Elevated Serum C-Reactive Protein and Free Fatty Acids Among Nondiabetic Carriers of Missense Mutations in the Gene Encoding Lamin A/C (*LMNA*) With Partial Lipodystrophy

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Objective—Dunnigan-type familial partial lipodystrophy (FPLD) due to mutant *LMNA* is a monogenic form of insulin resistance. Affected subjects, especially women, are at increased risk of early coronary heart disease (CHD). Although common insulin resistance is associated with several biochemical perturbations, including elevated C-reactive protein (CRP), the biochemical profile in subjects with mutant *LMNA* is incompletely defined.

Methods and Results—We studied 35 nondiabetic adult FPLD subjects (of whom 24 were women) with either the *LMNA* R482Q or R482W missense mutations and 51 matched normal first-degree relatives (of whom 27 were women). Compared with normal controls, *LMNA* mutation carriers had significantly higher plasma insulin and more dyslipidemia, higher mean triglycerides and lower HDL cholesterol, significantly higher nonesterified free fatty acids and CRP, and significantly lower leptin and adiponectin than controls. Subgroup analyses showed that these differences were more pronounced in women. Other biomarkers such as resistin, fibrinogen, and plasminogen activator inhibitor-1 were not different between groups.

Conclusions—*LMNA* mutations in nondiabetic patients with FPLD are associated with several metabolic and biochemical changes, particularly in women. The unfavorable profile might contribute to the increased susceptibility to CHD seen in *LMNA* mutation carriers. **(*Arterioscler Thromb Vasc Biol*. 2003;23:111-116.)**

Key Words: insulin resistance ■ diabetes ■ DNA analysis ■ cytokines ■ risk factor

Dunnigan-type familial partial lipodystrophy (FPLD; MIM 151660) is a rare autosomal dominant form of insulin resistance caused by mutant *LMNA*, which encodes nuclear lamin A/C,[1–9] a structural component of the nuclear envelope. FPLD patients have a unique physiognomy due to loss of subcutaneous fat from the extremities and gluteal region after the onset of puberty, so that fat only accumulates centrally.[1–9] FPLD subjects also develop dyslipidemia, hypertension, and type 2 diabetes.[10] In Canadian FPLD kindreds, *LMNA* mutation carriers, particularly women, had a very high risk of early coronary heart disease (CHD) compared with family controls.[11] CHD in FPLD was associated with both hyperinsulinemia and type 2 diabetes, although hyperinsulinemia was present earlier in the disease course.[11] The common insulin resistance syndrome has been associated with elevated serum inflammatory markers, including C-reactive protein (CRP)[12–20] and fibrinogen (FBG).[21] Also, serum CRP in nondiabetic subjects was directly related to insulin resistance,[22] suggesting that chronic subclinical inflammation was part of the insulin resistance syndrome contributing to atherosclerosis. Because *LMNA*-related FPLD is a monogenic form of insulin resistance with early CHD, we hypothesized that serum markers of inflammation and coagulation would be abnormal in nondiabetic FPLD subjects. We examined a panel of markers related to atherosclerosis risk in nondiabetic FPLD subjects and in age- and sex-matched normal family controls. We also measured adipocyte hormones, such as leptin, adiponectin, and resistin.[23] We found that nondiabetic carriers of mutant *LMNA* had a distinctive cluster of analytes, including elevated CRP and nonesterified free fatty acids (FFAs), hyperinsulinemia, hypertriglyceridemia, and depressed HDL cholesterol.

Methods

Study Subjects

Subjects from 3 extended Canadian FPLD kindreds were studied.[6,10,11,24,25] Only nondiabetic subjects aged 18 years and older were included, according to current diagnostic criteria of the American Diabetes Association. Cigarette smokers, subjects with a current acute illness (including clinically significant infectious disease), and subjects with CHD[11] were excluded. Mutation carriers were matched by age within 6 years and by sex with normal family control subjects without *LMNA* mutations. This left a total sample size of 86 subjects: 35 were FPLD subjects with mutant *LMNA*, of whom 27 and 8 had

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TABLE 1. Clinical Attributes of FPLD Nondiabetic *LMNA* Mutation Carriers And Normal Family Controls

	All Subjects			Women			Men		
	FPLD	Controls	*P* (Difference)	FPLD	Controls	*P* (Difference)	FPLD	Controls	*P* (Difference)
Number	35	51		24	27		11	24	
Age, y	39.7±3.0	42.5±2.3	NS	39.2±3.8	39.2±3.3	NS	41.0±4.8	46.2±3.0	NS
BMI, kg/m^2	25.5±0.8	27.2±0.72	NS	24.4±0.8	26.2±1.1	NS	27.7±1.5	28.4±0.9	NS
Dyslipidemia	68.6%	7.8%	<0.0001	75.0%	7.4%	<0.0001	54.5%	8.3%	0.0026
Blood pressure, mm Hg									
Systolic	142±15	132±23	NS	138±20	129±25	NS	144±16	135±21	NS
Diastolic	87±11	80±10	NS	87±11	77±10	0.033	87±10	84±11	NS
Hypertension treatment	21.2	8.3%	0.030	31.8%	15.4%	NS (0.07)	9.9%	4.4%	NS
Glucose, mmol/L	5.13±0.17	4.96±0.08	NS	4.93±0.15	4.79±0.13	NS	5.49±0.39	5.13±0.09	NS
HbA1c, %	5.63±0.16	5.53±0.10	NS	5.57±0.10	5.61±0.15	NS	5.79±0.54	5.47±0.13	NS

Values are mean±SEM.

BMI indicates body mass index; HbA1c, glycosylated hemoglobin; NS, not significant.

the R482Q and R482W mutations, respectively. Fifty-one family controls had a normal *LMNA* gene. Height, weight, body mass index (kg/m^2), and blood pressure were determined. The use of antihypertensive medications was recorded. Dyslipidemia was defined as an untreated plasma triglyceride >95th and/or HDL cholesterol below the 5th percentile for age and sex, according to thresholds from the normal Canadian population.[26] The study was approved by the University of Western Ontario Institutional Review Board.

Biochemical and Genetic Determinations

Blood was centrifuged at 2000 rpm for 30 minutes, and the plasma was stored at −70°C. Assays of fasting plasma concentrations of lipids, lipoproteins (including direct measurements of LDL cholesterol after ultracentrifugation), apolipoprotein (apo) A1 and B, glucose, glycosylated hemoglobin, insulin, C-peptide, and leptin were performed by using established procedures.[10,11] Fasting plasma insulin concentration was determined by radioimmunoassay (RIA, Pharmacia), which had a sensitivity of 0.4 ng/mL and intra-assay and interassay coefficients of variation of 5.2% and 8.7%, respectively. C-peptide concentration was determined by RIA (Diagnostic Products) with a minimal detection limit of 43 pmol/L and 0% cross-reactivity with insulin. Serum nonesterified FFA concentration was measured by using the ACS-ACOD kit (Wako), which had a detection limit of 0.01 mmol/L and a maximum coefficient of variation of 2.7%. Fasting plasma leptin concentration was determined by quantitative ELISA (Quantikine Human Leptin, R&D Systems), which had a minimal detectable concentration of 0.5 ng/mL, a limit of linearity of 100 ng/mL, and an interassay coefficient of variation of 8.3%. Fasting plasma adiponectin concentration was determined by RIA (Linco), which had a detection limit of 1 ng/mL and an interassay coefficient of variation of 5%. Plasma acylation-stimulating protein (ASP) concentration was determined by ELISA (Quidel), which had a detection limit of 200 ng/mL and an interassay coefficient of variation of 5%. Fasting plasma resistin concentration was determined by ELISA (BioVendor), which had a detection limit of 2 ng/mL and an interassay coefficient of variation of 7.2%. Serum tumor necrosis factor (TNF)-α concentration was determined by ELISA (Biosource International), which had a maximum coefficient of variation of 8.2%. Serum CRP concentration was measured using a high-sensitivity automated nephelometric method (Beckman Coulter), which had a detection limit of 0.05 mg/L and a maximum coefficient of variation of 6%. Serum plasminogen activator inhibitor-1 (PAI-1) concentration was measured with the Stachrom PAI kit (Diagnostica Stago) that had a detection limit of 5 IU/mL and a maximum coefficient of variation of 4%. Serum FBG was analyzed according to the von Clauss method,[27] which had a maximum coefficient of variation of 9.5%. DNA extraction and *LMNA* genotyping were performed as described,[10,11] using sequence-proven DNA standards.

Statistical Analyses

Clinical and biochemical traits for *LMNA* mutation carriers were compared with matched family controls. Differences between genotypes in qualitative traits were compared with Fisher's exact test in SAS version 6.12 (SAS Institute). All continuous variables assumed a distribution that was not significantly different from normal after logarithmic transformation. Differences between genotypes in logarithmically transformed quantitative traits were compared by Student's *t* tests from the general linear models procedure in SAS. For the purpose of presentation, untransformed variables are shown in the tables. For all quantitative variables, the entire sample size afforded >75% power to detect a 1.6-fold difference and >90% power to detect a 2.0-fold difference (α=0.05). Post hoc subgroup analyses according to sex were also performed. A value of $P<0.05$ was taken as the nominal level of significance.

Results

Baseline Clinical Attributes of the Study Sample

Baseline clinical and biochemical attributes (unadjusted mean±SE for quantitative traits) are shown in Table 1. FPLD subjects and controls were well matched by sex, age, and glycemic variables. Body mass index was not different. Post hoc analysis showed that the ratio of waist-to-hip circumference was higher in FPLD subjects (data not shown). Although systolic blood pressure and the use of antihypertensive medications were not significantly increased in the FPLD subjects, diastolic blood pressure was significantly increased in female FPLD subjects (Table 1). FPLD subjects with mutant *LMNA* also had significantly more dyslipidemia defined by the age- and sex-specific population cut points specified above,[26] and these differences were significant in both sexes (Table 1).

Biochemical Phenotypes

Biochemical traits related to atherosclerosis (unadjusted mean±SE) are shown in Table 2. In the entire study sample, there were numerous significant differences between FPLD subjects and normal family controls. For instance, among the plasma lipoproteins, mean triglyceride was significantly higher and mean HDL cholesterol was significantly lower in

TABLE 2. Biochemical Attributes of FPLD *LMNA* Mutation Carriers and Normal Family Controls

	All Subjects			Women			Men		
	FPLD	Controls	*P* (Difference)	FPLD	Controls	*P* (Difference)	FPLD	Controls	*P* (Difference)
Lipoprotein variables									
Cholesterol, mmol/L									
Total	5.12±0.18	5.29±0.16	NS	5.13±0.22	5.43±0.27	NS	5.11±0.29	5.14±0.15	NS
HDL	1.09±0.04	1.34±0.05	0.0004	1.16±0.05	1.45±0.06	0.0007	0.97±0.06	1.23±0.07	0.014
LDL	3.11±0.17	3.48±0.14	NS	3.07±0.31	3.46±0.25	NS	3.21±0.30	3.50±0.14	NS
Triglycerides, mmol/L	2.45±0.26	1.26±0.11	<0.0001	2.52±0.31	1.33±0.19	0.0012	2.29±0.47	1.17±0.11	0.0021
Lipoprotein(a), mg/dL	3.46±0.91	5.95±1.21	NS	2.75±0.82	6.57±1.84	NS	4.88±2.21	5.26±1.57	NS
ApoB, g/L	1.09±0.05	1.04±0.04	NS	1.14±0.05	1.03±0.07	NS	1.03±0.09	1.05±0.04	NS
Metabolic variables									
Insulin, U/L	16.6±2.0	12.5±1.8	0.020	19.2±2.5	12.0±2.1	0.0045	11.1±2.5	13.1±3.2	NS
C-peptide, pmol/L	1014±76	714±43	0.0004	1084±92	685±63	0.0007	883±129	748±56	NS
Free fatty acids, mol/L	0.66±0.05	0.43±0.03	<0.0001	0.72±0.06	0.46±0.05	0.0012	0.56±0.08	0.40±0.03	0.025
TNF-α, pg/mL	7.43±0.63	9.99±0.90	NS	7.43±0.63	10.5±1.46	NS	7.43±0.63	9.55±1.11	NS
Leptin, ng/mL	6.89±0.63	16.1±2.18	0.0025	7.26±0.58	23.3±3.3	0.0003	6.18±1.47	7.54±1.12	NS
Adiponectin, ng/mL	5.21±0.74	11.8±1.1	0.0042	5.05±0.82	13.8±2.1	0.016	5.51±1.80	10.1±1.0	NS (0.09)
ASP, ng/mL	585±337	748±296	NS	783±604	1061±518	NS	320±87	349±76	NS
Resistin, ng/mL	16.7±2.8	19.2±2.2	NS	17.0±2.5	18.7±2.5	NS	15.8±7.2	19.7±4.1	NS
Atherosclerosis markers									
CRP, mg/L	4.86±1.18	2.69±0.38	0.035	5.93±1.78	3.07±0.39	NS (0.06)	2.97±0.48	2.39±0.60	NS
PAI-1, IU/mL	12.3±4.8	13.4±1.8	NS	12.3±4.8	15.7±3.4	NS	12.3±5.2	11.4±1.4	NS
Fibrinogen, g/L	2.26±0.08	2.35±0.08	NS	2.33±0.09	2.35±0.09	NS	2.13±0.15	2.35±0.14	NS

Values are mean±SEM.

the FPLD subjects than in family controls. These significant differences were also seen in subgroup analysis according to sex and were consistent with the findings in Table 1. Twenty-four of 24 dyslipidemic subjects defined by age- and sex-matched cut points had high triglyceride and low HDL cholesterol levels, whereas only 4 of 24 had elevated LDL cholesterol values (data not shown). A post hoc subgroup analysis showed that dyslipidemic FPLD subjects also had significantly lower leptin but higher FFA and insulin levels than did normolipidemic subjects (data not shown). Plasma concentrations of lipoprotein(a) and apo B were not different between FPLD subjects and controls (Table 2).

Among metabolic variables, mean insulin and C-peptide were significantly higher in FPLD subjects than in family controls (Table 2). In the subgroup analysis according to sex, insulin and C-peptide were significantly higher in female FPLD subjects than in controls, but there was no difference in males. These findings suggest that insulin resistance is more pronounced in nondiabetic FPLD women than men. In the overall sample, serum FFA concentrations were significantly higher in FPLD subjects than in family controls. In the subgroup analysis according to sex, this difference was significant in both men and women.

As previously observed, mean leptin[28] and adiponectin[29] concentrations were significantly lower in FPLD subjects than in family controls. In the subgroup analyses according to sex, these analytes were both significantly lower in female FPLD subjects than in controls, but there was no difference in men. Again, this was consistent with a more severe biochemical phenotype related to insulin resistance in nondiabetic FPLD women than men. No differences were seen for ASP, resistin, or TNF-α between FPLD subjects and controls, both in the entire sample and in the subgroups divided by sex. Among inflammatory and coagulation markers, only mean CRP concentration was significantly higher in FPLD subjects than in family controls. Trends to higher mean CRP concentrations were seen in FPLD subjects of both sexes, but again the magnitude of the difference appeared to be more pronounced in nondiabetic FPLD women. Post hoc pairwise correlation analysis showed significant correlations between insulin or C-peptide and both FFA and CRP. For instance, in the overall sample, C-peptide was correlated with both FFA and CRP, with Pearson coefficients of 0.21 ($P=0.038$) and 0.50 ($P<0.0001$), respectively. Similar correlations were seen between insulin and these analytes, both overall and in subgroups divided by sex (data not shown). There were no differences in mean concentrations of PAI-1 or FBG in the entire sample or in the subgroups.

Discussion

FPLD due to mutant *LMNA* is a monogenic human model of insulin resistance with increased CHD risk, particularly in women.[11] Although CHD onset occurred later in the clinical course of FPLD (mean age, ≈47 years) and was associated with the presence of type 2 diabetes, hyperinsulinemia and dyslipidemia were seen in FPLD at younger ages, before the onset of hyperglycemia and diabetes.[11] The present findings extend the spectrum of proatherogenic metabolic abnormali-

ties found in younger hyperinsulinemic nondiabetic FPLD subjects with mutant *LMNA*. Specifically, the metabolic phenotype in FPLD includes increased serum concentrations of both FFA and CRP, with no changes in concentrations of TNF-α, PAI-1, or FBG. Leptin and adiponectin were significantly lower in FPLD subjects, whereas ASP and resistin were not significantly different. Overall, the distinctive cluster of proatherogenic metabolic abnormalities in nondiabetic FPLD subjects included elevated insulin, triglyceride, FFA, and CRP levels and low HDL cholesterol. Generally, these differences were more pronounced in the nondiabetic FPLD women than in men (Table 2). Subgroup analysis by sex showed that diastolic blood pressure was increased in female FPLD subjects. While early CHD in FPLD, especially in affected females, could simply reflect the risk seen in common type 2 diabetes, it is also possible that early metabolic changes present before the onset of diabetes may contribute to atherogenesis in FPLD. Because years may elapse between the onset of insulin resistance and the onset of frank diabetes, the presence of proatherosclerotic metabolic changes in nondiabetic FPLD subjects is consistent with the reported increase in CHD predisposition, especially in women.[11]

The association with CHD in FPLD is consistent with the epidemiological association of CHD with common hyperinsulinemia.[30] The basis for the increased risk of CHD in the common insulin resistance syndrome is complex, with contributions from several different component metabolic abnormalities.[30] The cardinal plasma lipoprotein abnormality in both common insulin resistance and FPLD is elevated triglycerides and depressed HDL cholesterol, with little or no increase in plasma LDL cholesterol or apo B.[10,11] Absence of an association of FPLD with lipoprotein(a) was consistent with the absence of an association of lipoprotein(a) with common insulin resistance. Alternatively, because lipoprotein(a) is so strongly genetically determined and widely variable in concentration, the sample size might have been too small to detect a difference. Future analyses of lipoprotein subfractions are planned for these kindreds. The presence of the high triglyceride/low HDL cholesterol profile in a monogenic syndrome of high CHD risk is relevant, because fibrate treatment to normalize this profile does not affect LDL cholesterol and still reduces CHD risk.[31]

Because an elevated FFA level is a feature of common insulin resistance,[32,33] it was important to document that FFA was elevated in FPLD, both in the overall sample and the subgroups divided by sex. FFA was correlated with insulin and C-peptide concentrations in this study. Elevated FFA underlies the insulin resistance syndrome through various mechanisms,[32,33] although the basis for increased FFA in FPLD is probably different from that in common insulin resistance. In this regard, it may be of interest that FFA was increased in male FPLD subjects, whose insulin and C-peptide concentrations were not significantly increased. Nonesterified FFA acts as both a metabolic substrate and a signal controlling glucose utilization in muscle and liver and insulin metabolism in pancreatic beta cells.[32,33] In FPLD, the absence of peripheral subcutaneous fat results in lipids being targeted for deposition in central fat stores. FFA is inextricably associated with the insulin resistance associated with central obesity, because central adipocytes are resistant to insulin action and portal FFA delivery to the liver increases when visceral triglyceride stores increase.[32,33] In addition, the increased muscle triglyceride stores in FPLD could interfere with insulin action or glucose utilization, although such a causal relationship has not been demonstrated.[32,33] Furthermore, increased FFA and prolonged dyslipidemia might be toxic to pancreatic beta cells, and this could predispose to pancreatic failure, leading to diabetes. In the subgroup analysis of men, the presence of significantly increased FFA in FPLD, with no significant difference in insulin or C-peptide, suggests that FFA elevation precedes the onset of hyperinsulinemia in affected subjects.

The adipocyte plays a key role in the development of insulin resistance.[34] The finding of decreases in both leptin and adiponectin in FPLD subjects, particularly women, confirms previous observations.[28,29] The significant decrease in these 2 adipocyte-derived hormones in FPLD is consistent with evidence that links leptin and adiponectin to the metabolic consequences of lipodystrophy. For instance, leptin and adiponectin administered together corrected insulin resistance in a mouse model of lipodystrophy.[35] Also, a decrease in adiponectin often precedes the development of insulin resistance.[36] A differentiating feature between FPLD and common insulin resistance is that although both are characterized by low adiponectin, FPLD is characterized by low leptin due to an absolute deficiency of subcutaneous adipose stores, whereas common insulin resistance is characterized by increased leptin due to increased subcutaneous adipose stores.[37] The absence of differences in ASP and resistin between FPLD and controls suggests that these analytes might be less strongly related to human insulin resistance.[23]

The other novel finding was the presence of a significantly increased serum CRP concentration in nondiabetic FPLD subjects in the overall sample. These trends were also seen in the subgroup analyses according to sex, particularly in women. CRP was positively correlated with insulin and C-peptide concentrations in this study. Prospective epidemiological studies identified high-normal or increased serum CRP as an independent predictor of CHD.[12–20] Furthermore, among healthy, nondiabetic subjects, elevated CRP was associated with measures of insulin sensitivity, suggesting that chronic subclinical inflammation was part of the insulin resistance syndrome.[22] However, until now there had been little information about serum inflammatory markers, particularly CRP, in monogenic syndromes of insulin resistance. The present findings suggest that increased serum CRP can be associated with central fat exclusively, because peripheral subcutaneous fat is absent in FPLD subjects and total body fat content was reduced, as indicated by decreased serum leptin. It is also possible that CRP is primarily elevated in FPLD owing to defective *LMNA* and its subsequent effects on adipocyte biology and chronic inflammation.

The specific association between increased CRP and FPLD is highlighted by the absence of significant differences for other acute-phase response markers. For instance, TNF-α, a cytokine produced by adipose tissue that may also play an important role in insulin resistance,[38] was not increased in FPLD subjects. Also, interleukin-6, which plays a key role in

adipocyte biology,[39] was not different in FPLD subjects compared with controls in a preliminary analysis (data not shown). Furthermore, PAI-1 and FBG, procoagulant markers whose concentrations were previously shown to be elevated in common insulin resistance,[21,40,41] were not elevated in FPLD. However, in FPLD, only CRP was significantly increased, to levels consistent with those associated with increased CHD risk in populations,[42] with no differences in the measured cytokines, such as TNF-α, or procoagulants, such as PAI-1 and FBG. Studies in populations have indicated that abdominal obesity with insulin resistance is associated with increases in both CRP[43,44] and PAI-1,[40] which contrasts with the increase in CRP only in FPLD. Although elevated CRP is also associated with specific clinical components of the common insulin resistance syndrome, such as blood pressure,[45] any independent association of elevated CRP with particular biochemical and/or clinical characteristics of FPLD would be difficult to ascertain in this relatively small study sample.

In summary, nondiabetic FPLD resulting from mutant *LMNA* was associated with hyperinsulinemia, high triglycerides, low HDL, hypoleptinemia, hypoadiponectinemia, elevated FFAs, and elevated CRP but not with changes in LDL cholesterol, apo B, lipoprotein(a), resistin, ASP, TNF-α, PAI-1, or FBG. The metabolic disturbances in nondiabetic FPLD subjects, particularly in women, were all deleterious, proatherosclerotic, and consistent with the increased CHD risk seen in Canadian FPLD families with mutant *LMNA*,[10,11] particularly in women. Our findings indicate that elevations in specific analytes, such as FFA and CRP, can occur relatively early in the clinical course of FPLD. Furthermore, the elevated FFA in FPLD was consistent with the elevated FFA in common insulin resistance. However, the specific elevation in CRP, without changes in the other cytokine and procoagulant markers typically associated with common insulin resistance, suggests that alterations in such variables are not always correlated and interdependent. Perturbations in each variable might follow a unique time course under the influence of distinct determinants, at least in monogenic syndromes such as FPLD.

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