyceride, HDL-C, and LDL-C levels.

Nevertheless, whether plasma insulin should or should not be considered an independent risk factor for the development of IHD remains a matter of considerable debate. It is well accepted, however, that elevated plasma insulin concentrations are most frequently associated with deteriorations in other cardiovascular risk factors.[39] Hyperinsulinemia and insulin resistance also appear to have direct effects on the arterial wall and contribute to a reduced fibrinolytic potential.[40] Plasma insulin levels may therefore provide a crude but global description of a number of additional metabolic abnormalities that may, in turn, be associated with an increased risk of IHD, but that may not be adequately assessed by the traditional triad of lipid risk factors. It is important to emphasize that results of the present study apply to nondiabetic men, particularly because patients with type 2 diabetes mellitus were excluded from the analyses. Although inclusion of men with type 2 diabetes mellitus in the study sample essentially had no impact on the results, whether results of the present study can be applied to other populations such as persons with type 2 diabetes mellitus, women, or the elderly population will have to be established more specifically in future studies.

Conclusions

Beyond the mechanisms underlying the atherogenicity of hyperinsulinemia, hyperapobetalipoproteinemia, and small, dense LDL, and irrespective of whether these mechanisms share common paths, results of the present study suggest that the risk of IHD is increased substantially when these metabolic abnormalities cluster. The synergistic contribution of the nontraditional cluster of risk factors to IHD risk and the fact that almost 1 of every 2 IHD cases had these abnormalities simultaneously reflect the multifactorial etiology of IHD. It also emphasizes the importance of defining the risk of IHD based on more than 1 risk factor.

There are a number of critical issues that have to be considered before any decision can be made toward the measurement of these nontraditional risk factors on a routine basis. Among others, results of this prospective case-control study will have to be confirmed through larger population-based studies, as the relatively low number of IHD cases allowed only a gross assessment of risk. The relatively large CIs associated with the estimated risk in some of the subgroups reflect this phenomenon. Population reference values such as those used for LDL-C, triglycerides, and HDL-C also will be needed before critical levels of fasting insulin, apolipoprotein B levels,

Risk Factors in Ischemic Heart Disease—Lamarche et al

and LDL particle size or density at which a person becomes at greater risk for IHD are identified. Means to achieve effective treatment of the nontraditional risk factors is also a critical issue that deserves a great deal of scrutiny before decisions can be made toward use of these variables in the risk management of IHD. There are data to suggest that LDL particle size can be modulated by changes in plasma triglyceride levels.[41] Studies have shown that triglyceride-lowering therapy with fibric acid derivatives can lead to a significant increase in LDL particle size.[42,43] There is also a large body of evidence demonstrating that LDL particle size, apolipoprotein B level, and insulin resistance and/or hyperinsulinemia can be effectively altered by diet and exercise-induced weight loss.[44,45] Thus, the ability to favorably modify the nontraditional risk factors by diet, exercise, and appropriate pharmacotherapy provides further support for the use of these risk factors in the management of IHD risk. Finally, the cost-effectiveness of implementing and using new risk factors as a basis for screening and treatment in primary and secondary prevention of IHD should be established. Irrespective of these important considerations, we hope that these results will help stimulate research aimed at identifying means that could substantially improve the early diagnosis and treatment of individuals at risk for IHD.

References

1. Castelli WP. Epidemiology of coronary heart disease: the Framingham Study. *Am J Med*. 1984;76: 4-12.
2. Stamler J, Wentworth D, Neaton JD. Is the relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? findings in 356 222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA*. 1986;256:2823-2828.
3. Genest JJ, McNamara JR, Ordovas JM, et al. Lipoprotein cholesterol, apolipoprotein A-I and B and lipoprotein (a) abnormality in men with premature coronary heart disease. *J Am Coll Cardiol*. 1992;19: 792-802.
4. Ginsburg GS, Safran C, Pasternak RC. Frequency of low serum high-density lipoprotein cholesterol levels in hospitalized patients with desirable total cholesterol levels. *Am J Cardiol*. 1991;68: 187-192.
5. Superko HR. Beyond LDL cholesterol reduction. *Circulation*. 1996;94:2351-2354.
6. Austin MA. Plasma triglyceride and coronary heart disease. *Arterioscler Thromb Vasc Biol*. 1991; 11:2-14.
7. Lamarche B, Després JP, Moorjani S, Cantin B, Dagenais GR, Lupien PJ. Triglycerides and HDL-cholesterol as risk factors for ischemic heart disease: results from the Québec Cardiovascular Study. *Atherosclerosis*. 1996;119:235-245.
8. Manninen V, Tenkanen L, Koskinen P, et al. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study: implications for treatment. *Circulation*. 1992;85:37-45.
9. Assmann G, Schulte H. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease: the PROCAM experience. *Am J Cardiol*. 1992;70:733-737.
10. Assmann G, Schulte H. Identification of individuals at high risk for myocardial infarction. *Atherosclerosis*. 1994;110(suppl):S11-S21.
11. Després JP, Lamarche B, Mauriège P, et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med*. 1996;334: 952-957.
12. Lamarche B, Moorjani S, Lupien PJ, et al. Apolipoprotein A-I and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Québec Cardiovascular Study. *Circulation*. 1996;94:273-278.
13. Lamarche B, Després JP, Moorjani S, Cantin B, Dagenais GR, Lupien PJ. Prevalence of dyslipidemic phenotypes in ischemic heart disease: prospective results from the Québec Cardiovascular Study. *Am J Cardiol*. 1995;75:1189-1195.
14. Lamarche B, Tchernof A, Dagenais GR, Cantin B, Lupien PJ, Després JP. Small, dense LDL particles and the risk of ischemic heart disease: prospective results from the Québec Cardiovascular Study. *Circulation*. 1997;95:69-75.
15. Lamarche B, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Després JP. Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men: prospective results from the Québec Cardiovascular Study. *Arterioscler Thromb Vasc Biol*. 1997;17:1098-1105.
16. Dagenais GR, Robitaille NM, Lupien PJ, et al. First coronary heart disease event rates in relation to major risk factors: Québec Cardiovascular Study. *Can J Cardiol*. 1990;6:274-280.
17. Moorjani S, Dupont A, Labrie F, et al. Increase in plasma high density lipoprotein concentration following complete androgen blockage in men with prostatic carcinoma. *Metabolism*. 1987;36:244-250.
18. Albers JJ, Warnick GR, Wiebe D, et al. Multilaboratory comparison of three heparin-MnCl$_2$ precipitation procedures for estimating cholesterol in high-density lipoproteins. *Clin Chem*. 1978;24:323-338.
19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502.
20. Laurell CB. Electroimmunoassay. *Scand J Clin Lab Med*. 1972;124:23-27.
21. Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J Lipid Res*. 1982;23:97-104.
22. McNamara JR, Campos H, Ordovas JM, Peterson J, Wilson PWF, Schaefer EJ. Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution: results from the Framingham Offspring Study. *Arterioscler Thromb Vasc Biol*. 1987;7:483-490.
23. Tchernof A, Lamarche B, Nadeau A, et al. The dense LDL phenotype: association with plasma lipoprotein levels, visceral obesity and hyperinsulinemia in men. *Diabetes Care*. 1996;19:629-637.
24. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willet WC, Krauss RM. Low density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA*. 1988;260:1917-1921.
25. Campos H, Genest JJ, Blijlevens E, et al. Low density lipoprotein particle size and coronary artery disease. *Arterioscler Thromb Vasc Biol*. 1992; 12:187-195.
26. Coresh J, Kwiterovich PO, Smith HH, Bachorik PS. Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women. *J Lipid Res*. 1993;34:1687-1697.
27. Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. *JAMA*. 1996;276:875-881.
28. Stampfer MJ, Krauss RM, Ma J, et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA*. 1996;276:882-888.
29. de Graaf J, Hak Lemmers HL, Hectors MP, Demacker PNM, Hendriks JC, Stalenhoef AF. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arterioscler Thromb Vasc Biol*. 1991;11: 298-306.
30. Nigon F, Lesnik P, Rouis M, Chapman MJ. Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. *J Lipid Res*. 1991;32:1741-1753.
31. Hodis HN, Mack WJ, Azen SP, et al. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin. *Circulation*. 1994;90:42-49.
32. Davignon J, Cohn JS. Triglycerides: a risk factor for coronary heart disease. *Atherosclerosis*. 1996;124(suppl):S57-S64.
33. Sniderman AD, Cianflone K. Measurement of apoproteins: time to improve the diagnosis and treatment of the atherogenic dyslipoproteinemias. *Clin Chem*. 1996;42:489-491.
34. Reaven GM. Role of insulin resistance in human disease. *Diabetes*. 1988;37:1495-1507.
35. Yarnell JWG, Sweetnam PM, Marks V, Teale JD, Bolton CH. Insulin in ischaemic heart disease: are associations explained by triglyceride concentrations? the Caerphilly Prospective Study. *Br Heart J*. 1994;71:293-296.
36. Pyörälä K. Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. *Diabetes Care*. 1979;2:131-141.
37. Eschwège E, Richard JL, Thibult N, et al. Coronary heart disease mortality in relation with diabetes, blood glucose and plasma insulin levels: the Paris prospective study, ten years later. *Horm Metab Res*. 1985;15:41-46.
38. Perry IJ, Wannamethee SG, Whincup PH, et al. Serum insulin and incident coronary heart disease in middle-aged British men. *Am J Epidemiol*. 1996; 144:224-234.
39. Haffner SM, Mykkänen L, Stern MP, Valdez RA, Heisserman JA, Bowsher RR. Relationship of proinsulin and insulin to cardiovascular risk factors in nondiabetic subjects. *Diabetes*. 1993;42:1297-1302.
40. Juhan-Vague I, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels: a possible link between insulin resistance and atherothrombosis. *Diabetologia*. 1991;34:457-462.
41. McNamara JR, Jenner JL, Li Z, Wilson PW, Schaefer EJ. Change in LDL particle size is associated with change in plasma triglyceride concentration. *Arterioscler Thromb Vasc Biol*. 1992;12:1284-1290.
42. Yuan JN, Tsai MY, Hunninghake DB. Changes in composition and distribution of LDL subspecies in hypertriglyceridemic and hypercholesterolemic patients during gemfibrozil therapy. *Atherosclerosis*. 1994;110:1-11.
43. Guerin M, Bruckert E, Dolphin PJ, Turpin G, Chapman MJ. Fenofibrate reduces plasma cholesteryl ester transfer from HDL to VLDL and normalizes the atherogenic, dense LDL profile in combined hyperlipidemia. *Arterioscler Thromb Vasc Biol*. 1996;16:763-772.
44. Williams PT, Krauss RM, Vranizan KM, Wood PD. Changes in lipoprotein subfractions during diet-induced and exercise-induced weight loss in moderately overweight men. *Circulation*. 1990;81:1293-1304.
45. Després JP, Lamarche B. Effects of diet and physical activity on adiposity and body fat distribution: implications for the prevention of cardiovascular disease. *Nutr Res Rev*. 1993;6:137-159.