

Adiponectin and Leptin Concentrations May Aid in Discriminating Disease Forms in Children and Adolescents With Type 1 and Type 2 Diabetes

ALBA MORALES, MD¹
CLIVE WASSERFALL, MS²
TODD BRUSKO, BS²
CAROLYN CARTER, MD¹

DESMOND SCHATZ, MD¹
JANET SILVERSTEIN, MD¹
TAMIR ELLIS, PHD²
MARK ATKINSON, PHD²

OBJECTIVE — The incidence of pediatric type 2 diabetes has recently seen an alarming increase. To improve our understanding of pediatric type 2 diabetes and identify markers that discriminate these subjects from those with type 1 diabetes, we performed a multivariate analysis associating serum adiponectin and leptin levels with anthropometrical parameters and disease state.

RESEARCH DESIGN AND METHODS — Samples from children and adolescents with type 1 diabetes ($n = 41$) and type 2 diabetes ($n = 17$) and from nondiabetic individuals of similar age from the general population ($n = 43$) were investigated. An analysis included the parameters of matching for BMI and Tanner stage. Receiver-operator characteristic (ROC) curves were established to assess these analytes' association with disease.

RESULTS — Contrary to studies of adult type 1 diabetes, adiponectin levels in our pediatric type 1 diabetic subjects (10.2 $\mu\text{g/ml}$ [95% CI 8.6–11.7]) did not differ from those of healthy control subjects (10.6 $\mu\text{g/ml}$ [9.2–12.0]; $P = \text{NS}$). Children with type 2 diabetes (5.5 $\mu\text{g/ml}$ [4.8–6.2]) had significantly lower adiponectin levels than both of those groups. Conversely, type 2 diabetic subjects showed marked elevations in serum leptin concentrations (24.3 ng/ml [17.1–31.5]) compared with healthy control subjects (2.7 ng/ml [1.3–4.1]; $P < 0.001$) and type 1 diabetic subjects (5.1 ng/ml [3.5–6.7]; $P < 0.001$). Importantly, each of the properties ascribed to pediatric type 2 diabetes was present when the comparison was restricted to healthy children or type 1 diabetic patients whose BMI was >85 th percentile or who had Tanner stage 4 and 5. The evaluation of adiponectin-to-leptin ratios revealed a striking difference between children with type 1 diabetes (6.3 [3.8–8.8]) and type 2 diabetes (0.3 [0.2–0.5]; $P < 0.001$).

CONCLUSIONS — In pediatric diabetes, where diagnosis of disease is often difficult, these studies suggest that the adiponectin-to-leptin ratio may provide additional help in the discrimination between type 1 and type 2 diabetes.

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A worldwide epidemic exists with respect to type 2 diabetes, primarily because of increased rates of obesity (1,2). Recent studies have established adipose tissue as an endocrine organ capable of hormone and cytokine secretion (3).

One such secreted molecule, adiponectin, is an anti-inflammatory and antiatherogenic hormone exclusively synthesized in adipose tissue (4,5). Serum adiponectin levels are decreased in obese adults, including those with type 2 diabetes, and

increase during weight loss or treatment with thiazolidinediones (6). Indeed, adiponectin has been proposed to independently protect against type 2 diabetes (7). Conversely, increased plasma levels of adiponectin were observed in adult Japanese type 1 diabetic patients compared with BMI-matched healthy control subjects (8). Adiponectin appears to increase insulin sensitivity by regulating glucose and lipid metabolism. Indeed, a major effect of adiponectin involves the enhancement of insulin action in liver and, hence, the regulation of hepatic glucose output.

Another obesity-related hormone, leptin, is a molecule critical to the regulation of energy balance and body weight (9). Like adiponectin, it is secreted mainly by adipocytes. However, unlike adiponectin, which is inversely proportional to body fat, leptin levels have a direct correlation with total body fat and with increased serum levels in those with type 2 diabetes (10). Although previous studies have evaluated leptin and adiponectin production in diabetes, such studies have predominantly focused on adult populations (9–12). Therefore, we investigated children and adolescents with diabetes for production of these hormones not only for the pathogenic information that may be gleaned from their assessment, but also to identify any diagnostic value these markers might provide in discriminating between type 1 and type 2 diabetes, metabolic disorders that are sometimes difficult to distinguish in this age-group.

RESEARCH DESIGN AND METHODS

— We measured adiponectin and leptin levels in a single serum sample (nonfasting, stored at -80°C) from children and adolescents with type 1 diabetes and type 2 diabetes and from nondiabetic individuals of similar age from the general population (demographics in Table 1). Type 1 and type 2 diabetes were diagnosed according to American

From the ¹Department of Pediatrics, University of Florida, Gainesville, Florida; and the ²Department of Pathology, University of Florida, Gainesville, Florida.

Address correspondence and reprint requests to Dr. Mark A. Atkinson, PhD, University of Florida, College of Medicine, Department of Pathology, ARBR3-128, 1600 SW Archer Rd., Gainesville, Florida 32610-0275. E-mail: atkinson@ufl.edu.

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Abbreviations: ROC, receiver-operator characteristic.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—Anthropometrical and ethnic variables in the study population

| | Type 2 diabetes | Type 1 diabetes | Healthy control subjects |
|--------------------------|--|------------------|--------------------------|
| <i>n</i> | 17 | 41 | 43 |
| Age (years) | 14.4 (10–20) | 13.2 (9–20) | 13.7 (6–21) |
| Men/women | 4/13 | 21/20 | 22/21 |
| Tanner stage | 4.4 (2–5) | 3.9 (1–5) | 3.3 (1–5) |
| BMI (kg/m ²) | 36 (32.6–39.4) | 21.7 (20.4–23.1) | 20.5 (18.8–22.3) |
| Disease duration (years) | 2.4 (0.1–7) | 3.9 (0.1–10) | NA |
| Ethnicity (%) | | | |
| Caucasian | 18 | 73 | 62 |
| African American | 75 | 20 | 33 |
| Latino | 6 | 7 | 5 |
| Treatment | Combination of oral hypoglycemic agents with/without insulin | Insulin | NA |

Data are mean (range) unless otherwise indicated. Data for BMI are mean (95% CI). No healthy control subject (or any type 2 diabetic subject by their differential diagnosis) was identified with any of three type 1 diabetes-associated autoantibodies. In contrast, frequencies of 50–70% positivity were observed for those autoantibodies in the type 1 diabetic group. NA, not applicable.

Diabetes Association criteria (13). Specifically, type 1 diabetes was diagnosed through a clinical evaluation of a number of factors including a symptomatic history (e.g., polydipsia, polyphagia, polyuria), weight loss, BMI, ketoacidosis, and the presence of a type 1 diabetes-associated autoantibody (described below). For cases of pediatric type 2 diabetes, a diagnosis was established by historical (e.g., family history of type 2 diabetes), symptomatic history, physical (e.g., BMI, race, acanthosis nigricans), and laboratory data including the absence of type 1 diabetes-associated autoantibodies (13,14). All healthy control subjects were also autoantibody negative.

Since this study also involved subjects with an extended duration of either disease, in addition to the need for disease diagnosis to be uninfluenced by the knowledge of an individual's adiponectin or leptin level, all persons with either form of diabetes enrolled in this study were subjected to a blinded chart review by two pediatric endocrinologists. Additional information about the aforementioned clinical data made available to those two individuals for categorization of subjects to the type 1 or type 2 diabetes groups included that of C-peptide production and pharmacologic history. It is important to note that for this study, all subjects having any type 1 diabetes-associated autoantibodies were classified as having type 1 diabetes. However, 12 of the 41 (29%) individuals classified as having type 1 diabetes were autoantibody negative, which was most likely due to their extended duration of disease and the expected declines in positivity that occur

with increasing disease duration. Only after this process of independent diagnosis were adiponectin and leptin levels matched to the subject, thereby avoiding the potential for ascertainment bias to classify a subject to a particular disease category. For reasons of subject availability, individuals were also in subanalyses that were divided into groups based on being Tanner stage 4 or 5 or using the Centers for Disease Control and Prevention (Atlanta, GA) sex- and age-based definitions of >85th percentile to include those defined as “at risk for overweight” and “overweight.” Tanner staging was performed by standardized methods. Institutional review board–approved informed consents and assents were obtained.

Serum analyte and autoantibody detection

LINCOplex (Linco Research, St. Louis, MO) kits were used for the measurement of human leptin (sensitivity 0.01 ng/ml; interassay coefficient of variation [CV] 5.0%), while B-Bridge International (San Jose, CA) human adiponectin enzyme-linked immunosorbent assay kits were used for monitoring serum adiponectin levels (lower limit 0.02 ng/ml; interassay CV 3.2%). To reduce the potential for interfering heterophile or natural antibodies, a serum matrix diluent was provided by the manufacturer of the assay kits. Tests for autoantibodies against three type 1 diabetes-associated autoantigens were performed in all study participants, including those against insulin autoantibody, GAD antibody, and insulinoma-associated protein 2 antigen. Assays were

performed as previously described (15). The investigators are regular participants in workshops, and the proficiency tests were sponsored by the Immunology of Diabetes Society and the Centers for Disease Control and Prevention to validate assay performance. At the most recent effort (Diabetes Autoantibody Standardization Program [DASP] 2003), our performance for GAD antibody assay indicated 86% sensitivity and 96% specificity for type 1 diabetes, while our insulinoma-associated protein 2 antigen assay provided 68% sensitivity and 100% specificity.

Statistics

All statistical analyses were undertaken with GraphPad Prism 4.0 (GraphPad, San Diego, CA) using Fisher's exact test, receiver-operator characteristic (ROC) analysis, linear regression, *t* testing, or ANOVA (Kruskal-Wallis) with Dunn's posttesting. *P* < 0.05 was deemed significant.

RESULTS

Adiponectin levels in children and adolescents

Adiponectin levels were inversely correlated with BMI for the entire pediatric population studied ($r^2 = -0.2$; *P* < 0.0001). Analysis of subjects with BMI >85th percentile indicated that control subjects had higher adiponectin levels than type 2 diabetic subjects (Fig. 1A; control versus type 2 diabetic subjects, *P* < 0.01). Type 1 diabetic subjects were not significantly different from healthy control subjects (*P* = NS), yet levels in

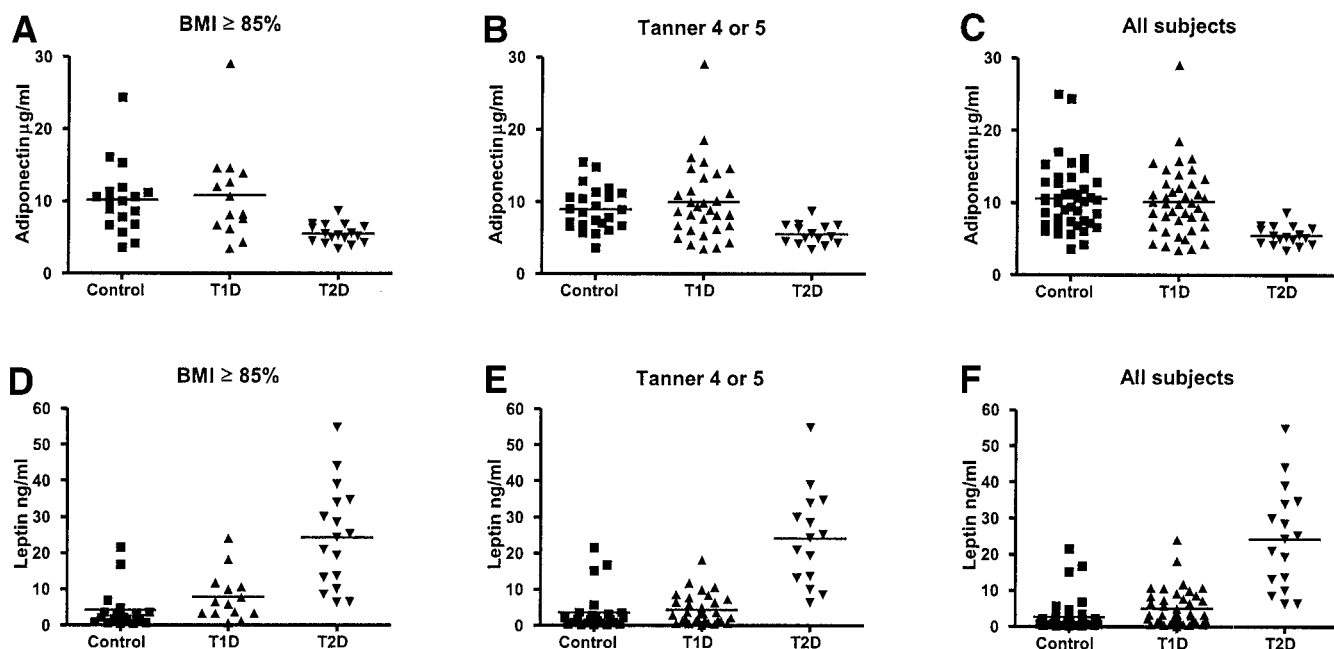


Figure 1—Adiponectin and leptin levels in healthy pediatric subjects and those with diabetes. Serum adiponectin levels in indicated subject groups with BMI >85th percentile (A), those with Tanner stage 4 or 5 (B), or all study participants (C). Serum leptin levels in study subject groups with BMI >85th percentile (D), those with Tanner stage 4 or 5 (E), or all study participants (F). Bar represents mean value. T1D, type 1 diabetes; T2D, type 2 diabetes.

type 1 diabetic subjects were higher than in those with type 2 diabetes ($P < 0.01$). There was no correlation between adiponectin levels and sex, but levels were lower in subjects with type 2 diabetes who were Tanner stage 4 or 5 (Fig. 1B; control versus type 1 diabetic subjects, $P = \text{NS}$; control versus type 2 diabetic subjects, $P < 0.01$; and type 1 diabetic versus type 2 diabetic subjects, $P < 0.01$). The adiponectin levels in our pediatric type 1 diabetic subjects (Fig. 1C; $10.2 \mu\text{g/ml}$ [95% CI $8.6\text{--}11.7$]) did not differ from those of healthy control subjects ($10.6 \mu\text{g/ml}$ [9.2–12.0]; $P = \text{NS}$). Children with type 2 diabetes ($5.5 \mu\text{g/ml}$ [4.8–6.2]) had significantly lower adiponectin levels than both of those groups (control versus type 2 diabetic subjects, $P < 0.001$; type 1 diabetic versus type 2 diabetic subjects, $P < 0.01$).

Childhood and adolescent leptin levels

Leptin levels were directly correlated with BMI for the entire pediatric population studied ($r^2 = 0.60$; $P < 0.0001$). This association was also observed when analyzed as a function of disease status. An analysis of subjects whose BMI was >85th percentile (Fig. 1D; control versus type 2 diabetic subjects, $P < 0.001$; type 1 diabetic versus type 2 diabetic subjects,

$P < 0.01$) or those with Tanner stage 4 and 5 (Fig. 1E; control versus type 2 diabetic subjects, $P < 0.001$; type 1 diabetic versus type 2 diabetic subjects, $P < 0.001$) indicated that children with type 2 diabetes had significantly higher leptin levels than healthy children and those with type 1 diabetes. However, leptin levels were not different between type 1 diabetic and control subjects if evaluating those >85th percentile (control versus type 1 diabetic subjects; $P = \text{NS}$) or with Tanner stage 4 and 5 (control versus type 1 diabetic subjects; $P = \text{NS}$). Leptin concentrations were somewhat higher (albeit not statistically significant) among girls (7.1 ng/ml [95% CI $5.5\text{--}8.7$]) than boys

(5.2 ng/ml [3.8–6.6]; $P = 0.061$), regardless of their diagnosis and BMI. Without accounting for BMI (i.e., all subjects), a trend was observed wherein mean leptin levels were elevated in type 2 diabetic subjects (Fig. 1F; 24.3 ng/ml [17.1–31.5]) versus all healthy control (2.7 ng/ml [1.3–4.1]; $P < 0.001$) or type 1 diabetic (5.1 ng/ml [3.5–6.7]; $P < 0.001$) subjects. Leptin levels were also modestly elevated in type 1 diabetic subjects compared with control subjects ($P < 0.05$).

Adiponectin-to-leptin ratios

An exploration of adiponectin-to-leptin ratios revealed an even more striking difference between type 1 and type 2 dia-

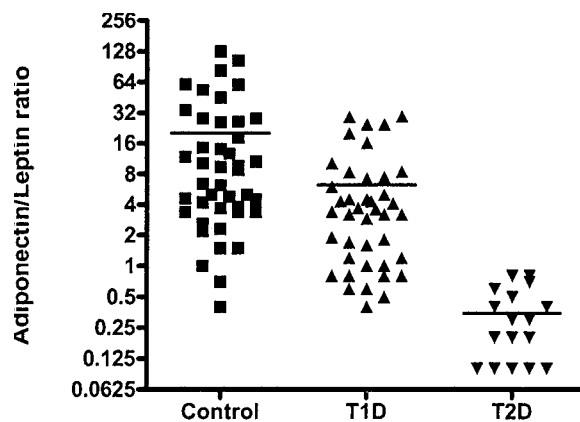


Figure 2—Adiponectin and leptin levels in healthy pediatric subjects and those with diabetes. Serum adiponectin-to-leptin ratios in indicated subject groups. Bar represents mean value; y-axis, \log_2 scale. T1D, type 1 diabetes; T2D, type 2 diabetes.

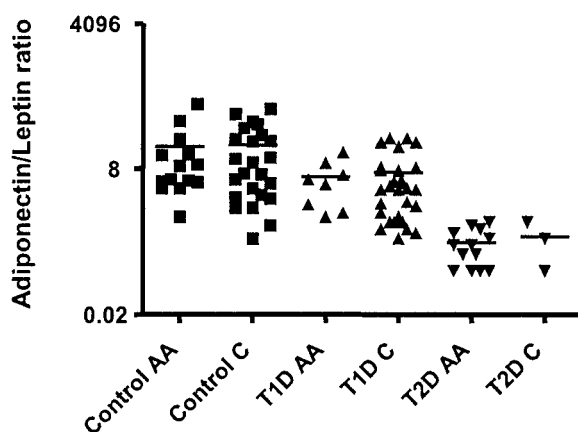


Figure 3—Adiponectin and leptin ratios in healthy pediatric subjects and those with diabetes as a function of race. Bar represents mean value; y-axis, log₂ scale. AA, African American; C, Caucasian; T1D, type 1 diabetes; T2D, type 2 diabetes.

betic children. Adiponectin-to-leptin ratios were dramatically different among healthy (20.2 [95% CI 11.3–29.0]) and type 1 diabetic (6.3 [3.8–8.8]) children compared with those with type 2 diabetes (0.3 [0.2–0.5]) (Fig. 2; control versus type 1 diabetic subjects, $P = NS$; control versus type 2 diabetic subjects, $P < 0.001$; type 1 diabetic versus type 2 diabetic subjects, $P < 0.001$). As anticipated, when restricting analysis to include only those subjects with BMI >85th percentile (control versus type 1 diabetic subjects, $P = NS$; control versus type 2 diabetic subjects, $P < 0.001$; type 1 diabetic versus type 2 diabetic subjects, $P < 0.01$) or Tanner stage 4 and 5 (control versus type 1 diabetic subjects, $P = NS$; control versus type 2 diabetic subjects, $P < 0.001$; type 1 diabetic versus type 2 diabetic subjects, $P < 0.001$), the ratios decrease because increases in BMI positively associate with increasing leptin or pubertal stage and decreases in adiponectin. Despite this, the ratio for type 1 diabetic and control subjects was significantly elevated versus type 2 diabetic subjects ($P < 0.001$). To ascertain potential influences of ethnicity, an analysis was performed that compared adiponectin-to-leptin ratios as a function of race (Fig. 3). Interestingly, no differences were observed in this ratio when comparing Caucasian and African-American subjects within the same disease group (all $P = NS$).

Diagnostic value

The key to these types of studies are issues of specificity and sensitivity. ROC plots were constructed comparing type 1 with type 2 diabetic subjects (i.e., area under the ROC curve of 0.969 [95% CI 0.93–1.00]; $P < 0.0001$) to determine

an appropriate cutoff value for the adiponectin-to-leptin ratio (Fig. 4). At a ratio cutoff of <0.9, the sensitivity was 100% (range 80–100%), with a specificity of 80% (65–91%) for type 2 as opposed to type 1 diabetes. At a ratio cutoff of <0.7, sensitivity was 88% (64–99%), with a specificity of 90% (77–97%).

CONCLUSIONS— While marked increases in the percentage of obese Americans have been the subject of much attention over the last decade, somewhat less well-discussed, but nonetheless equally significant, increases have been those involving the number of obese children and adolescents being diagnosed with type 2 diabetes (16,17). In retrospect, earlier efforts to remove the term “juvenile” from classifications used to describe diabetes in children may have been prophetic in that age alone cannot be as readily utilized to distinguish a particular form of diabetes. Even with alterations in terminology used for disease classification, major gaps remain in our understanding of the pathogenic mechanisms underlying both type 1 and type 2

diabetes, factors that especially hold true as they apply to studies of children. A large and rapidly growing body of research has been directed at improving our understanding of the role of obesity-related hormones, a principle one being leptin, in the pathogenesis of type 2 diabetes. Much attention has also been recently directed at the adipocyte hormone adiponectin. However, as suggested earlier, one common feature to studies of both of these hormones has been the propensity for investigations in adults. This does represent an unfortunate situation in that type 1 and type 2 diabetes are, in particular, often difficult to distinguish and diagnose in pediatric populations. These needs and interests formed the foundation for our investigations.

As suggested by previous literature, adiponectin levels in our study participants were inversely correlated with BMI when subjected to evaluation in all subjects. However, contrary to the aforementioned study in adult Japanese type 1 diabetic populations (8), adiponectin levels in our pediatric type 1 diabetic subjects did not differ from healthy control children. While the reasons for this difference are unclear, answers may reside in differing ages of onset, genetic factors, or other ethnicity-related facets that are commonly known to be distinctive between type 1 diabetes in Euro-Caucasian versus Asian subjects (e.g., different HLA and autoantibody frequencies). A key facet of our studies, and one applied throughout the entire investigation, was to perform two levels of analysis. The first being comparisons between groups subjected to relative sex and age matching, while the second-level analyses compared subjects based on similar pubertal stage (i.e., Tanner) and BMI. Specifically, as our pediatric type 2 diabetic population was

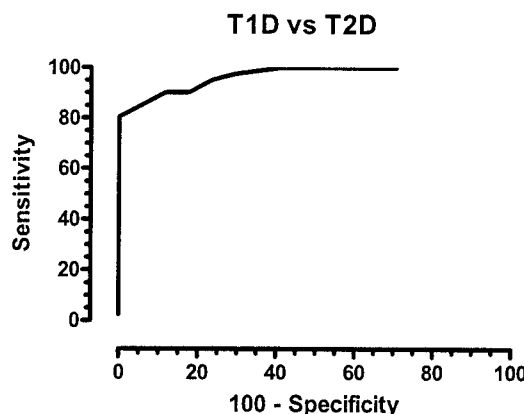


Figure 4—ROC curve for specificity and sensitivity of adiponectin-to-leptin ratio in those with type 1 diabetes (T1D) versus type 2 diabetes (T2D).

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entirely comprised of subjects with BMI >85th percentile, and nearly all subjects were of Tanner stage 4 or 5, we therefore performed separate analyses comparing type 2 diabetic subjects with control and type 1 diabetic subjects of like anthropomorphic measures.

Using these criteria, adiponectin levels in control and type 1 diabetic patients having BMI >85th percentile or Tanner stage 4 and 5 were similar, yet they were also higher than those observed in type 2 diabetic patients. Hence, while it is clear that adiponectin levels appear to be markedly diminished in children with type 2 diabetes, the genetic or pathogenic mechanisms underlying this insufficiency remain unclear. As expected, leptin levels correlated well with BMI in all subjects, regardless of their disease state and were modestly higher among girls than boys, with the latter facet thought to be the result of body fat distribution as a function of age in adolescent development (9).

However, most striking in our studies was the observation that ascertainment of adiponectin-to-leptin ratios revealed a distinct difference between type 1 and type 2 diabetic children. To our knowledge, this approach has not previously been applied. As already indicated, the development of diagnostic markers requires statistical ascertainment of specificity and sensitivity and not mere distribution analyses (i.e., mean + 2 SDs of control populations). To achieve this, ROC plots were constructed to compare type 1 with type 2 diabetic subjects, investigations that indicated a beneficial profile for these parameters. Indeed, at varying degrees of sensitivity (88–100%), specificities for disease of 80–90% could be achieved.

In terms of future directions, cohort studies, especially those prospective in nature, will be needed to validate our findings and uncover potential predictive values for disease development above the diagnostic values suggested by the aforementioned analyses. Indeed, most informative, but not attempted here due to limited subject numbers, would be a comparison of subjects at diagnosis of diabetes. Another question surrounds the stability of this phenotype (i.e., serum adiponectin and leptin levels) over time, apart from that associated with obvious changes in BMI. Our limited experience with longitudinal investigation of these analytes (data not shown) indicates a rel-

ative level of stability over short intervals of time, but expanded efforts are needed. Studies have also suggested (albeit not uniformly supporting) the notion that insulin therapy in children with type 1 diabetes can influence serum leptin levels (18,19). As such, future studies designed to separate issues of cause from effect are needed. Finally, additional studies must be designed to dissect the effect of obesity from the effect of the disease process.

In summary, we believe this study represents a unique and practical effort with regard to the findings, suggesting that ascertainment of adiponectin and leptin concentrations, as well as determination of their ratios, may aid in the differential diagnosis of pediatric diabetes beyond those offered by BMI or autoantibodies. While indirect, these studies would also support the notion that modulation of adipokines may represent a novel means for targeted therapies aimed at the prevention or reversal of diabetes.

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