CME

Mexican American Children Have Differential Elevation of Metabolic Biomarkers Proportional to Obesity Status

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ABSTRACT

Objectives: There is a health disparity for obesity among Mexican Americans compared with other racial/ethnic groups. In particular, Mexican American children who are obese are likely to become obese adults. The purpose of this study was to examine traditional and nontraditional risk factors in a subset of Mexican American children before their participation in a larger clinical weight loss study.

Methods: Venous blood samples were collected from self-identified Mexican American children (12–14 years old) who were assigned to 1 of 3 weight groups based on their standardized body mass index; normal weight (N = 66), overweight (N = 23), or obese (N = 39). Serum was analyzed for interleukin-6, tumor necrosis factor-α, C-peptide, ghrelin, glucagon-like protein, gastric inhibitory polypeptide-1, glucagon, insulin, leptin, macrophage chemoattractant protein 1, and pancreatic polypeptide using a Luminex MagPix-based assay. Total cholesterol, high-density lipoprotein-cholesterol, triglycerides, and glucose were analyzed using enzymatic assays. Data were analyzed for significance using separate analysis of variance tests, with significance set at $P < 0.05$.

Results: Relative to normal weight and overweight children, obese children had significantly elevated C-peptide ($P < 0.0001$), insulin ($P < 0.0001$), leptin ($P < 0.0001$), macrophage chemoattractant protein 1 ($P = 0.005$), and tumor necrosis factor-α ($P = 0.006$).

Conclusions: We observed that Mexican American children as a function of body weight had elevated serum concentrations of several biomarkers that have been linked to chronic disease development in adults. More research is needed to understand how these differences affect disease risk in adulthood.

Key Words: chronic disease, health, inflammation, poor nutrition

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Obesity is an independent risk factor for the development of many chronic diseases, such as type 2 diabetes mellitus and cardiovascular disease (CVD) and is a major concern within the Hispanic community (1). Not only is CVD the leading cause of death for Hispanics (1), but Hispanics also are more likely to die of CVD than other ethnic groups. Early prevention is critical because risk factors of CVD are shown to manifest at a younger age in Hispanic children (2–5). Presently, 77% of Hispanic adults and 38% of Hispanic children are overweight or obese (1), putting this population at greater risk for associated diseases including CVD, type 2 diabetes mellitus, hypertension, and hyperlipidemia (2–5). Although clinically accepted measures exist, such measures often fail to detect early disease onset; thus, the evaluation of novel disease risk markers in this understudied population is needed.

Our laboratory has spent the last decade examining the interplay among obesity, physical inactivity, and disease (6–10). Within this work, we have focused on the health disparity for diseases that exists among individuals from different racial/ethnic groups. The present study contributes to our ongoing research agenda because it aimed to compare a panel of nontraditional metabolic risk factors among Mexican American children of differing body weights. We have previously published reports detailing the other aspects of disease risk in this population (6–10). Our long-term goal is to identify and characterize new risk factors for disease in overweight and obese children. We hypothesized that Mexican American children would experience an increase in metabolic risk factors that was proportionate to their obesity status. Based on previous research from our laboratory with other biomarkers in this population, we also hypothesized that the target biomarkers would differentially increase with increased level of obesity.

The purpose of this study was to examine the nature of the relation between obesity status in Mexican American children and the serum concentration of interleukin (IL)-6, tumor necrosis factor-α (TNF-α), C-peptide, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, macrophage chemoattractant protein 1 (MCP-1), and pancreatic polypeptide (PP).

METHODS

Subjects

All of the procedures involving human subjects were reviewed and approved by the committee for the protection of human subjects at the Baylor College of Medicine. The subjects attended a charter school, whose population was predominantly Mexican American (>95%). Before participating in the study, both parental consent and child assent were obtained using approved forms. The subjects in this study were participating in a larger clinical weight loss study that has been in existence since 2003 and has been described in a number of previous publications (6,8–11). The present investigation included baseline data collected from students participating in this study in the autumn of 2010.

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Group Assignment

Body weight (digital scale) and height (stadiometer) were measured with the subjects wearing light clothing and no footwear. Body mass index (BMI) percentile was calculated using height, weight, age, and sex normative data from the Centers for Disease Control and Prevention (3). Using the Centers for Disease Control and Prevention guidelines, subjects were grouped according to their BMI percentile into normal weight (<85th percentile; N = 66), overweight (85th–95th percentiles; N = 23), or obese (>95th percentile; N = 39). There was no significant group difference in maturity status, as determined by a Tanner test administered by the school nurse. Descriptive statistics, stratified by group, are presented in Table 1.

Venous Blood Collection

A peripheral arm vein was used to collect whole blood samples (10 mL) into a vacutainer designed for separating serum (SST Vacutainer, Becton Dickinson, Franklin Lakes, NJ). All of the samples were collected in the morning (7–9 AM) following overnight fast and abstention from exercise (>8 hours). After collection, samples were placed on ice and serum was isolated by centrifugation within 2 hours of collection. Serum was stored frozen (−80°C) in sealed library tubes until analysis.

Cholesterol Profile and Glucose

Total cholesterol, high-density lipoprotein-cholesterol, triglyceride, and glucose concentration were measured in duplicate using separate enzymatic assays (Pointe Scientific, Canton, MI). Absorbance was measured using an automated microplate reader (Molecular Devices, Sunnyvale, CA) and unknown values were calculated using a single standard point method as described by the manufacturer. All of the samples were analyzed on the same day for a given analyte to minimize the influence of interassay variability. The measured interassay (<10%) and intraassay (<8%) variability were within the normal limits reported by the manufacturer.

Metabolic Biomarkers

Serum was analyzed in duplicate to determine the concentration of IL-6, TNF-α, C-peptide, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, MCP-1, and PP using a commercially available multiplex assay (HMHMAG-34K, EMD Millipore, Billerica, MA). Manufacturer-supplied controls were included to measure assay variation, and all of the samples were analyzed on the same day to minimize day-to-day CVs. A minimum of 100 beads were collected for each analyte using a Luminex MagPix System (Austin, TX), which was calibrated and verified before sample analysis. Unknown sample values were calculated offline using Milliplex Analyst Software (EMD Millipore). The measured interassay (<8%) and intraassay (<5%) variabilities were within the normal limits reported by the manufacturer.

Statistical Analysis

All of the statistical testing was completed using SPSS Statistics (version 19.0, SPSS Inc, Chicago, IL). Before analysis of variance testing, data were individually analyzed using the EXPLORE function in SPSS to identify potential outliers. If a subject's mean value was >3 standard deviations from the mean, then that subject was excluded from the dataset. We also tested for normality and completed log transformation as needed (data not shown). Details of excluded subjects or transformations are presented in the Results section with each variable. Separate analysis of variance tests were used to evaluate the biomarker data. Statistical significance was set at $P < 0.05$. Location of significant effects was completed using a separate t test with a Bonferoni correction.

RESULTS

Cholesterol Profile and Glucose

We only found significant group differences for triglycerides ($F = 7.203$, $P = 0.001$; Table 1), but no group differences for total cholesterol, high-density lipoprotein-cholesterol, or glucose (Table 1). Specifically, the overweight group had 26% more and the obese group had 43% more triglycerides than the normal weight group.

Metabolic Biomarkers

During initial screening of samples, we excluded 4 subjects from the final dataset because their value was >3 standard deviations from the mean for at least 2 variables. The number of subjects in each group (see above and Table 1) reflects the 4 subjects that were outliers and excluded. We found group differences for

TABLE 1. Subject characteristics stratified by weight group

Characteristic	Normal weight, N = 66	Overweight, N = 23	Obese, N = 39
Age, y	11.75 ± 0.56	11.74 ± 0.938	11.73 ± 0.67
Sex, % female	67.1	68.1	64.0
BMI, kg/m^2	18.65 ± 1.57	22.61 ± 1.49*	30.69 ± 5.53*,†
zBMI, percentile	59.80 ± 20.55	91.14 ± 2.99	98.34 ± 1.10
Waist circumference, cm	65.70 ± 4.61	77.71 ± 5.15*	97.52 ± 13.52*,†
Systolic blood pressure, mmHg	104.64 ± 8.90	107.86 ± 7.90	118.06 ± 8.38*,†
Diastolic blood pressure, mmHg	59.63 ± 7.58	63.71 ± 6.42*	70.26 ± 6.36*,†
Fasting glucose, g/mL	90.6 ± 8.8	90.9 ± 7.2	92.8 ± 9.3
Total cholesterol, mg/dL	117.9 ± 22.5	118.6 ± 20.5	120.7 ± 19.5
Triglycerides, mg/dL	60.7 ± 13.5	74.1 ± 15.3*	88.0 ± 26.7*,†
HDL-cholesterol, mg/dL	60.3 ± 16.0	58.1 ± 19.8	61.2 ± 19.2

BMI = body mass index; HDL = high-density lipoprotein.
* Indicates significantly greater than normal weight ($P < 0.05$).
† Indicates significantly greater than overweight ($P < 0.05$).

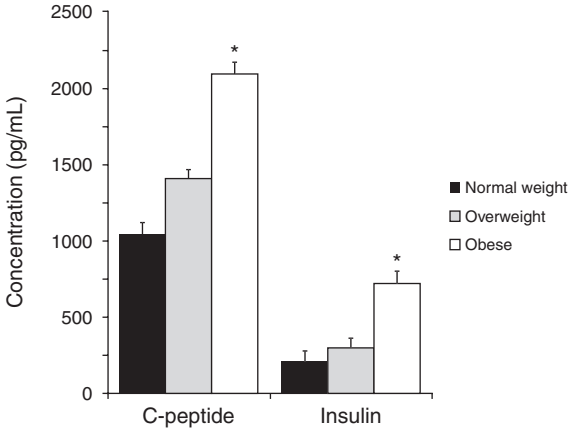

FIGURE 1. Serum C-peptide and insulin concentrations stratified by body weight group in Mexican American children. Body weight groups were determined according to standardized body mass index (zBMI). C-peptide and insulin concentrations were determined using a multiplex magnetic bead assay. *Indicates obese significantly greater than overweight and normal weight ($P < 0.05$).

C-peptide ($F = 31.937$; $P < 0.0001$; Fig. 1), insulin ($F = 22.900$; $P < 0.0001$; Fig. 1), leptin ($F = 37.037$; $P < 0.0001$; Fig. 2A), MCP-1 ($F = 5.629$; $P = 0.005$, Fig. 2B), and TNF-α ($F = 5.331$; $P = 0.006$; Fig. 2C). For C-peptide (↑50%) and insulin (↑62%), the obese group had significantly greater levels than the overweight and normal-weight groups, which did not differ from each other. For leptin, increased obesity status was associated with a progressive increase in leptin. The highest leptin concentration was found in the obese group (21,874 ± 2675 pg/mL) and the lowest leptin concentration was in the normal-weight group (3839 ± 474 pg/mL). For MCP-1, both the obese (↑20%) and overweight (↑23%) groups were significantly greater than normal weight, but did not differ from each other. For TNF-α, the obese group (↑24%) was greater than both the overweight and normal-weight groups. We did not find any significant group differences for PP, IL-6, GLP-1, glucagon, or ghrelin. Also, none of these variables appeared to demonstrate any potential trends toward significance group differences.

DISCUSSION

This study was completed as part of a larger, long-term study, the objective of which was to understand and develop methods to counter the behavioral and physiological consequences of overweight and obesity in Mexican American children (6–8,11). The target blood biomarkers for the present study were selected because of information on them in children and other data linking them as contributors of disease in obese adults (8,9,12–18). The key finding of the present study was that obese/overweight children had greater serum concentration of C-peptide, insulin, leptin, MCP-1, and TNF-α than normal-weight children. One novel finding of the present study was the differential nature of the relation between the various target biomarkers and obesity status. Specifically, we observed 3 patterns of metabolic biomarker response: elevated in overweight subjects and not further elevated with obesity (MCP-1 and TNF-α); normal in overweight subjects, but elevated in obese subjects (C-peptide and insulin); and elevated in overweight subjects and further elevated in obese subjects (leptin). To our knowledge, the present study is the first to report differential elevation of metabolic biomarkers in Mexican American children of differing obesity status. Although more research will be needed to understand the clinical significance of this finding, it is reasonable to speculate that some biomarkers may be more effective early indicators of disease, whereas others may better represent active disease or long-term risk.

Weight gain results in metabolic and physiologic changes in adipose tissue that contribute to adipose tissue inflammation (9,19,20). Inflamed adipose tissue is known to release leptin, MCP-1, and TNF-α, which are recruitment signals for proinflammatory monocytes (9). We have previously demonstrated that weight gain and obesity alter the profile of monocytes in both humans and mice (9,21). Although all 3 of our markers were elevated in obese compared with normal-weight children, only leptin and MCP-1 were also elevated in overweight compared with normal-weight children. This finding is interesting because it appears that leptin and MCP-1 may be more responsive to smaller differences in body fat than TNF-α. Given this hypothesis, it is plausible that during a weight loss intervention, there would be a cyclic change in these biomarkers such that leptin and MCP-1 would precede TNF-α. Our findings with respect to leptin, MCP-1, and TNF-α are consistent with previous reports from our laboratory and others (9,10). Collectively, these differences may contribute to increased adipose

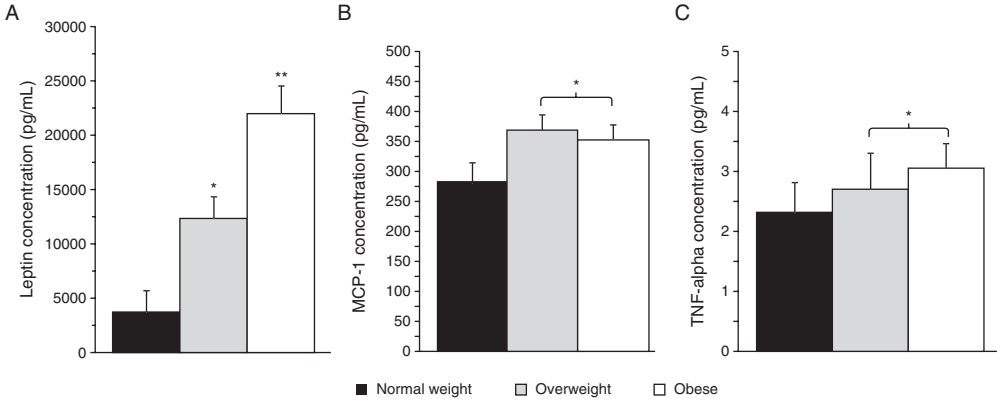

FIGURE 2. Serum leptin (A), macrophage chemoattractant protein 1 (MCP-1) (B), and tumor necrosis factor-α (TNF-α) (C) stratified by body weight group in Mexican American children. Body weight groups were determined according to standardized BMI (zBMI). Leptin, MCP-1, and TNF-α concentrations were determined using a multiplex magnetic bead assay. *Indicates obese and overweight significantly greater than normal weight ($P < 0.05$). **Indicates obese significantly greater than overweight and normal weight ($P < 0.05$).

tissue inflammation because biomarkers are generally regarded as the recruitment signal for the transmigration of proinflammatory monocytes from the blood into hypertrophied adipose tissue.

Metabolic disturbances of glucose sensitivity are a common, early stage in the onset of type 2 diabetes mellitus. Differences in blood insulin and to a lesser extent C-peptide are accepted measures of diabetes risk (16,22,23). The differences we observed with respect to these risk factors are consistent with the risk reported by others in that obese children had elevated insulin and C-peptide compared with normal-weight and overweight children (4,12). Similar to the outcome described above with TNF-α, there was no significant difference between obese and overweight children. Further interpretation of this finding suggests that insulin and C-peptide may not be as sensitive to differences in body weight as other disease risk factors. Given this to detect early differences in disease risk, it may be more appropriate to use leptin or MCP-1 because in this population, these biomarkers seem more responsive to small differences in body weight. Overall, the observed differences in insulin and C-peptide mirror that of MCP-1, leptin, and TNF-α; thus, it is reasonable to speculate that all of these outcomes are suitable measures of disease risk in this population of Mexican American children.

Mexican American children are a traditionally understudied population that has an elevated risk of obesity and subsequent disease development. Overweight/obese children represent a unique group to study because although they have elevated risk factors, unlike adults, children rarely present with active cardiovascular disease or type 2 diabetes mellitus (4,12,22,23). Comparing children and adults is difficult because children are not just small adults. For adults to reduce their obesity status, they must lose weight; however, children could reduce their obesity status by simply growing taller, whereas their weight remains constant. These differences between children and adults complicate our ability to fully evaluate our findings with respect to the existing literature. Despite the lack of active disease, we believe that the results presented in this study demonstrate an increased risk toward the development of obesity-associated disease in adulthood. Future, longitudinal tracking should strive to link childhood risk and adult risk in the same group of subjects; however, such experiments are complex and well beyond the scope of the present study.

Although we observed potential novel differences, in 5 biomarkers, we did not observe differences in PP, IL-6, GLP-1, glucagon, or ghrelin. Others have reported differences in some of these biomarkers with obesity in adults, but they do not appear to be altered in the Mexican American children that were tested as part of this study. More research would be needed to describe whether these biomarkers are only elevated in adults compared with children. Given the lack of significance in this childhood population, it is reasonable to speculate that an individual may need to have fully matured to alter these risk factors.

In summary, the key finding of the present study was that in Mexican American children, obesity was associated with a differential change in insulin, C-peptide, leptin, MCP-1, and TNF-α. Future clinical research should strive to evaluate the different response potential of the observed disease risk factors. Also, epidemiological studies will be needed to assess the long-term consequences of these elevated risk factors as children transition to adulthood. One limitation of the present study is that it was only cross-sectional in nature. In future studies, we will assess the ability to alter these biomarkers following a period of reduced zBMI. In this traditionally understudied population that has elevated obesity risk, we have found that several biomarkers that are commonly elevated in diseased adults are also elevated in overweight and obese children. Differences were either direct markers of disease (C-peptide and insulin) or associated with immunological changes in adipose tissue that favor increased inflammation (leptin, MCP-1, and TNF-α).

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