Alterations in Plasma Lipids in the Presence of Mild Glucose Intolerance in the Offspring of Two Type II Diabetic Parents

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Plasma lipids and oral glucose tolerance were determined in 67 normal control subjects (age range 19–67 yr) and 150 individuals (17–69 yr) who were offspring of two type II diabetic parents. Age- and weight-adjusted analyses of covariance were performed for lipids and for glucose and insulin responses. For both men and women, the mean concentrations of total, low-density-, and high-density-lipoprotein–cholesterol and of triglycerides in the offspring with normal glucose tolerance (N = 109) were similar to respective controls. For offspring with abnormal glucose tolerance (N = 41), the mean levels of total cholesterol, LDL-cholesterol, and triglycerides were significantly elevated (P = 0.02 or less) in women but not in men. The mean HDL-cholesterol levels were 20% lower and LDL/HDL-cholesterol ratios 60% greater in women with abnormal glucose tolerance, whereas no significant differences existed for any of the lipid fractions in men, compared with respective controls. Both men and women with abnormal glucose tolerance had a comparable magnitude of hyperglycemia as well as hyperinsulinemia. These observations indicate that (1) significant alterations in plasma lipids exist in individuals with mild, asymptomatic glucose intolerance and (2) there are important sex differences in lipid metabolism in the early stage of diabetes, despite comparable degrees of glucose intolerance and insulin responses.
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In the multifactorial pathogenesis of accelerated macrovascular disease in diabetic patients, abnormalities of lipid metabolism play a major role.[1–3] Recently, several investigators have reported a reduction in high-density lipoprotein (HDL)-cholesterol levels in association with diabetes, particularly non-insulin-dependent diabetes (NIDDM; type II diabetes).[4–7] The mechanism of this alteration in HDL metabolism is uncertain, but it may contribute to accelerated atherogenesis in the diabetic person.[3,8] Epidemiologic observations indicate a strong inverse correlation between the HDL-cholesterol level and macrovascular disease.[9–11] In view of the positive correlation between low-density-lipoprotein (LDL)–cholesterol and macrovascular disease,[9] a few studies have suggested that the LDL/HDL-cholesterol ratio might be an even more sensitive risk indicator than either lipid fraction alone.[8,12]

In individuals with impaired glucose tolerance or asymptomatic, mild hyperglycemia, several long-term prospective studies have shown an increased frequency of clinically significant atherosclerotic events, in the absence of overt diabetes mellitus.[13–17] The mechanism underlying this intriguing observation is unclear. In an earlier study from this laboratory, a 19% prevalence of type IV hyperlipidemia was found in a mildly abnormal ("chemical" diabetes) group of offspring of two diabetic parents.[18] However, the significance of hypertriglyceridemia as a possible risk factor for vascular disease remains controversial.[3,8]

Studies have now been extended to include assessment of LDL- and HDL-cholesterol and LDL/HDL-cholesterol (L/H) ratios in nondiabetic, asymptomatic individuals, metabolically characterized by oral glucose tolerance and hemoglobin A_{1c} determinations. These observations indicate that mild abnormalities of glucose tolerance can be associated with significant alterations in lipid metabolism, particularly in women.

MATERIAL AND METHODS

Subjects. The study population consisted of 67 normal control subjects (38 men, 29 women) with no family history of diabetes and 150 offspring of two type II diabetic parents (ODP) of whom 109 (63 men, 46 women) had normal oral glucose tolerance (ODP-N), and 41 (14 men, 27 women) had ab-

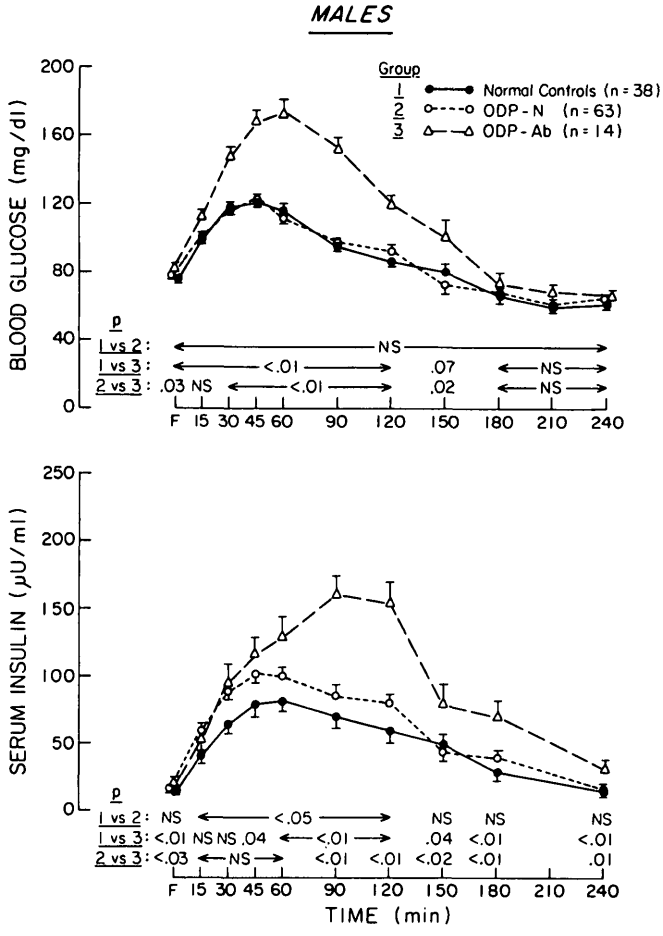

FIG. 1. Blood glucose and serum immunoreactive insulin responses to oral glucose (100 g p.o.) in normal controls, offspring of diabetic parents with normal glucose tolerance (ODP-N), and ODP with abnormal glucose tolerance (ODP-Ab) in men.

normal oral glucose tolerance (ODP-Ab). The oral glucose tolerance tests (100 g p.o.) were performed under standardized conditions as described earlier.[19,20] The ODP were divided into normal and abnormal glucose-tolerant (ODP-N, ODP-Ab) groups, based on mean + 2 SD glucose responses in a large group of normal controls (N = 295) as reported earlier.[19] Only 17% (7 of 41) of subjects classified as ODP-Ab by our criteria would be classifiable as impaired glucose tolerant (IGT) by the National Diabetes Data Group.[21] Thus, the type of glucose intolerance in ODP-Ab was indeed mild, but statistically significant. None of the subjects in any of the three major groups was hypertensive or taking medications known to affect carbohydrate or lipid metabolism.

The mean age (± SEM) (and range) of normal controls, ODP-N, and ODP-Ab was 34.2 ± 1.6 (19–67), 38.7 ± 1.8 (14–69), and 44.4 ± 1.5 (37–59) yr in men; and 38.1 ± 2.0 (23–60), 37.5 ± 1.8 (17–65), and 42.5 ± 2.0 (22–60) yr in women, respectively. The corresponding percent ideal body weights (Metropolitan Life Insurance Tables, 1959) for the male and female subjects in the three study groups were 103 ± 2.2% (85–153%), 113 ± 3.0% (82–210%), and 118 ± 4.0% (90–168%) in men and 96 ± 2.4% (74–132%), 107 ± 3.0% (84–177%), and 117 ± 5.6% (80–206%) in women, respectively. The prevalence of obesity, defined as >120% ideal body weight, was 8%, 22%, and 29% in men and 7%, 24%, and 41% in women, respectively.

Methods. Whole blood glucose (BG) determinations were performed by the ferricyanide method[22] and serum insulin (IRI) by double-antibody technique.[23] Plasma cholesterol and triglycerides were determined in fresh, fasting blood samples (obtained on the same day as the OGTT in each individual) by enzymatic techniques,[24,25] employing the Technicon Auto-Analyzer II system (Technicon Corp., Tarrytown, New York). For HDL-cholesterol determination, HDL was separated from other lipoproteins by the sodium phosphotungstate and magnesium chloride precipitation method[26] as described earlier.[27] The LDL-cholesterol was calculated by the method of Friedwald et al.[28] None of the individuals had triglyceride concentrations >500 mg/dl. Hemoglobin A_{1c} was determined by high-performance liquid chromatography as described elsewhere.[29]

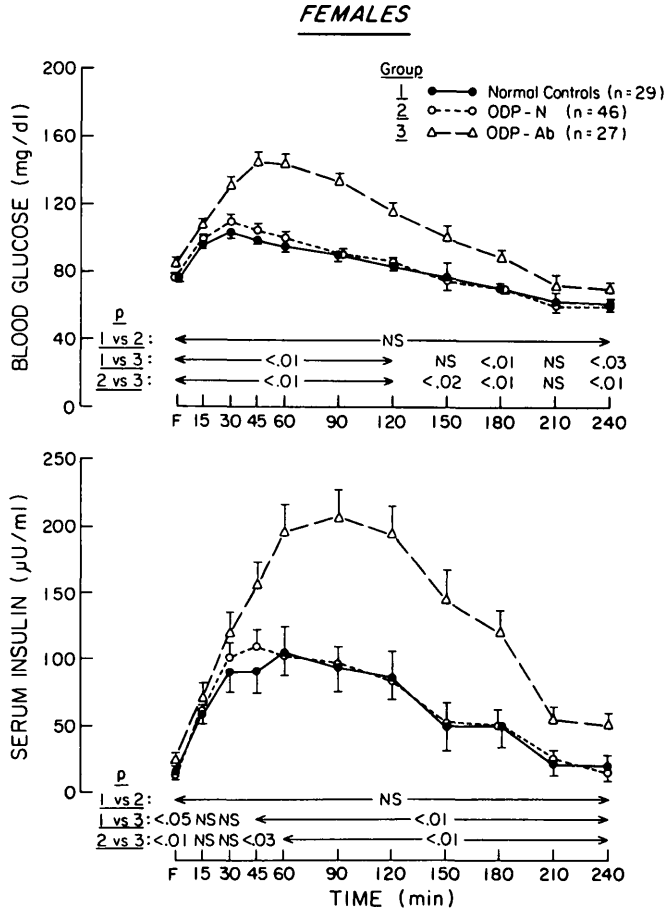

FIG. 2. Blood glucose and serum immunoreactive insulin responses to oral glucose (100 g p.o.) in normal controls, offspring of diabetic parents with normal glucose tolerance (ODP-N), and ODP with abnormal glucose tolerance (ODP-Ab) in women.

TABLE 1
Age- and weight-adjusted mean (± SEM) fasting blood glucose (FBG), hemoglobin (A_{1c}), fasting insulin (FIRI), and aggregate BG and IRI responses following oral glucose (100 g) in normal controls (N) and offspring of diabetic parents with normal (ODP-N) or abnormal (ODP-Ab) glucose tolerance

	FBG (mg/dl)	A_{1c} (%)	FIRI (μU/ml)	Peak BG (mg/dl)	Peak IRI (μU/ml)	BG 0-240 (mg/dl × min)	IRI 0-240 (μU/ml × min)
Men							
Group (N)							
1 N (38)	77 ± 1.2	5.2 ± 0.09	13 ± 1.2	131 ± 4	98 ± 10	3,810 ± 358	8,396 ± 1039
2 ODP-N (63)	78 ± 0.9	5.5 ± 0.06	16 ± 0.9	132 ± 3	130 ± 8	3,827 ± 269	11,480 ± 779
3 ODP-Ab (14)	82 ± 1.9	5.4 ± 0.16	21 ± 2.0	186 ± 6	195 ± 16	8,626 ± 583	18,330 ± 1691
P							
1 vs. 2	—*	0.02	—	—	<0.01	—	0.02
1 vs. 3	0.01	—	<0.01	<0.001	<0.001	<0.001	<0.001
2 vs. 3	0.03	—	<0.03	<0.001	<0.001	<0.001	<0.001
Women							
Group (N)							
1 N (29)	76 ± 1.3	5.4 ± 0.10	16 ± 3	112 ± 3	124 ± 20	2,663 ± 379	12,534 ± 2760
2 ODP-N (46)	76 ± 1.0	5.3 ± 0.08	14 ± 2	117 ± 2	136 ± 16	3,025 ± 291	12,999 ± 2115
3 ODP-Ab (27)	84 ± 1.4	5.3 ± 0.11	24 ± 3	156 ± 3	230 ± 21	7,283 ± 399	27,469 ± 2893
P							
1 vs. 2	—	—	—	—	—	—	—
1 vs. 3	<0.001	—	<0.05	<0.001	<0.001	<0.001	<0.001
2 vs. 3	<0.001	—	<0.01	<0.001	<0.001	<0.001	<0.001

*Not significant.

Statistical analyses. The early and total blood glucose and serum insulin responses during OGTT were determined by calculating the areas under the respective curves, above baseline, for the initial 60 min (BG 0–60, IRI 0–60) or for the entire 240-min period (BG 0–240, IRI 0–240), respectively. Since the study subgroups differed with regard to age and percent ideal body weight, the data were analyzed by analyses of covariance using these two variables as the covariates. Post hoc tests of significance among adjusted subgroup means were performed by the least-squares-means option of the General Linear Models (GLM) procedure in the SAS (Statistical Analysis System) program package.[30] Data from men and women were analyzed separately and all values are expressed as means ± standard error of the mean (SEM) unless otherwise indicated. Distributions of L/H ratios were compared using chi-square analysis with Yates correction for continuity. Pearson's correlation coefficients were calculated for regression equations between various parameters. All analyses were done on an IBM 4341 computer at the Health Sciences Computing Facility, Harvard School of Public Health.

RESULTS

Blood glucose (BG) and insulin (IRI) responses. Figures 1 and 2 present the BG and IRI responses during OGTT in men and women, respectively. The fasting BG and IRI levels were significantly elevated in the ODP-Ab group compared with normals and ODP-N, although mean BG levels were clearly in the "nondiabetic" range in each group. The mean hemoglobin A_{1c} levels were virtually identical in all three subgroups in each sex category (Table 1). However, the BG and IRI responses at each time interval following oral glucose ingestion were clearly higher in ODP-Ab compared with normal controls and ODP-N group in both sexes.

The age- and weight-adjusted, integrated BG and IRI responses (0–240-min area) revealed an approximately 2–3-fold greater glycemic response and corresponding hyperinsulinemic response in the ODP-Ab group compared with normal controls, whereas minor or nonsignificant differences existed between ODP-N and normal controls (Table 1). Similarly, the initial (0–60 min) BG and IRI areas were also approximately 1.5–2-fold greater in the ODP-Ab group in either sex (data not shown).

Age- and weight-adjusted lipids (Table 2). Striking sex differences emerged when plasma lipid levels were analyzed after age and weight adjustment by analysis of covariance. In men, the total cholesterol and LDL-cholesterol concentrations were virtually identical in the three groups. The mean triglycerides in ODP-Ab were 40% greater than normal controls and HDL-cholesterol 9% lower, but none of these differences were significant. Similarly, mean LDL/HDL-cholesterol ratios were very similar in the three groups.

On the other hand, mean cholesterol, LDL-cholesterol, as well as triglyceride levels were significantly higher in ODP-Ab women compared with normal controls or ODP-N group. The mean HDL-cholesterol levels were 20% lower in ODP-Ab compared with controls. Finally, the LDL/HDL-cholesterol ratio was 60% greater in ODP-Ab (3.5 versus 2.2) compared with controls and 35% greater compared with ODP-N. There was no significant difference between normal controls and ODP-N for any of the lipid parameters.

Table 3 depicts the frequency of abnormal LDL/HDL-cho-

TABLE 2
Age- and weight-adjusted fasting lipids (mean ± SEM) in normal controls (N) and offspring of diabetic parents with normal (ODP-N) or abnormal (ODP-Ab) glucose tolerance

Group		Chol (mg/dl)	Trig (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	L/H ratio
	Men*					
1	N (N = 38)	210 ± 7	123 ± 16	45 ± 2.0	140 ± 6	3.5 ± 0.3
2	ODP-N (N = 63)	205 ± 5	133 ± 12	41 ± 1.5	137 ± 5	3.8 ± 0.2
3	ODP-Ab (N = 14)	211 ± 11	176 ± 26	41 ± 3.3	134 ± 10	3.6 ± 0.5
	Women					
1	N (N = 29)	193 ± 7	72 ± 10	57 ± 3.0	122 ± 6	2.2 ± 0.2
2	ODP-N (N = 46)	199 ± 5	91 ± 7	53 ± 2.3	127 ± 5	2.6 ± 0.14
3	ODP-Ab (N = 27)	218 ± 8	135 ± 10	46 ± 3.1	145 ± 7	3.5 ± 0.2
	P					
	1 vs. 2	NS	NS	NS	NS	NS
	1 vs. 3	0.02	0.001	0.01	0.01	<0.001
	2 vs. 3	0.04	<0.001	NS	0.03	<0.001

NS = not significant.
*P = not significant for all comparison between groups 1, 2, 3.

lesterol (L/H) ratios in women, according to an arbitrary "cutoff" limit of 3.5. Thirteen of 27 (48%) women with ODP-Ab exceeded this "cutoff" limit, whereas only 1 of 29 (3%) normal controls and 5 of 46 ODP-N (11%) had a ratio greater than 3.5. There was no significant difference between those above or below the L/H ratio of 3.5 for mean levels of total cholesterol or triglycerides. Furthermore, the mean percent ideal body weight in women with a L/H ratio of >3.5 (111 ± 4%) was not significantly different from that in those with a ratio of ≤3.5 (123 ± 10%).

Correlations between age and L/H ratio. Regression equations between age, weight, and lipids were computed for the three groups in men and women separately. Only women with abnormal glucose tolerance revealed a significant correlation between age and L/H ratio ($r = 0.41$; $P < 0.04$) (Figure 3). No significant relationship existed between age or weight and the lipid parameters in the other groups.

DISCUSSION

In this study, the plasma lipid status was evaluated for individuals who are genetically at high risk for developing diabetes but who presently do not demonstrate evidence of overt diabetes. Although the offspring with "abnormal" glucose tolerance were so defined on the basis of results in 295 normal tests of glucose tolerance, only 4 of 14 men and 3 of 27 women in the ODP-Ab group would be classified as IGT by the NDDG criteria, indicating mild but significantly abnormal glucose tolerance in these subjects. Of major importance is our observation that there were significant alterations in plasma lipid concentrations in women with this degree of mild glucose intolerance (ODP-Ab), even after analysis of covariance, which adjusted for the age and weight differences between the three subgroups. The reason for this sex difference is presently unclear. The ODP-Ab men did reveal a trend toward increasing triglyceride concentrations (176 ± 26 versus 123 ± 16) and decreasing HDL-cholesterol levels (41 ± 3.3 versus 45 ± 2.0), as compared with controls, but these differences were not statistically significant. The number of ODP-Ab men in this study was relatively small (N = 14) and the nonsignificant nature of these differences in men could be due to this reason. However, another likely explanation may be some inherent sex difference that renders women more susceptible to atherogenic lipid alterations once mild glucose intolerance supervenes. The menopausal status of women was not clearly defined in our population. However, it is unlikely to account for the differences observed, since the age distribution in ODP-Ab was similar to that in ODP-N (Figure 3), whose lipid concentrations were indeed identical to those in controls.

Studies from the Framingham prospective survey[31,32] have consistently indicated a higher risk of atherosclerosis in diabetic women than in men. Interestingly, in a recent large series of patients studied by Walden et al.,[33] diabetic women had significantly greater elevations in triglycerides and LDL-cholesterol and lower HDL-cholesterol concentrations, compared with diabetic men and the corresponding controls, thus explaining the higher atherogenic risk in the diabetic women.

TABLE 3
Distribution of age- and weight-adjusted LDL/HDL-cholesterol (L/H) ratio in control women (N) and offspring of diabetic parents with normal (ODP-N) or abnormal (ODP-Ab) glucose tolerance

	L/H Ratio ≤3.5 (% of total)	L/H Ratio >3.5 (% of total)
Normal (N) (N = 29)	28 (97)	1 (3)
ODP-N (N = 46)	41 (89)	5 (11)
ODP-Ab (N = 27)	14 (52)	13 (48)*†

*X^2 = 12.61 ($P < 0.0005$) with Yates correction, compared with N.
†X^2 = 10.79 ($P < 0.0005$) with Yates correction, compared with ODP-N.

Our data suggest that this increased risk may be present even when the extent of glucose intolerance is rather mild and the glucose-intolerant women are otherwise healthy and asymptomatic. Several epidemiologic studies, including the Bedford survey,[13] the Whitehall study,[14] the Chicago Peoples Gas Company,[15] and the Busselton study,[17] have shown an increased frequency of clinically significant atherosclerotic events in individuals with persistent evidence of "impaired glucose tolerance" in the absence of overt diabetes. It is not clear, however, if there is a "threshold" level of glycemia that initiates and perpetuates the atherogenic events or, perhaps more likely, the mild glucose intolerance in susceptible individuals reflects the presence of other concomitant factors underlying atherogenesis in the diabetic individual or in individuals with asymptomatic, mild hyperglycemia.[13,31]

Despite the heterogeneity within type II diabetes, the offspring of two type II diabetic persons with normal glucose tolerance revealed virtually identical lipid profiles in both men and women, as compared with normal controls with no known family history of diabetes. This would support the suggestion of Brunzell et al.[34] that the genes for diabetes and familial hyperlipidemia segregate independently, although both of these phenotypes often coexist because of high prevalence of both disorders in the population. However, the possibility of a genetic linkage between glucose intolerance and atherogenic factors such as hyperlipidemia in the genetically homogeneous subgroups cannot be conclusively excluded by the available data.

The mechanism of dyslipidemia in the individuals with glucose intolerance has received considerable attention. Obesity, hyperglycemia, and hyperinsulinemia have all been associated with increased VLDL-synthesis,[2,3,8] and a reciprocal relationship between VLDL and HDL has been documented.[1-3] However, the differences in triglyceride levels, LDL-cholesterol, HDL-cholesterol, and LDL/HDL-cholesterol ratios persisted in our study after age and weight adjustments by analysis of covariance. Moreover, the extents of hyperglycemia and hyperinsulinemia in the "ODP-Ab" group in men and women were similar compared with the other two groups in each sex. This would suggest that factors other than the ambient blood glucose and insulin excursions might determine the sex differences in the observed lipid alterations. On the other hand, it was reported by Schmitt et al.[12] that, in type II diabetes, a stronger correlation exists between LDL/HDL ratio and the extent of glycemia in women than in men. In any case, from their studies, and ours, it appears that the LDL/HDL-cholesterol ratio might provide a more sensitive indicator of an abnormal lipid profile than either lipid fraction alone. Direct measurements of LDL-, HDL-, and HDL-subfractions were not performed in our studies and would be required in patients with more marked hypertriglyceridemia. Similarly, in the recently reported results of the National Heart, Lung and Blood Institute (NHLBI)

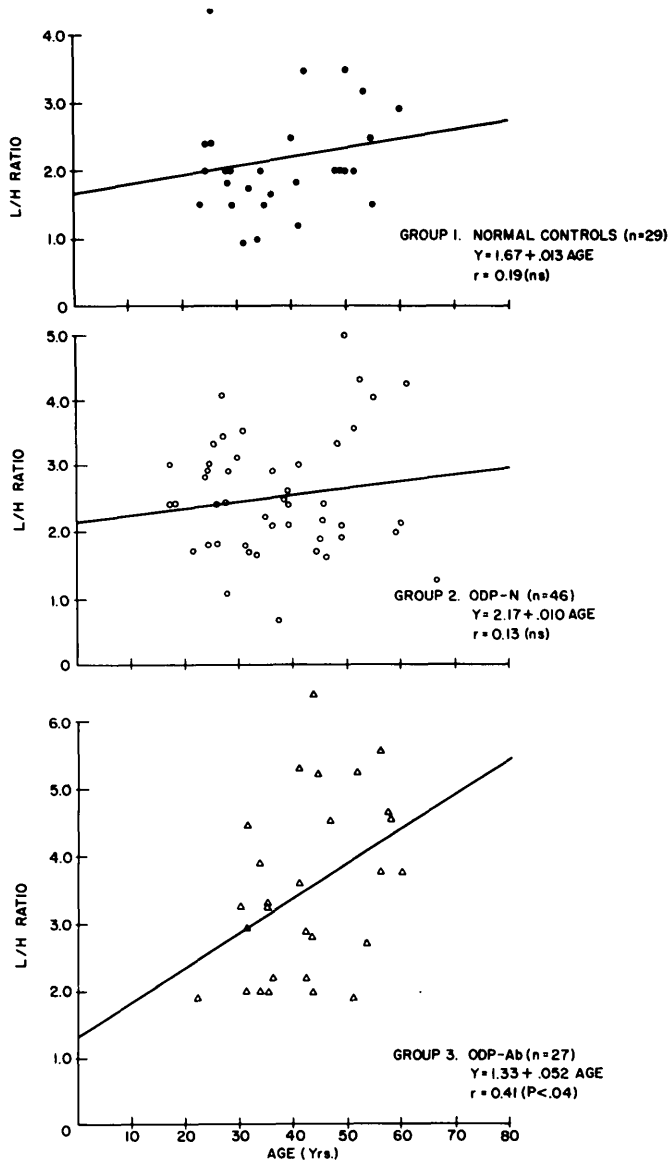

FIG. 3. Regression equations between age and low-density/high-density lipoprotein-cholesterol (L/H) ratio in women. Abbreviations ODP-N and ODP-Ab correspond to those in Figure 2.

Coronary Intervention Study, changes in HDL/LDL-cholesterol were better predictors for progression of coronary artery disease, compared with either of these lipid parameters considered alone.[35] Additional studies are required to explore the prospective interrelationships between the future progression of glucose intolerance and lipid alterations in individuals with mild glucose intolerance.

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