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Original Contribution

Metreleptin therapy lowers plasma angiopoietin-like protein 3 in patients with generalized lipodystrophy

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KEYWORDS:
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BACKGROUND: Reduced triglyceride clearance due to impaired lipoprotein lipase–mediated lipolysis contributes to severe hypertriglyceridemia in lipodystrophy. Angiopoietin-like protein 3 (ANGPTL3) and 4 (ANGPTL4) impair clearance of triglycerides by inhibiting lipoprotein lipase. Whether circulating ANGPTL3/4 levels are altered in lipodystrophy and the effects of leptin replacement on these ANGPTLs are unknown.

OBJECTIVE: To examine if ANGPTL3/4 levels are elevated in patients with generalized lipodystrophy and assess the effects of leptin replacement on these ANGPTLs.

METHODS: Preleptin treatment plasma levels of ANGPTLs in patients with generalized lipodystrophy (n = 22) were compared with healthy controls (n = 39) using a post hoc case-control study design. In a prospective open-label study, we studied the effects of metreleptin therapy (16–32 weeks) on plasma ANGPTL3/4 in patients with generalized lipodystrophy.

RESULTS: Plasma ANGPTL3 (geometric mean [95% confidence interval]; 223 [182–275] vs 174 ng/mL [160–189], $P = .02$) but not ANGPTL4 levels (55 [37–81] vs 44 ng/mL [37–52], $P = .26$) were higher in patients with lipodystrophy compared with healthy controls. There was a significant decrease in total cholesterol, triglycerides, and glycosylated hemoglobin (A1C) levels following metreleptin therapy. After metreleptin, ANGPTL3 concentrations decreased significantly (223 [182–275] vs 175 ng/mL [144–214], $P = .01$) with no change in ANGPTL4 (55 [37–81] vs 48 ng/mL [32–73], $P = .11$).

CONCLUSIONS: These findings suggest that elevated plasma levels of ANGPTL3 in leptin-deficient states is attenuated with leptin therapy.

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Introduction

Lipodystrophy, a rare disorder characterized by hypoleptinemia and partial or complete (generalized) absence of adipose tissue, is associated with insulin resistance and hypertriglyceridemia.[1] Inefficient storage of circulating triglycerides due to the lack of adipose tissue, increased lipolysis, elevated hepatic fat synthesis, reduced activity of lipoprotein lipase (LPL), and impaired clearance of chylomicrons have all been proposed to play a causal role in hypertriglyceridemia.[2,3] In fact, hypertriglyceridemia is a frequent and consistent (>70%) manifestation in patients with generalized lipodystrophy.[4] In these patients, leptin replacement with recombinant human methionyl leptin (metreleptin), in addition to improving glycemia, causes an impressive reduction in triglycerides (>70%).[4–6] However, the mechanisms mediating the beneficial effects of leptin are not well understood.

Circulating angiopoietin-like proteins 3 (ANGPTL3) and 4 (ANGPTL4) are secreted glycoproteins that modulate triglyceride metabolism by directly inhibiting LPL.[7–9] In humans, ANGPTL3 is predominantly expressed in the liver, whereas ANGPTL4 is expressed in both liver and adipose tissue.[10] Elegant studies in mice and genetic studies in humans confirm the important role of these proteins in triglyceride metabolism. Mice deficient in ANGPTL3 or ANGPTL4 have reduced atherosclerosis and a favorable lipid phenotype.[11,12] In humans, nonsense mutations in ANGPTL3[13] and ANGPTL4[14] are associated with lower triglyceride levels and reduced risk for cardiovascular disease.

Circulating levels and hepatic expression of ANGPTL3 are increased in leptin-resistant db/db mice and leptin-deficient ob/ob mice.[15] Administration of leptin to ob/ob mice decreases hepatic ANGPTL3 messenger RNA expression and plasma ANGPTL3 levels.[15] Based on these studies and the key role played by these ANGPTLs in triglyceride metabolism, we hypothesized that ANGPTL3 and ANGPTL4 would be elevated in patients with lipodystrophy before metreleptin replacement compared with healthy controls and that ANGPTL3 and ANGPTL4 would decrease upon metreleptin replacement. To that end, in this study, we examined the levels of plasma ANGPTL3 and ANGPTL4 in healthy controls and patients with lipodystrophy before and during metreleptin replacement therapy.

Subjects and methods

Study design and study subjects

The study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases and conducted at the Clinical Center of the National Institutes of Health, Bethesda, Maryland. Written informed consent/assent was obtained from all participants/guardians. We used a cross-sectional case-control design to compare plasma levels of ANGPTL3 and ANGPTL4 in healthy volunteers recruited from a study of the phenotype of overweight and obese adults (ClinicalTrials.gov identifier NCT00428987) with patients with generalized lipodystrophy enrolled in a study evaluating the effects of metreleptin therapy in patients with lipodystrophy (ClinicalTrials.gov identifier NCT00025883). The study design, rationale, and inclusion criteria for this study have been described previously.[5] Patients with human immunodeficiency virus–associated lipodystrophy were not studied in this protocol. The effects of leptin replacement on plasma ANGPTL3 and ANGPTL4 levels as an exploratory outcome were examined in generalized lipodystrophy patients (n = 22) treated with metreleptin (Bristol-Myers Squibb and AstraZeneca). These patients received self-administered subcutaneous metreleptin injections.

Study procedure

In healthy and lipodystrophic patients at baseline and at 16–32 weeks after initiation of metreleptin, fasting blood samples were obtained to measure ANGPTL3, ANGPTL4, insulin, glucose, A1C, lipid panel, liver and renal function tests. A 75-g oral glucose tolerance test after an overnight fast was performed in patients with lipodystrophy. Samples for determination of plasma glucose, insulin, C-peptide at 0, 30, 60, 90, 120, and 180 minutes after the oral glucose load were collected. Oral Glucose Insulin Sensitivity (OGIS), a surrogate marker for insulin sensitivity was derived as previously described.[16] Body composition was measured by dual energy x-ray absorptiometry using a total body scanner (Lunar iDXA; GE Healthcare, Madison, WI). Plasma ANGPTL3 and ANGPTL4 were measured by using commercially available ELISA kit (BioVendor, Asheville, NC). Leptin was measured by radioimmunoassay using a commercial kit (Linco Research). All other measurements were done in the NIH Clinical Center laboratory using standard methodology.

Statistical analysis

After testing for normality, data were logarithm transformed where appropriate. Data are presented as means ± standard deviation for normally distributed variables or median (interquartile range) for nonsymmetric distributions. Comparison of various parameters were performed using the unpaired or paired Student *t* test for parameters with normal distribution and nonparametric equivalent of Student *t* tests for those with nonnormal distribution. In tests comparing plasma ANGPTL3 and ANGPTL4 levels, values are presented as geometric mean and 95% confidence intervals. $P < .05$ was considered to represent statistical significance. The statistical software JMP, version 8.1 (SAS Institute Inc, Cary, NC), was used for data analysis.