Low-Density Lipoprotein Particle Size Is Not a Discriminating Marker for Atherogenic Risk in Male Offspring of Parents With Early Coronary Artery Disease

Arnold H. Slyper, Svetlana Zvereva, Gordon Schectman, Raymond G. Hoffmann, and John A. Walker

The aim of this study was to assess the importance of low-density lipoprotein (LDL) particle size as a marker of atherogenic risk in male offspring of a parent with early coronary artery disease (CAD) before the age of 60 years. CAD-positive (CAD^+) offspring were recruited into two groups based on age, 15 to 30 years (n = 20) and 31 to 45 years (n = 41), and matched to CAD-negative (CAD^-) offspring by age and body mass index (BMI) (n = 20 and 21 per group). LDL peak particle diameter was assessed by polyacrylamide gradient gel electrophoresis. There was no significant difference in LDL peak particle diameter between CAD^+ and CAD^- offspring (26.2 ± 0.1 *v* 26.2 ± 0.1 nm, mean ± SE). There was also no difference between CAD^+ offspring and CAD^- offspring when comparisons were made within their own age group (26.5 ± 0.1 nm in younger CAD^+ offspring *v* 26.2 ± 0.1 nm in younger CAD^- offspring, and 26.0 ± 0.1 nm in older CAD^+ offspring *v* 26.1 ± 0.2 nm in older CAD^- offspring). Peak particle diameter was significantly greater in younger CAD^+ offspring than in older CAD^+ offspring (26.5 ± 0.1 *v* 26.0 ± 0.1 nm, $P < .05$). We conclude that small LDL particle size is not a discriminating marker for early atherogenic risk, and that measurement of LDL particle size has limited value in the assessment of coronary risk, at least in the age ranges we studied.

LOW-DENSITY LIPOPROTEIN (LDL) particles are nonhomogeneous in terms of their size, density, and composition. LDL has been categorized into two phenotypes based on the peak particle diameter of LDL particles following nondenaturing gradient gel electrophoresis: type A, in which peak particle diameter is greater than 25.5 nm, and type B, with a peak particle diameter less than 25.5 nm.[1] An intermediate pattern with a peak particle diameter of 25.2 to 25.5 nm has also been suggested.[2] Austin et al[3] established that the type B LDL subclass containing small, dense LDL particles is a marker for an "atherogenic" lipoprotein phenotype, consisting of increased concentrations of triglyceride, apolipoprotein B, LDL, and intermediate-density lipoprotein and decreased concentrations of high-density lipoprotein (HDL) cholesterol, HDL_2, and apolipoprotein A-1. A strong genetic component to LDL subclass phenotype has also been proposed.[4,5]

Atherogenesis may begin early in life. Fatty streaks are common in postmortem adolescent coronary vessels.[6] More advanced lesions continue to form throughout early adulthood, and by age 29 years 34% of autopsy specimens show advanced atherosclerotic lesions.[6] In the Pathobiological Determinants of Atherosclerosis in Youth Study in which autopsy specimens of 15- to 34-year-old males were examined, coronary lesions were also shown to increase significantly with age.[7]

There is a strong genetic tendency to coronary artery disease (CAD),[8-10] and family studies have been useful for elucidating the importance of the major genetic coronary risk factors.[11-17] To assess the significance of LDL particle size as a marker for atherogenic risk, we examined LDL peak particle size in male offspring of a parent with significant CAD at or before age 60 years. Because of the known influence of age on LDL particle size,[18,19] we recruited CAD-positive (CAD^+) offspring into two age groups, younger offspring aged 15 to 30 years and older offspring aged 31 to 45 years. These subjects were matched to CAD-negative (CAD^-) offspring who had no parental history of CAD by age and body mass index (BMI).

SUBJECTS AND METHODS

Subjects

The subjects were male offspring of a parent with documented evidence of major CAD on or before age 60 years. All parents had a history of either coronary angioplasty, coronary bypass surgery, or myocardial infarction (including fatal myocardial infarction). Exclusion criteria for the parents were an LDL cholesterol level and family history suggestive of familial hypercholesterolemia, and treatment of diabetes mellitus with an oral hypoglycemic agent or insulin within 1 year of diagnosis of coronary disease. No attempt was made to exclude parents with impaired glucose tolerance. Seven of 61 CAD^+ offspring (11.5%) had a parent with non–insulin-dependent diabetes that had developed at least 1 year after diagnosis of the parent's coronary disease, and often many years. Subjects were recruited into two groups based on age: 20 CAD^+ offspring aged 15 to 30 years inclusive (younger subjects) and 41 CAD^+ offspring aged 31 to 45 years inclusive (older subjects). All but three CAD^+ offspring had familial CAD, with a history of coronary disease in both a parent and grandparent. All adolescents were at Tanner stage V of pubertal development. Subjects were recruited into the study irrespective of previously known lipid values. Excessive body weight was not an exclusion criterion, although none of the subjects were morbidly obese. All subjects were healthy without evidence of gastrointestinal, renal, cardiac, endocrine, or other significant chronic disease. All CAD^- offspring were male and were matched to CAD^+ offspring by age and BMI, matching one CAD^- offspring to two CAD^+ offspring of similar age and BMI. There were 20 CAD^- offspring in the younger age group and 21 CAD^- offspring in the older age group. All CAD^- offspring met the following criteria: (1) absence of a history of parental CAD, (2) absence of a history in the grandparents suggestive of CAD if either of the subject's parents were less than 60 years of age at the time of the study, and (3) absence of parental diabetes. All CAD^+ offspring

From the Medical College of Wisconsin, Milwaukee; and St. Luke's Medical Center, Milwaukee, WI.

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Address reprint requests to Arnold H. Slyper, MD, MACC Fund Research Center, 8701 Watertown Plank Rd, Milwaukee, WI 53209.

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were Caucasians, one of the younger CAD$^-$ offspring was Asian, and one of the older CAD$^-$ offspring was African-American.

Study Protocol

The study was approved by the Institutional Review Board of the Medical College of Wisconsin. At a preliminary examination and after provision of informed consent, the subject's height, weight, and blood pressure were measured. The blood pressure recorded was the average of three readings from the right arm after the subject had been resting for 10 minutes in the supine position. The waist to hip ratio was determined as the ratio of the waist measurement at the level of the umbilicus and hip measurement at the level of the greater trochanter. Blood for lipid measurements was collected into EDTA-containing tubes following a 12-hour overnight fast.

In addition, for each parent with CAD, the maximum fasting triglyceride value was extracted from their medical records. In 10 parents (19.6%), these data were not available.

Laboratory Procedures

Serum lipoproteins were separated by sequential flotation ultracentrifugation into very–low-density lipoprotein ([VLDL] d < 1.019 g/mL), LDL (d = 1.019 to 1.063 g/mL), and HDL (d > 1.063 g/mL).[20] Cholesterol level was measured using a commercial kit (Boehringer Mannheim, Indianapolis, IN). Interassay and intraassay coefficients of variation for cholesterol measurements were 3.8% and 3.2%, respectively. Triglyceride level was measured using a commercial kit (Stanbio Laboratory, San Antonio, TX) with interassay and intraassay coefficients of variation of 3.2% and 2.0%, respectively.

Measurement of LDL Peak Particle Diameter

An aliquot of LDL was subjected to nondenaturing polyacrylamide gradient gel electrophoresis using 2.5% to 16% polyacrylamide gradient gels (Isolab, Akron, OH), Tris-borate buffer at pH 8.35, and an electrophoresis time of 24 hours at 175 V as previously described.[21] Gels were stained for protein using 0.05% Coomassie R-250. LDL particle size was measured using high–molecular-weight protein standards (Electrophoresis Calibration Kit; Pharmacia Biotech, Piscataway, NJ) and a LDL standard kindly calibrated by Dr Ronald Krauss (Lawrence Berkeley National Laboratory, Berkeley, CA), and these were both assayed on each gel. An internal protein standard (apoferritin), part of the Electrophoresis Calibration Kit, was used to monitor reproducibility of the assay. Another LDL specimen previously measured in the laboratory of Dr Krauss was also added to each assay as a control, and was used to measure the coefficients of variation. Each LDL sample was measured in duplicate. The gels were scanned at 600 nm with a computer-assisted densitometer using I-D Analyst II data analysis software adapted for the Model 620 Densitometer (Bio-Rad Laboratories, Hercules, CA). The area under each LDL peak was calculated, and the peak particle diameter recorded was that of the peak with the maximum area. This peak was also used to classify samples as type A (peak particle diameter > 25.5 nm), type B (peak particle diameter < 25.2 nm), or intermediate (peak particle diameter 25.2 to 25.5 nm). Interassay and intraassay coefficients of variation for LDL particle size were 2.2% and 1.7%, respectively, with an intraassay standard deviation of 0.4 nm.

Statistics

Offspring and controls were compared using a two by two factorial ANOVA, the factors being age group and offspring versus controls. Results are presented as the mean ± SEM. Logarithmic transformation was used to normalize the data where appropriate. There was no evidence of bimodality for LDL peak particle diameter, and the Anderson-Darling normality test showed no significant difference between our data and the normal distribution (P = .238; Fig 1).

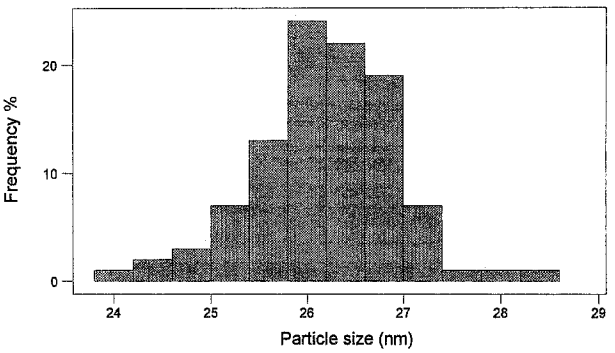

Fig 1. Histogram showing the frequency distribution of LDL peak particle diameter.

Multiple regression analysis was used to evaluate factors that could predict membership in either the CAD$^+$ or CAD$^-$ offspring group. Statistical analyses were performed using the Minitab statistical package (Minitab, State College, PA).

RESULTS

Fasting Triglyceride Concentrations of Affected Parents

In 80.4% of parents of CAD$^+$ offspring, a maximum fasting triglyceride concentration could be extracted from medical records (234 ± 18.2 mg/dL, mean ± SE). For parents of younger CAD$^+$ offspring (n = 20), the maximum triglyceride concentration was 231.3 ± 30.7 mg/dL, and for older CAD$^+$ offspring (n = 31), 236.3 ± 23.4 mg/dL.

Characteristics and Lipid Concentrations of Subjects and Controls

Characteristics of CAD$^+$ and CAD$^-$ offspring and their fasting lipoprotein concentrations are presented in Table 1. CAD$^+$ offspring were similar to CAD$^-$ offspring in age, BMI, waist to hip ratio, systolic blood pressure, triglyceride, VLDL cholesterol, VLDL triglyceride, LDL cholesterol, and HDL cholesterol, but had significantly higher levels for diastolic blood pressure (80.5 ± 1.6 *v* 74.8 ± 1.8 mm Hg, $P < .01$) and LDL cholesterol (98.9 ± 3.5 *v* 85.0 ± 3.7 mg/dL, $P < .01$). Results are the mean ± SE. Comparison of CAD$^+$ and CAD$^-$ offspring within age groups showed that the younger CAD$^+$ offspring were well matched to younger CAD$^-$ offspring in terms of BMI and waist to hip ratio. There were also no significant differences in systolic blood pressure, VLDL cholesterol, VLDL triglyceride, LDL cholesterol, and HDL cholesterol, but CAD$^+$ offspring had significantly higher diastolic blood pressure (73.6 ± 3.0 *v* 68.1 ± 2.5 mm Hg, $P < .05$) and lower triglyceride (log-transformed, 82.9 ± 15.8 *v* 98.1 ± 8.0 mg/dL, $P < .05$). There were no significant differences in these variables between older CAD$^+$ and older CAD$^-$ offspring.

There were significant differences between younger and older CAD$^+$ offspring in terms of BMI (23.4 ± 0.7 *v* 26.6 ± 0.5, $P < .01$), waist to hip ratio (0.845 ± 0.001 *v* 0.937 ± 0.008, $P < .001$), diastolic blood pressure (73.6 ± 3.0 *v* 83.9 ± 1.6 mm Hg, $P < .01$), fasting triglyceride concentration (log-transformed, 82.9 ± 15.8 *v* 126.7 ± 9.6 mg/dL, $P < .01$), VLDL cholesterol (log-transformed, 15.8 ± 1.8 *v* 25.4 ± 2.4 mg/dL, $P < .05$), and VLDL triglyceride (log-transformed, 53.6 ± 13.1

Table 1. Characteristics and Fasting Lipoprotein Concentrations of CAD$^+$ and CAD$^-$ Offspring

Parameter	All Offspring		Younger Offspring		Older Offspring	
	CAD$^+$	CAD$^-$	CAD$^+$	CAD$^-$	CAD$^+$	CAD$^-$
No. of subjects	61	41	20	20	41	21
Age (yr)	32.7 ± 1.1	30.4 ± 1.4	21.9 ± 1.0	22.1 ± 0.9	38.0 ± 0.7	38.3 ± 1.0
BMI (kg/m^2)	25.5 ± 0.5	24.5 ± 0.5	23.4 ± 0.7§	23.3 ± 0.5§	26.6 ± 0.5§	26.1 ± 0.8§
Waist to hip ratio	0.907 ± 0.009	0.886 ± 0.011	0.845 ± 0.011‖	0.861 ± 0.013‡	0.937 ± 0.008‖	0.909 ± 0.013‡
Diastolic BP (mm Hg)	80.5 ± 1.6†	74.8 ± 1.8†	73.6 ± 3.0*§	68.1 ± 2.5*§	83.9 ± 1.6§	79.7 ± 2.0§
Systolic BP (mm Hg)	127.3 ± 1.9	126 ± 1.8	125.6 ± 3.4	127.4 ± 2.5	128.2 ± 2.3	124.7 ± 2.6
LDL cholesterol (mg/dL)	98.9 ± 3.5†	85.0 ± 3.7†	93.9 ± 6.7	82.1 ± 4.1	101.3 ± 4.2	87.7 ± 6.1
Triglyceride (mg/dL)¶	112.1 ± 8.6	101.3 ± 8.5	82.9 ± 15.8*§	98.1 ± 8.0*	126.7 ± 9.6§	104.3 ± 14.9
VLDL cholesterol (mg/dL)¶	22.3 ± 1.8	18.3 ± 1.8	15.8 ± 1.8‡	14.5 ± 1.5‡	25.4 ± 2.4‡	21.9 ± 2.9‡
VLDL triglyceride (mg/dL)¶	63.6 ± 5.7	59.8 ± 6.4	53.6 ± 13.1§	54.5 ± 5.6	68.5 ± 5.6§	64.8 ± 11.3
HDL cholesterol (mg/dL)	45.6 ± 1.4	46.6 ± 2.2	46.9 ± 2.5	42.7 ± 2.3	45.0 ± 1.7	50.3 ± 3.6

NOTE. Younger subjects were aged 15 to 30 years and older subjects 31 to 45 years inclusive. Results are the mean ± SEM.
CAD$^+$ *v* CAD$^-$ offspring: *$P < .05$, †$P < .01$.
Younger *v* older CAD$^+$ offspring and younger *v* older CAD$^-$ offspring: ‡$P < .05$, §$P < .01$, ‖$P < .001$.
¶Log-transformed for purposes of comparison.

v 68.5 ± 5.6 mg/dL, $P < .01$). There were also significant differences between younger and older CAD$^-$ offspring in terms of BMI (23.3 ± 0.5 *v* 26.1 ± 0.8 kg/m^2, $P < .01$), waist to hip ratio (0.861 ± 0.013 *v* 0.909 ± 0.013, $P < .05$), diastolic blood pressure (68.1 ± 2.5 *v* 79.7 ± 2.0 mm Hg, $P < .01$), and VLDL cholesterol (log-transformed, 14.5 ± 1.5 *v* 21.9 ± 2.9 mg/dL, $P < .05$).

Comparison of LDL Peak Particle Diameter Between Groups

There was no significant difference in LDL peak particle diameter between CAD$^+$ and CAD$^-$ offspring (26.2 ± 0.1 nm in CAD$^+$ offspring *v* 26.2 ± 0.1 nm in CAD$^-$ offspring; Fig 2). There were also no differences evident when LDL peak particle size was analyzed by age group: 26.5 ± 0.1 nm in younger CAD$^+$ offspring versus 26.2 ± 0.1 nm in younger CAD$^-$ offspring, and 26.0 ± 0.1 nm in older CAD$^+$ offspring versus 26.1 ± 0.2 nm in older CAD$^-$ offspring. However, a significant difference was noted between younger and older CAD$^+$ offspring (26.5 ± 0.1 *v* 26.0 ± 0.1 nm, $P < .05$), although not between younger and older CAD$^-$ offspring (26.2 ± 0.1 *v* 26.1 ± 0.2 nm). The significant difference between younger and older CAD$^+$ offspring was eliminated after correcting for fasting triglyceride concentration (26.3 ± 0.1 *v* 26.2 ± 0.1 nm). Using the type A, B, and intermediate phenotype classification, phenotype distribution was as follows: all CAD$^+$ offspring, type A 82%, type I 6.5%, and type B 11.5%; all CAD$^-$ offspring, type A 87.8%, type I 4.9%, and type B 7.3%; younger CAD$^+$ offspring, type A 100%; younger CAD$^-$ offspring, type A 90% and type I 10%; older CAD$^+$ offspring, type A 73.2%, type I 9.8%, and type B 17.0%; and older CAD$^-$ offspring, type A 85% and type B 15%.

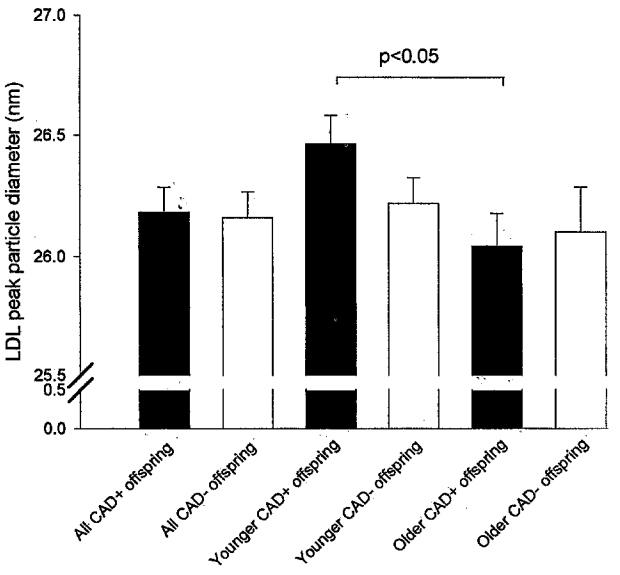

Fig 2. LDL peak particle diameter in CAD$^+$ and CAD$^-$ offspring.

Correlation Coefficients and Regression Analysis for Risk of CAD

LDL peak particle diameter was significantly correlated with triglyceride concentration (log-transformed, $r = -.566$, $P < .001$), the relationship being described best by an exponential curve. Multiple stepwise regression analysis was performed to evaluate if any factors were able to predict membership in either the CAD$^+$ or CAD$^-$ offspring group. The following were entered into the regression analysis: LDL particle size, BMI, waist to hip ratio, diastolic blood pressure, systolic blood pressure, fasting triglyceride (log-transformed), LDL cholesterol, HDL cholesterol, VLDL cholesterol (log-transformed), and VLDL triglyceride (log-transformed). Only diastolic blood pressure and LDL cholesterol had significant predictive power at *P* less than .05, although the predictive power was weak for both variables.

DISCUSSION

In this study of male offspring of parents with early CAD, we were unable to demonstrate a difference in LDL peak particle diameter between offspring with a history of parental CAD and those without. This was also the case when CAD$^+$ and CAD$^-$ offspring were compared within their own age group, ie, a group aged 15 to 30 years and a group aged 31 to 45 years. The probability of detecting a difference in LDL peak particle diameter of 0.3 nm or greater (the power) in this study was 80%, and for a difference of 0.4 nm, 96%. In addition, on multiple regression analysis, LDL particle size was unable to provide a

prediction as to membership in either the CAD$^+$ or CAD$^-$ group. In light of these results, it is difficult to conceive that dense LDL has a role as an early atherogenic risk factor. Nor does LDL particle size appear useful as a discriminating marker for atherogenic risk in young adults.

It could be argued that by chance we selected proband parents with a low incidence of small LDL particles. Based on maximum fasting triglyceride levels extracted from the parents' medical records and a previously determined linear relationship between triglyceride and LDL particle size,[22] we calculated a theoretical particle size of 25.4 nm for the parents in whom triglyceride levels were available (80.4%). This is within the range of the type B phenotype, so this was clearly not the case.

There is a significant increase in small LDL particles in patients with CAD.[2,12,23-26] A 55% incidence of the type B pattern was noted by Austin et al[2] in 366 post–myocardial infarction men and women, compared with a 31% prevalence in age- and sex-matched controls. This implied a threefold increased coronary risk for individuals with small, dense LDL particles. Adjusting for the use of β-blockers, Campos et al[23] found a 48% incidence of small LDL particles (diameter < 26.0 nm) in 280 male CAD survivors younger than 60 years of age, compared with a 30% incidence in controls. In a prospective study, small LDL size was shown to be a strong predictor of CAD.[27] In addition, small LDL particles are increased in many conditions predisposing to atherosclerosis.[28] Men with non–insulin-dependent diabetes mellitus have more than a twofold increase in dense LDL.[29] Small LDL size is also a feature of familial combined hyperlipidemia and familial dyslipidemic hypertension.[18,30]

Because of the strong association between LDL size and coronary disease, it has been suggested that small LDL is a more atherogenic form of LDL. Attention has been focused particularly on the process of lipid peroxidation, which may have a major role in atherogenesis.[31] LDL consisting of small-sized particles readily undergoes lipid peroxidation.[32,33] LDL from coronary disease patients is also more susceptible to lipid peroxidation.[34]

However, there are many observations that cast doubt as to the importance of dense LDL as an independent atherogenic risk factor. Fasting triglyceride concentrations are strongly correlated with LDL density,[29,35-37] and changes in LDL pattern are correlated with changes in fasting triglyceride concentration.[19] After accounting for fasting triglyceride levels, Austin et al[2] found that dense LDL was no longer a significant risk factor in their coronary disease patients. Campos et al[23] concluded that small LDL particle size was not an independent discriminator for CAD in their coronary patients after accounting for conventional risk factors and lipoproteins. In a prospective study, Stampfer et al[38] found that LDL size no longer appeared as a risk factor for coronary disease after adjusting for triglyceride levels.

How does one reconcile data from our study that downplay the importance of LDL size in the development of coronary disease and other studies suggesting that LDL particle size has an important role in the atherogenesis equation? The influence of maturation on LDL particle size seems likely to be important in this respect. Austin et al[3] established that the type B LDL subclass pattern is a marker for an atherogenic lipoprotein phenotype consisting of an increase in triglyceride, apoprotein B, and VLDL and IDL mass and a decrease in HDL cholesterol, HDL_2 mass, and apoprotein A-1. The type B LDL pattern is also strongly associated with aspects of the insulin resistance syndrome, namely increased fasting and postload insulin, triglyceride, waist to hip ratio, and systolic blood pressure and decreased HDL cholesterol.[39] However, when LDL size is considered as a continuous rather than as a dichotomous variable, the strongest association with LDL particle size is triglyceride concentration. Visceral obesity and non–insulin-dependent diabetes are two major causes of increased triglyceride concentration and dense LDL, and the incidence of both visceral obesity and non–insulin-dependent diabetes increases with age.[40-43] A study in Mormon families found that younger family members had an exclusive type A LDL pattern.[18] In our study, we found a significant difference in LDL size between younger and older CAD$^+$ offspring, coincident with a significant increase in fasting triglyceride. However, this difference was no longer apparent after correcting for triglyceride concentration. The age dependence of LDL particle size may also account for our failure to see a difference between older CAD$^+$ and older CAD$^-$ offspring, since none of these subjects were over age 45 years. One could speculate that should the older CAD$^+$ offspring become more obese and hypertriglyceridemic in the future, a decrease in LDL particle size between CAD$^+$ and CAD$^-$ offspring may become manifest.

In conclusion, our data suggest that small LDL particle size is not an early atherogenic risk factor or a discriminating marker for atherogenesis in adolescent and young adult offspring at risk for CAD because of family history. The maturational changes that occur in LDL particle size limit the value of LDL particle size as an indicator of coronary risk in young adults.

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