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Stress and Body Mass Index Each Contributes Independently to Tumor Necrosis Factor- α Production in Prepubescent Latino Children

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Abstract

This investigation extended prior work by determining if stress and body mass index (BMI) contributed independently to tumor necrosis factor- α (TNF- α) levels among prepubescent Latino children and if sex and family history of type 2 diabetes mellitus (T2DM) modified these relationships. Data were collected in South Florida from 112 nondiabetic school-aged Hispanic children, of whom 43.8% were obese (BMI $\geq$ 95th percentile) and 51.8% presented with a family history of T2DM. Stressful life events were assessed via parental report using a life events scale. Plasma TNF- α levels were determined with enzyme-linked immunosorbent assay. The relative contributions of stress and BMI with TNF- α levels and the potential interaction effects of sex and family history of T2DM were analyzed with multiple linear regression analyses. Stress and BMI each accounted for a significant proportion of the unique variance associated with TNF- α . The association between stress and TNF- α was not modified by sex or family history of T2DM. These findings implicate BMI and stress as independent determinants of TNF- α (an inflammatory cytokine and adipocytokine) among Latino children. Future investigations should examine the potential roles of exercise, nutritional status, age, and growth hormone in explicating the relationship between TNF- α production and psychosocial distress and risk for infection among obese children.

Keywords

TNF- α ; Children; BMI; Stress

Obesity has reached pandemic proportions worldwide. Recent data had indicated that obesity has doubled in developed and developing nations (Ogden, Flegal, Carroll, & Johnson, 2002). In the United States, 16% of children and teenagers between 6 and 19 years old (more than 9 million) have been determined as obese, defined as placing above the 95th reference percentiles of body mass index (BMI), adjusted for age and sex (Ogden et al., 2002), with a disproportionate representation of Latino and African American children (Goran, 2001). These numbers remain concerning in light of the associations that have been established between childhood overweight and obesity (as reflected by greater BMI), with increased childhood morbidity, including type 2 diabetes mellitus (T2DM) and insulin resistance syndrome, hypertension, dyslipidemia, left ventricular hypertrophy, atherosclerosis, proteinuria,

obstructive sleep apnea and exacerbation of asthma, nonalcoholic fatty liver disease and steatohepatitis, slipped capital femoral epiphysis and Blount's disease, pseudotumor cerebri, and depression (Daniels et al., 2005). Although obesity has been recognized as one of the easiest medical conditions to recognize, it has remained one of the most difficult to treat, with an annual cost estimated at 100 billion dollars in the United States alone (Wang & Dietz, 2002). These significant costs, with regard to actual dollars as well as the significant noted physical and psychological burden, have provided the impetus to identify pathophysiological markers associated with childhood obesity.

As an inflammatory cytokine, tumor necrosis factor- α (TNF- α) has been implicated in the pathogenesis of medical complications secondary to obesity, including insulin resistance and T2DM (Hotamisligil, 2000), coronary morbidity/mortality (Bacon, 2005; Miller, Freedland, & Carney, 2005), and depression (Toni, Malaguti, Castorina, Roti, & Lechan, 2004), at least among adult populations. In fact, insulin resistance has been characterized as a proinflammatory "condition" involving increased TNF- α and other inflammatory cytokines (Toni et al., 2004) among adult populations. Importantly, the molecular mechanisms remain poorly understood, and relatively few studies have examined these effects in children (Dixon, Goldberg, Schneiderman, & Delamater, 2004; Grohmann et al., 2004; Grohmann et al., 2005; Halle, Korsten-Reck, Wolfarth, & Berg, 2004; Hotamisligil, 2000; Hotamisligil, Arner, Atkinson, & Spiegelman, 1997), with inconsistent findings noted. Some research had found increasing levels of TNF- α with increased BMI (Halle et al., 2004; Reinehr, de Sousa, Toschke, & Andler, 2006), some others had found no relationship (Aeberli et al., 2006; Nemet et al., 2003), and still some (including our own) had found higher TNF- α levels with *decreased* BMI in child populations (Aeberli et al., 2006; Dixon et al., 2004; Zinman, Hanley, Harris, Kwan, & Fantus, 1999). Thus, the role of this important inflammatory cytokine as well as identified "adipocytokine" in the development and maintenance of obesity and associated comorbidities in children has remained largely undetermined.

Other factors, including cachexia (Argiles, Lopez-Soriano, Busquets, & Lopez-Soriano, 1997; Holden & Pakula, 1996), febrile and other infectious illnesses (Finck & Johnson, 2000; Wilson et al., 2000), acute injury and increased pain (Fernandez-Real & Ricart, 1999), growth hormone (GH) levels (Andiran & Yordam, 2007; Bozzola, De Amici, Zecca, Schimpff, & Rapaport, 1998), and greater psychosocial stress levels (Lalive, Burkhard, & Chofflon, 2002; Maes et al., 1998; Owen & Steptoe, 2003; Steptoe, Willemsen, Owen, Flower, & Mohamed-Ali, 2001), have been known to contribute to increased circulating TNF- α levels. In fact, differential effects have been noted for men versus women with regard to the relationship between greater psychosocial stress and higher circulating levels of TNF- α (Grossi, Perski, Evengard, Blomkvist, & Orth-Gomer, 2003; Steptoe, Owen, Kunz-Ebrecht, & Mohamed-Ali, 2002). However, this relationship has not been studied in children. In addition, albeit limited, research had demonstrated indirect associations between greater psychosocial stress and increased obesity, most commonly via eating behaviors (Bjorntorp, 2001; Cartwright et al., 2003; Dallman et al., 2003; Kivimaki et al., 2006; Nishitani & Sakakibara, 2005). Interestingly, stress has been specifically implicated in the pathogenesis of central (or abdominal) obesity and related comorbidities (Dallman et al., 2003). The mechanism postulated for this relationship has included the activation of the hypothalamic-pituitary-adrenal (HPA) axis, with increased adrenal hormones associated directly with the centralization of body fat and increased glucocorticoids associated with increased food intake, otherwise defined as "stress eating" (Dallman et al., 2003). However, the relationship of stress with cytokines associated with activation of the HPA axis, including TNF- α , has remained unstudied among child populations.

One of the most striking results from our prior work that studied TNF- α levels among obese versus nonobese Latino children regarded the observed clinically significant elevated TNF- α

levels in most of the sample, in the absence of any febrile illness or infection (Dixon et al., 2004). In fact, the children had been examined by a pediatrician prior to their entrance into the study and determined to be in good general health condition (Dixon et al., 2004). Given that TNF- α has been determined to play a critical role in the development of T2DM, this particular finding prompted the current investigation, which sought to determine potential predictors of TNF- α levels within a population of children who remained at increased risk for both insulin resistance syndrome and T2DM (Butte et al., 2005). Recognizing that prior work with adults had demonstrated significant associations between stress and TNF- α levels, our first main objective was to determine if elevated psychosocial stress would help explain the observed elevations in TNF- α levels among these children. In this regard, we hypothesized that greater stress would remain associated with increased TNF- α levels in these children. Our second main objective was to tease apart the potential independent effects of BMI and stress on TNF- α within this population of Latino children. We hypothesized that increased stress (consistent with prior work with adults) and decreased BMI (consistent with our prior findings) would demonstrate independent relative effects on TNF- α levels among these children. Our third main objective was to determine if these associations were modified by sex and/or family history of T2DM within these same children. We hypothesized that sex and family history of T2DM would modify the effects of BMI and stress on the outcome variable of TNF- α levels among this population of children.

Methods

Participants

The recruitment procedures and participants for this study have been described in detail previously (Delamater et al., 2001; Dixon et al., 2004). In brief, 122 school-aged children were randomly selected and subsequently enrolled into the study. Study participants underwent a physical examination (including Tanner staging) by a pediatrician and a blood test, while their parents were interviewed regarding health habits as well as history and provided with feedback regarding their child's health. Children were excluded from the study if they had a chronic disease (e.g., diabetes, renal disease, liver disease), a diagnosed endocrine or hormonal cause for obesity, or medications that could affect their glucose tolerance (e.g., dilantin, corticosteroids, diuretics, β -blockers). Ten children were not able to complete the blood sampling procedure. As was consistent with the demographics of this geographical region of South Florida, the final sample consisted of 112 prepubertal Latino children between 5 and 10 years old (kindergarten to Grade 3) who completed all study procedures. The study protocol was approved by the University of Miami School of Medicine Institutional Review Board, and informed child assent and parental consent were obtained from the participants and their parents, respectively.

Measurements

The construct of psychosocial stress was assessed via parental completion of the Life Events Checklist (LEC) for children, a measure that has been well validated for the assessment of stress among child populations (Johnson & McCutcheon, 1980). The LEC includes 46 specified items focusing primarily on external events, such as school failure, fights, arguments with peers, and family problems, and 4 additional optional items to note "other" events. For all items, the scale permits each research participant to indicate whether or not the events have occurred over the preceding year, to characterize endorsed events as "good" or "bad," and to then rate the degree of impact on a 4-point scale (0 = *no effect*; 1 = *some effect*; 2 = *moderate effect*; 3 = *great effect*). Because negative life event scores have been found to correlate with poorer psychological adjustment (Johnson & Bradlyn, 1988; Johnson & McCutcheon, 1980), this study focused on the 27 items that described negative events. Because no evidence supports that weighting remains preferable to simple counts of experienced events (Brand & Johnson,

1982; Johnson & Bradlyn, 1988), unweighted total negative stressful event scores were summed such that higher scores indicated greater negative stress. The test–retest stability for the negative stress subscore of the LEC over a 2-week interval has been demonstrated as $r = .72$, and the total and subscore scales have demonstrated good validity (Brand & Johnson, 1982; Johnson & Bradlyn, 1988; Johnson & McCutcheon, 1980).

Body composition was assessed with several measures, including height (centimeters), weight (kilograms), and BMI (weight in kilograms divided by height in meters squared). Fasting plasma TNF- α levels were determined from fasting baseline blood collections from a standardized oral glucose tolerance test (OGTT) administration (Owens et al., 1979). Specifically, intravenous catheters were placed into antecubital veins starting between 7:30 and 8:30 a.m. (the insertion of the catheter represented “time –30 minutes”). At baseline (Time 0), subjects ingested a 75-g glucose load. Blood samples were collected at –30, 0, 30, 60, 90, and 120 minutes for measurements of glucose, insulin, and TNF- α levels. This complete protocol consisted of five blood samples taken in a 120-minute period immediately following glucose ingestion. Upon each blood collection, plasma was immediately separated and placed on ice. Upon completion of the OGTT, all samples were immediately stored at -70°C and then batch assayed at a later date. The TNF- α levels for the current analyses were assayed from the Time 0 draw to allow for any acute inflammatory processes secondary to the venipuncture to subside. Enzyme-linked immunosorbent assay was used to determine fasting plasma TNF- α concentrations according to recommendations from the manufacturer (Immunotech, Beckman Coulter, Westbrook, ME), with standards assayed in duplicate. The sensitivity for the cytokine determinations was 10 pg ml^{-1} .

Statistical Analysis

All statistical analyses were performed using STATA software (Stata Statistical Software: Release 9. College Station, TX: StataCorp). We first examined the distributional characteristics of the continuous variables (TNF- α , BMI, and stressful life events). Because the distributions of all three variables were positively skewed, we used the ladder-of-powers method proposed by Hamilton (1992) to determine the appropriate transformation method, thus ensuring that the normality assumptions for the linear regression models were satisfied. Therefore, BMI was log transformed, and the variables of TNF- α and stressful life events were square-root transformed, prior to entrance into the univariate and multivariate analyses. We tested our first hypothesis by performing zero-order bivariate linear correlation analysis to examine the relationships between BMI, parental report of stressful life events (hereto “stress”), and TNF- α , followed by multiple linear regression analysis to determine the potential independent effects of BMI and stress (independent variables, expressed as continuous data) on TNF- α (dependent variable, expressed as continuous datum) when entered simultaneously into the same equation. We met our second objective by using linear regression to model the main effects of each of the independent (BMI and stress) and selection (sex and family history of T2DM, expressed as categorical data) variables on the dependent variable of level of TNF- α . In a separate model, we added two (interaction) terms to test the interaction between BMI and sex and that between sex and stress (Aiken & West, 1991). We examined the residual plots of the two models to ensure that the assumptions (of linearity, normality, homoscedasticity, and independence) of the linear regression model were met. We also calculated R^2 and the link test (Pregibon & DiClemente, 1980) to evaluate the goodness of fit of the models.

Results

This study included 55 girls and 57 boys, of whom 43.8% ($n = 49$) were found to meet criteria for obesity at the 95th percentile (adjusted for age and sex; Hammer, Kraemer, Wilson, Ritter, & Dornbusch, 1991), 52.7% ($n = 59$) presented with a family history of T2DM (for both first-

and second-degree relatives), as determined by physician interview (Mitchell et al., 1993), and 25% ($n = 28$) were obese *and* had a positive family history of T2DM. The detailed clinical and laboratory characteristics of the obese versus nonobese children have been described in detail elsewhere (Delamater et al., 2001; Dixon et al., 2004). As expected, obese children demonstrated greater insulin resistance, higher diastolic and systolic blood pressures, higher fasting glucose, triglyceride, and very-low-density lipoprotein cholesterol levels, and lower high-density lipoprotein cholesterol levels compared with nonobese children. With one exception, all children demonstrated glucose tolerance within normal limits. Higher circulating TNF- α levels were associated with nonobese status, female sex, and decreased insulin resistance (Dixon et al., 2004). Of the participants, 87.5% had detectable levels of TNF- α , also within the range of the TNF- α standardization curve. The rest of the participants (12.5%) had TNF- α levels below the assay limits that were determined as “0” values. These “0” values were preserved in the analyses by adding a constant of “10” to all values prior to entrance into the data analyses. Therefore, “0” values were treated as “real” versus “missing” values in the data analyses.

Stress, TNF- α , and BMI

The zero-order relationships between BMI, stress, and plasma TNF- α levels demonstrated that higher plasma TNF- α levels were associated with lower BMI ($r = -.31, p < .01$) and greater stress ($r = .26, p = .01$) in the sample. BMI and stress were positively correlated, but the relationship did not reach statistical significance ($r = .17, p = .09$). Multiple regression analyses revealed that BMI and stress each contributed a significant proportion of unique variance associated with TNF- α levels (Table 1).

Family History of T2DM

Table 2 characterizes the sample on the variables of age, BMI, sex, stress, and TNF- α , each as a function of family history of T2DM. The groups were equitable on all of these variables. When multiple regression analyses were conducted by entering family history of T2DM (selection variable) simultaneously into the equation with BMI, stress, and sex, no main effect was determined. Therefore, the interaction terms of Family History $\times$ BMI and Family History $\times$ Stress were not tested further. In addition, family history was not entered into any of the subsequent analyses.

Sex

Table 3 characterizes the sample on the variables of age, BMI, family history, stress, and TNF- α , each as a function of sex. The groups were equitable on all of these variables, with the exceptions of stress and TNF- α . When multiple regression analyses were conducted by entering sex (selection variable) simultaneously into the equation with BMI and stress, no main effect was determined, although a trend toward female sex with higher level of TNF- α was noted (Table 4).

Discussion

The results from this investigation provide important additional data regarding factors related to TNF- α levels (a potent inflammatory cytokine and adipocytokine) in a group of prepubertal children who remained at risk for insulin resistance syndrome and T2DM. Consistent with prior work with adult populations, these data supported our first hypothesis in that greater stress was associated with higher plasma TNF- α levels among these children (Lalivie et al., 2002; Maes et al., 1998; Owen & Steptoe, 2003; Steptoe et al., 2001). In fact, stress and BMI represented independent predictors (according to the statistical model) of TNF- α levels in these children, thus providing support for our second hypothesis. The first finding may explain, at least in part, the clinically significant elevated TNF- α levels that had been observed among

most of the children in our prior work, despite the absence of injury, infection, and febrile or chronic illness (Dixon et al., 2004).

Increased risks for T2DM and insulin resistance syndrome (characterized by a constellation of metabolic perturbations, including glucose intolerance, hypertension, and dyslipidemia) have been observed among adult Latino populations (Butte et al., 2005; DiMartino-Nardi, 1999; Nemet et al., 2003). Recent published data have provided support for a genetic predisposition of TNF- α and production of other proinflammatory cytokines, with implications for adult populations identified as at risk of developing insulin resistance syndrome (Grunnet, Poulsen, Klarlund, Mandrup-Poulsen, & Vaag, 2006). However, family history of T2DM did not represent a unique determinant of TNF- α production among the prepubertal Latino children in the current study such that our third hypothesis was not supported. As such, TNF- α may not represent a reliable marker of the pathophysiological development of insulin resistance syndrome in younger children. Other proinflammatory cytokines, such as interleukin 6, hepatic synthesis of C-reactive protein, and leptin production, have been shown to correlate with components of the insulin resistance syndrome, including elevated BMI, high triacylglycerol concentrations, low high-density lipoprotein cholesterol concentrations, elevated systolic blood pressure, and impaired glucose tolerance, at least among older (e.g., pubertal and postpubertal) children (Aeberli et al., 2006). Future research should investigate the role of family history of T2DM and these other factors in the development of T2DM among prepubertal Latino children.

Sex failed to modify the relationships between stress, BMI, and TNF- α levels among these children, despite the observation that girls were reported to have experienced more stressful life events compared with boys; in addition, girls evidenced greater TNF- α levels compared with boys. Thus, the second part of our third hypothesis was not supported. Our findings may have been limited by sample size, as the main effect for sex and the predictor variables approached, but failed to reach, statistical significance. In fact, we tested the interaction effect after noting this trend and found that sex appeared to represent a potential moderating factor for both BMI and stress on the outcome of TNF- α levels. Therefore, further research is needed to test the potential moderating effect of sex on BMI and stress on TNF- α and other factors that have been implicated in the development of both insulin resistance and T2DM among children.

Importantly, the baseline specimen collection was drawn 30 minutes following venipuncture to allow any acute inflammatory processes to subside prior to the blood sample collection. Furthermore, and in accordance with our approved institutional review board protocol, 10 children were not able to complete the blood sampling procedures and were subsequently withdrawn from the study, whereas the insertion of the catheter and the OGTT procedure were well tolerated (i.e., without any observed or expressed pain) among the remaining 112 children. Furthermore, pain was formally assessed as a “fifth vital sign” for the OGTT procedure, and none of the children indicated pain levels greater than “0” on a scale of 0–10 (0 = *no pain*). Therefore, pain did not appear to contribute to elevated TNF- α levels among this group of children. Depression (Kim et al., 2007; Yang et al., 2007) and anxiety (Arranz, Guayerbas, & De la Fuente, 2007; Chandrashekara et al., 2007) have also been associated with increased TNF- α levels, at least among adult populations. Because we did not evaluate the effects of these fairly complex additional clinical constructs among our younger child population, this area of research warrants further investigation.

GH has been found to attenuate TNF- α levels among adult and child populations, although there have been some inconsistent findings (Andiran & Yordam, 2007; Bozzola et al., 1998). A number of studies have determined TNF- α levels in GH-deficient children as significantly higher (in the absence of any infection or injury) than those in control subjects and to decrease

significantly to the levels in control subjects following long-term treatment with recombinant human GH (Andiran & Yordam, 2007; Lanes, Paoli, Carrillo, Villaroel, & Palacios, 2005; Serri, St-Jacques, Sartippour, & Renier, 1999). The inhibitory effects seemed to be direct in that insulin-like growth factor 1 (IGF-1) levels did not correlate in the same manner as GH with TNF- α levels (Andiran & Yordam, 2007). These results have paralleled studies of children suffering from burns (Chrysopoulou, Jeschke, Ramirez, Barrow, & Herndon, 1999) and cystic fibrosis (Hardin et al., 2001) in that GH administration resulted in a marked decrease in TNF- α levels among both patient populations. Hypopituitary women without GH treatment have also demonstrated clinically elevated TNF- α levels per unit of fat mass as compared with control subjects (Bulow, Ahren, & Erfurth, 2001). Importantly, both IGF-1 and GH have been shown to increase throughout the course of normal childhood development, peaking at approximately Tanner stages (TSs) III and IV (i.e., during puberty; Veldhuis, Roemmich, & Rogol, 2000). After TS IV, both GH and IGF-1 tend to decline (Veldhuis et al., 2000). Although we did not measure GH levels in our study sample, all of the children had been assessed by a pediatrician at TS I; thus, it remains possible that decreased GH levels may have contributed to the elevated TNF- α levels observed in these children. Interestingly, some research had demonstrated increased TNF- α levels with younger age in children (Aeberli et al., 2006). Although we found a similar negative correlation between higher TNF- α levels and younger age (data not published), our analyses failed to reach statistical significance, perhaps owing to the fact that the age range in our study may have been more restricted compared with other published data. Future studies are needed to determine any potential age-dependent effects of GH and TNF in young (prepubertal) children.

The current investigation determined that the relationship between TNF- α and BMI (as a continuous variable vs. a dichotomous variable) was inverse in that increasing BMI was associated with decreasing TNF- α levels, a finding that remained consistent with a limited number of other research studies with prepubertal children (Aeberli et al., 2006; Zinman et al., 1999). Other research had determined higher TNF- α levels in obese children (Halle et al., 2004; Reinehr et al., 2006), whereas others had not determined any association between TNF- α concentrations and BMI (or any other measure of adiposity, such as percentage of body fat or waist-hip ratio) in children (Aeberli et al., 2006; Nemet et al., 2003). The disparity in these findings may reflect, at least in part, some small sample sizes used, varying age groups across studies, and variability in cytokine production that was not controlled for by age within these studies. Nonetheless, the elevated TNF- α levels that have been observed among obese adult populations (Hotamisligil, Arner, Caro, Atkinson, & Spiegelman, 1995; Kern, 1997; Kern, Di Gregorio, Lu, Rassouli, & Ranganathan, 2003; Kern et al., 1995) have not been consistently demonstrated among children. As such, TNF- α may not represent a reliable pathophysiological marker of the subclinical inflammation that has been associated with greater risk for T2DM or cardiovascular disease among adult overweight and obese populations (Pearson et al., 2003; Pradhan, Manson, Rifai, Buring, & Ridker, 2001).

The conclusions gleaned from this study remain limited by a number of factors, including those limitations noted previously (Dixon et al., 2004). Given its correlational design, the results of this study should be considered preliminary in nature and therefore interpreted with caution. This study did not determine potential interaction effects associated with leptin, adiponectin, and resistin levels, among other factors, all of which have been implicated in TNF- α production among obese populations and which could represent additional explanatory factors for the noted disparities between research with adults and those with children (Ahima & Flier, 2000; Bokarewa, Nagaev, Dahlberg, Smith, & Tarkowski, 2005; Chu, Spiegelman, Rifai, Hotamisligil, & Rimm, 2000; Ferguson et al., 2004; Finck & Johnson, 2000; Fruhbeck, Gomez-Ambrosi, Muruzabal, & Burrell, 2001; Hinze-Selch et al., 2000; Kern et al., 2003; Mai, Bottcher, & Leijon, 2004; Martin-Romero, Santos-Alvarez, Goberna, & Sanchez-Margalet, 2000; Montalban, Del Moral, Garcia-Unzueta, Villanueva, & Amado, 2001; Wang et al.,

2000). In addition, the potential combined or separate effects of nutritional or exercise status on psychological stress, BMI, and/or TNF- α production were not determined among the children enrolled in this study; neither were they determined in other research that have studied adult or child populations. Finally, our findings may have been limited by the fact that stress was assessed using parental report of stressful life events, rather than each child's self-report of perceived stress—a limitation imposed by the fact that our population included children younger than 8–10 years. As a result, the children may not have perceived the endorsed life events as stressful. The assessment of psychological constructs remains a challenge when studying children younger than 8–10 years because no validated self-report questionnaire below that age range exists, owing to the fact that younger children tend to lack well-established reading comprehension skills. However, the measure of “objective” negative life events used for this study has been well validated to assess psychosocial stress among child populations.

Despite these limitations, this study provides important data regarding BMI and stressful life events as independent factors related to circulating TNF- α levels (an inflammatory cytokine and adipocytokine) in prepubertal children. Given the current paucity of available data with younger children, these findings provide a much needed contribution to the field of pediatric obesity. Further research needs to replicate these findings with other pediatric populations to determine if circulating TNF- α levels change in response to potential variations in BMI and/or stress levels among children. For example, research could investigate the potential effects of interventions designed to reduce perceived stress and/or BMI on modulating TNF- α levels. Additional research is needed to explore the relationships between stress, eating behaviors, depression, HPA axis and sympathetic nervous system activation, and TNF- α among children at risk for T2DM. Furthermore, the relationship between stress and TNF- α on resistance to infection in prepubertal children remains untested and warrants additional research. In addition, the relationship between elevated TNF- α levels, increased stress, and development of potential disease pathology in these children remains unclear, particularly given the inverse relationship between BMI and TNF- α among these same children. Finally, the effects of sex and positive or negative family history of T2DM on these relationships, as well as the implications for the potential development of insulin resistance syndrome and/or T2DM, need to be investigated.

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Appendix A

Previous presentations of this article at scientific congresses/meetings:

Dixon, D. (November 2007). Obesity, Stress and Immune Markers in Children: What Do We Understand Now, and Where Do We Go, From Here? Department of Psychiatry Division of Child Psychiatry and the Applied Behavioral Medicine Institute Invited Speakership, Stony Brook University Medical Center, Stony Brook, NY, USA.

Dixon, D. (October 2007). Obesity, Stress and Immune Markers in Children: What Do We Understand Now, and Where Do We Go, From Here? Department of Psychology Division of Social and Health Psychology Invited Speakership, Stony Brook University, Stony Brook, NY, USA.

Dixon, D. (August 2007). Obesity, Stress and Immune Markers in Children: What Do We Understand Now, and Where Do We Go, From Here? Virginia Treatment Center for Children

Department of Psychiatry Invited Speakership, Virginia Commonwealth University Medical Center, Richmond, VA, USA.

Dixon, D. (April 2007). Childhood Obesity: Current Perspectives and Directions. Department of Psychology Invited Speakership, Wayne Patterson University, Wayne, NJ, USA.

Dixon, D. (December 2006). Insight into Childhood Obesity. Department of Pediatrics Invited Grand Rounds Presentation, Stony Brook University Medical Center, Stony Brook, NY, USA.

Dixon, D. (February 2002). Implications of Child Obesity on Immune Function. Department of Psychiatry Invited Grand Rounds Presentation, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ, USA.

Dixon, D., Goldberg, R., & Delamater, A. (2001). Implications of Obesity, Stress, & TNF- α for Pediatric Insulin Resistance. Annual Research Day of the University of Medicine and Dentistry of New Jersey: Department of Psychiatry, New Jersey Medical School, Newark, NJ, USA.

Dixon, D., Goldberg, R., Schneiderman, N., & Delamater, A. (May–June 2006). Stressful Life Events, BMI and TNF-Alpha Levels in Children at Risk for Metabolic Syndrome. Annual Meeting of the Psychoneuroimmunology Research Society: Miami, FL, USA.

Dixon, D., Goldberg, R., Schneiderman, N., & Delamater, A. (May 2003). Stress and TNF-Alpha Levels in Obese versus Non-Obese Latino Children. Annual Meeting of the Psychoneuroimmunology Research Society: Amelia Island, FL, USA.

Dixon, D., Goldberg, R., & Delamater, A. (September 2001). Obesity, Stress and Immunity in Children. Annual Meeting of the European Health Psychology Society: St. Andrew's, Scotland, United Kingdom.

Dixon, D., Goldberg, R., Delamater, A., Fletcher, M., & Schneiderman, N. (May 2001). TNF-Alpha, Obesity, and Insulin Resistance in Children: Where Do We Go From Here? Annual Meeting of the Psychoneuroimmunology Research Society: Utrecht, The Netherlands.

Dixon, D., Delamater, A., Goldberg, R., Antoni, M., & Schneiderman, N. (November 2000). Implications of Stress & TNF-Alpha for Pediatric Insulin Resistance. Integrating Psychology and Medicine, Waiheke Island, New Zealand.

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Summary of Multiple Regression Analysis for Independent Contributions of Stressful Life Events and BMI to TNF- α Levels in the Children Studied ($n = 98$)

Variable	Coefficient	SE	β (sr^2)	t	95% confidence limits
LnBMI ^a	-4.11	1.54	-.26 (.07)	-2.66**	-7.17, -1.04
Stress_sq ^b	0.89	0.41	.21 (.05)	2.18*	0.08, 1.70

^aBMI, natural log transformed.

^bTotal score on the stressful life events scale via parental report, square-root transformed.

* Significant at the .05 α level.

** Significant at the .01 α level.

Table 2
Comparisons of Age, BMI, Sex, Stressful Life Events, and TNF- α by Family History of T2DM

Variable	Negative FHx ($M \pm SEM$)	Positive FHx ($M \pm SEM$)
Age (in months)	94.73 $\pm$ 2.33	93.97 $\pm$ 1.96
BMI	18.82 $\pm$ 0.60	19.69 $\pm$ 0.60
Sex	0.52 $\pm$ 0.07	0.48 $\pm$ 0.07
Stress ^a	3.36 $\pm$ 0.42	3.93 $\pm$ 0.28
TNF- α (pg/ml)	65.91 $\pm$ 9.07	62.30 $\pm$ 9.08

Note: FHx indicates family history of T2DM.

^aTotal score on the stressful life events scale via parental report.

Table 3
Comparisons of Age, BMI, Family History of T2DM, Stressful Life Events, and TNF- α by Sex

Variable	Female ($M \pm SEM$)	Male ($M \pm SEM$)
Age (in months)	94.22 $\pm$ 2.27	93.77 $\pm$ 1.98
BMI	19.09 $\pm$ 0.58	19.43 $\pm$ 0.60
Family history of T2DM	0.51 $\pm$ 0.07	0.55 $\pm$ 0.07
Stress ^a	4.02 $\pm$ 0.39	3.44 $\pm$ 0.39*
TNF- α (pg/ml)	73.01 $\pm$ 9.66	57.70 $\pm$ 8.27*

^aTotal score on the stressful life events scale via parental report.

* Significant at the .05 α level.

Table 4
Summary of Multiple Regression Analysis for Testing Main and Interaction Effects of Independent Variables as Contributing to TNF- α Levels ($n = 98$)

Variable	Model 1	Model 2	Model 3	Model 4
LnBMI	-4.08 **	-2.47	-2.94	-4.11 **
Stress_sqr	0.84 *	0.83 *	2.88	1.18 *
Sex ^a	0.92	11.12	0.9	2.09
BMI $\times$ Sex	—	-3.47		
BMI $\times$ Stress_sqr	—		-0.67	
Sex $\times$ Stress_sqr	—			-0.66
Constant	17.94	13.21	14.47	17.45
R^2	.148	.160	.149	.154

Note: Model 1 tested the main effects of BMI, stress, and sex (girls); Models 2–4, the interaction effects between BMI and sex, between BMI and stress, and between sex and stress, respectively.

^aFor girls, 1; for boys, 0.

* Significant at the .05 α level.

** Significant at the .01 α level.