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Parent reported nutritional risk and laboratory indices of cardiometabolic risk and in preschool-aged children

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Abstract

Background: Eating habits formed during childhood may contribute to the increasing prevalence of cardiometabolic disorders. Assessing nutritional risk in young children may help to prevent later cardiometabolic disease. The objective of this study was to determine whether parent-reported nutritional risk in preschool-aged children was associated with laboratory indices of cardiometabolic risk, namely leptin and insulin.

Methods: In this cross-sectional study, the relationship between nutritional risk as determined by the parent-completed NutriSTEP® questionnaire was assessed and compared to the serum leptin and insulin concentrations, hormones involved in regulation of food intake and biomarkers of adiposity and cardiometabolic risk. The community-based primary care research network for children in Toronto, Canada (TARGet Kids!) was used. The participants were children aged 3–5 years recruited from TARGet Kids! A total of 1856 children were recruited from seven primary care practices. Of these, 1086 children completed laboratory testing. Laboratory data for leptin and insulin were available for 714 and 1054 of those individuals, respectively.

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Results: The total NutriSTEP® score was significantly associated with serum leptin concentrations (p=0.003); for each unit increase in the total NutriSTEP® score, there was an increase of 0.01 ng/mL (95% confidence interval [CI] 0.004–0.018) in serum leptin concentrations after adjusting for potential confounders. The total NutriSTEP® score was not significantly associated with serum insulin concentration.
Conclusions: Parent reported nutritional risk is associated with serum leptin, but not insulin, concentrations in preschool-aged children. The NutriSTEP® questionnaire may be an effective tool for predicting future cardiometabolic risk in preschool-aged children.

Keywords: child; leptin; metabolism; nutrition assessment; preschool child.

Introduction

Eating habits are formed during childhood and poor habits may contribute to the increasing prevalence of obesity, heart disease, diabetes, and other chronic diseases [1]. Childhood obesity increases the likelihood of adulthood obesity [2] and is highly correlated with a variety of disorders including insulin resistance and hypertension [3]. Nutrition risk refers to the risk factors or characteristics that may result in impaired nutritional status [4]. However, identifying nutritional risk factors for cardiometabolic risk during childhood is challenging because medical illnesses associated with nutritional risk usually occur in adulthood [2, 5]. Potential targets affecting cardiometabolic outcome include levels and types of physical activity, nutritional intake and eating-related behaviors. These include the servings of specific food groups consumed per day, number of meals eaten per day, whether meals are eaten while watching television, and other eating behaviors [1].

Eating habits may be associated with the development of cardiometabolic consequences. For example, the Young Finns Study demonstrated that childhood vegetable consumption was inversely associated with metabolic syndrome after a 27-year follow-up period [6]. Indicators of metabolic syndrome are moderately stable throughout adolescence and early adulthood [7], and short-term diet and rigorous exercise interventions during childhood (ages 10–17 years) can promote reversal of metabolic syndrome [3]. These findings suggest that individuals with high cardiometabolic risk can be identified using indicators measured in childhood, and thus, early interventions may prevent cardiometabolic diseases.

We have previously shown that eating behaviors as assessed by the NutriSTEP® (Nutritional Screening for Every Preschooler) questionnaire, a valid and reliable parent-completed measure of nutritional risk [1, 8], is associated with serum non-high-density lipoprotein (HDL) levels, a surrogate marker for later cardiovascular risk [9]. Leptin, a hormonal protein released from adipocytes, is a non-traditional marker of cardiometabolic risk. Although the primary role of leptin is to signal satiety and regulate food intake, leptin has also been associated with a number of cardiometabolic disturbances [10]. Leptin is identified as a product of the "obesity gene" [11–13] and is considered a reliable marker of body fat mass and energy homeostasis [10, 14–16]. The predictive significance of leptin concentrations in the progression of obesity and cardiometabolic disorders has been demonstrated in several longitudinal studies in older children and adolescents but has not been evaluated in young children [17, 18].

Insulin is the primary hormone involved in glucose homeostasis [19]. It is positively related to obesity, blood pressure (systolic and diastolic) and low-density lipoprotein (LDL) cholesterol concentrations [20, 21]. Leptin is an emerging biomarker of insulin resistance through its effects on insulin-sensitive cells [11, 15, 19, 22]. Leptin and insulin have inhibitory effects on food intake and both increase energy expenditure [10, 14]. Individuals with higher abdominal obesity and BMI displayed higher than expected leptin concentrations in relation to their BMI [23], as well as resistance to the normal function of the hormone [12, 24]. Leptin resistance has been implicated in insulin resistance even after adjustment for BMI in obese children and in adults suggesting leptin dysregulation may be leading to cardiometabolic dysfunction [12, 19, 25]. It is unknown if nutritional intake or eating behaviors are associated with leptin or insulin in young children. The objective of our study was to investigate whether the parent-reported nutritional risk, measured by the NutriSTEP® score at 3–5 years of age [1, 8] is associated with leptin and insulin concentrations, two hormones that may be surrogate makers of cardiometabolic risk [16, 19, 26]. Elucidating a link between nutritional risk and cardiometabolic disorders may help determine which children would benefit from early interventions that promote cardiometabolic health.

Materials and methods

Subjects

Children aged 3–5 years were recruited from the community-based primary care research network for children in Toronto, Canada

(TARGet Kids!). There were seven primary care practices in the network, each of which had between 3 and 10 practicing physicians.

Study design

In this cross-sectional study, trained research assistants in each practice obtained survey data and physical measurements, and performed venous sampling.

Measurements

Our primary exposure was total NutriSTEP® score. This parent-completed questionnaire consists of 17 items (range of total scores 0–68), with questions divided a priori into the following five subscales: eating behaviors, dietary intake, parental concerns about food and activity, screen time duration (television, computer or video games use) and the use of vitamin/mineral supplements. Higher scores represent greater nutritional risk. The questionnaire has been validated for assessing nutritional risk in a population of multicultural Canadian preschool-aged children, with a detailed assessment by a registered dietitian that includes nutritional history and a 3-day dietary recall [1]. Some of the items constituting the NutriSTEP® questionnaire include whether children are allowed to decide how much they ate, the number of meals they ate per day, fast food consumption per week, and the servings of vegetables and fruit per day.

Our primary outcomes were non-fasting leptin and insulin. Non-fasting venous blood samples were obtained from the children on the same day as the NutriSTEP® questionnaire to assess leptin and insulin concentrations and were processed at the Mount Sinai Services Laboratory, Toronto, Ontario (mountsinaisservices.com).

We considered several potential confounders of the relationship between total NutriSTEP® score and insulin and leptin, which included child factors (age, sex, ethnicity, birth weight, zBMI, time of last meal) and parent factors (education level, ethnicity, history of gestational diabetes, BMI). Ethnicity data was obtained using open-ended survey questions from the Canadian Census [27]. Each participant was assigned an ethnicity risk category (elevated, average or reduced) based on the reported parental ethnicities. Risk for each parental ethnicity was categorized in the same way based on observational studies of metabolic risk [28, 29], and each participant was assigned the highest risk ethnicity of his or her parents for metabolic risk. Height and weight of the children and their parents were measured using established protocols [30] and standardized their body mass indices (BMIs) to z-score BMIs (zBMIs) using World Health Organization growth charts [31].

Statistical analysis

For our primary analysis we assessed the relationship between the total NutriSTEP® score and serum leptin concentrations using a multiple linear regression model adjusted for age, sex, ethnicity, birth weight, zBMI, parental BMI, history of gestational diabetes and mother's education status. All variables were a priori specified to be included in the model to avoid biased high R^2 values, biased low standard errors and p-values that are too small [32]. A similar model was constructed to evaluate the relationship between the total NutriSTEP® score and serum insulin concentrations, with the addition of time between participant's last meal and blood sampling. The distribution of leptin and insulin concentrations were skewed; thus, log-transformation of these variables was applied for use in the models. Missing data fit the missing-at-random criterion for both outcomes; multiple imputation methods using predictive mean matching to analyze these data were applied [32, 33]. A minimum of five datasets were imputed for each analysis and adjusted variances were computed. Imputation for the outcome variables of interest (leptin and insulin) was not performed. Multi-collinearity was not found to be a problem when examined using correlation matrices and variance inflation factors.

Ethical standards disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Hospital for Sick Children and St Michael's Hospital. Written informed consent was obtained from all participants.

Results

A total of 1856 children aged 3–5 years were recruited between 2008 and 2011 (Figure 1). Of these leptin and insulin data was available for 714 and 1054 participants, respectively, and were included in this analysis. Due to funding constraints, leptin testing was performed on fewer subjects. Subjects with laboratory testing were slightly younger with a lower birth weight, compared to subjects without laboratory tests (Tables 1 and 2). The mean (±standard deviation [SD]) laboratory indices for the subjects included in this study were as follows: leptin 2.2 (±2.4) ng/mL and insulin 64.2 (±56.1) pmol/L.

In the adjusted model, total NutriSTEP® score was significantly associated with serum leptin concentrations, ($p = 0.003$; Table 3). For each unit increase in total NutriSTEP® score, there was an increase of 0.01 ng/mL (95% CI 0.004–0.018) in log serum leptin levels. Birth weight and zBMI were positively associated with serum leptin concentrations and male sex was negatively associated with leptin concentrations (Table 3).

There was no statistically significant association between the total NutriSTEP® score and insulin concentrations. zBMI, age, sex and the time since last meal were statistically associated with insulin levels as indicated in Table 4. The greatest association was found in regards to the participant's sex, whereby for the male sex there

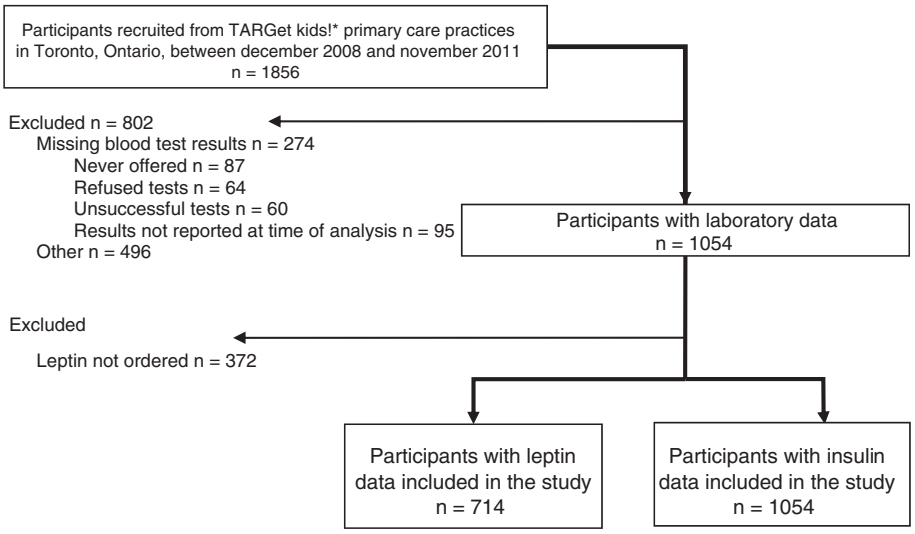

Figure 1: Recruitment of participants of the study.
*TARGetKids! is a primary care research network in Toronto, Ontario.

Table 1: Patient demographics for participants with leptin measurements.

	No. (%)[a]		p-Value
	Nonparticipants (n=714)	Participants (n=1142)	
Age, year, mean (±SD)	4.04 (±0.82)	4.19 (±0.83)	0.0002
Sex, male	358 (50%)	563 (49%)	0.72
BMI z-score, mean (±SD)	0.36 (±1.00)	0.31 (±1.05)	0.42
Birth weight, mean, kg (±SD)	3.30 (±0.65)	3.38 (±0.92)	0.05
Parental BMI, mean (±SD)	25.3 (±4.81)	25.1 (±5.79)	0.41
Maternal postsecondary education	613/695 (88%)	951/1067 (89%)	0.55
Gestational diabetes	27/669 (4%)	43/1016 (4%)	0.84
Ethnicity with high metabolic risk	254/592 (43%)	353/837 (42%)	0.78

BMI, body mass index; SD, standard deviation. [a]Unless otherwise indicated.

Table 2: Patient demographics for participants with insulin measurements.

	No. (%)[a]		p-Value
	Nonparticipants (n=1054)	Participants (n=802)	
Age, year, mean (±SD)	4.14 (±0.84)	4.13 (±0.82)	0.80
Sex, male	520 (49%)	401 (50%)	0.78
BMI z-score, mean (±SD)	0.35 (±1.00)	0.30 (±1.06)	0.44
Birth weight, mean, kg (±SD)	3.30 (±0.66)	3.42 (±1.01)	0.009
Parental BMI, mean (±SD)	25.27 (±4.77)	25.13 (±6.25)	0.65
Maternal postsecondary education	896/1011 (89%)	668/751 (89%)	0.83
Gestational diabetes	42/980 (4%)	28/705 (4%)	0.75
Ethnicity with high metabolic risk	363/862 (42%)	244/567 (43%)	0.73

BMI, body mass index; SD, standard deviation. [a]Unless otherwise indicated.

was a decrease in insulin concentrations, similar to leptin (Table 4). There was a significant negative association between the time since the participant's last meal and serum insulin concentrations. There was no association between the time since the participant's last meal and serum leptin measurements.

Table 3: Regression model showing the adjusted association between total NutriSTEP® score, patient demographics and log-transformation of serum leptin concentrations.

Characteristic	Adjusted[a] β estimate, 95% CI, n=714	p-Value
NutriSTEP® score	0.01 (0.004–0.018)	0.0033
Age	0.05 (–0.003–0.103)	0.067
Sex, male	–0.54 (–0.63 to –0.45)	<0.001
Birth weight	–0.06 (–0.28–0.16)	0.046
BMI z-score	0.35 (0.30–0.39)	<0.001
Parental BMI	0.0012 (–0.008–0.01)	0.79
Gestational diabetes	–0.06 (–0.28–0.16)	0.57
Maternal post-secondary education	0.01 (–0.13–0.16)	0.85
Ethnicity with high metabolic risk	0.04 (–0.05–0.14)	0.37

BMI, body mass index; CI, confidence interval. [a]Adjusted for all other variables in the table Model $R^2 = 0.37$.

Table 4: Regression model showing the adjusted association between total NutriSTEP® score, patient demographics and log-transformation of serum insulin concentrations.

Characteristic	Adjusted[a] β estimate, 95% CI, n=1054	p-Value
NutriSTEP® score	–0.002 (–0.01–0.007)	0.61
Age	0.11 (0.04–0.18)	0.002
Sex, male	–0.13 (–0.24 to –0.014)	0.028
Birth weight	–0.03 (–0.11–0.04)	0.37
BMI z-score	0.08 (0.03–0.14)	0.005
Parental BMI	–0.005 (–0.016–0.006)	0.37
Gestational diabetes	–0.05 (–0.35–0.25)	0.73
Maternal post-secondary education	0.03 (–0.16–0.22)	0.78
Ethnicity with high metabolic risk	0.0003 (–0.13–0.13)	1
Time between participant's last meal and blood sampling	–0.003 (–0.0034 to –0.0019)	<0.001

BMI, body mass index; CI, confidence interval. [a]Adjusted for all other variables in the table.

Discussion

In our study, nutritional risk as assessed by the parent-completed NutriSTEP® questionnaire was positively associated with serum leptin concentrations in children between the ages of 3 and 5 years. The association between the total NutriSTEP® score and leptin concentrations remained significant after adjusting for the following potential confounders: age, sex, birth weight, zBMI, parental BMI, history of gestational diabetes, parental ethnicity and mother's educational status. To the best of our knowledge, this study is the first to report on the association between nutritional risk and the hormone leptin in preschool-aged children. Several other studies have found leptin concentrations positively associated with BMI [10, 18, 34–36], but studies in this age group have not examined nutritional risk and leptin. If nutritional risk is shown to be associated with markers of current and future cardiometabolic risk, then nutritional risk measures such as the NutriSTEP® questionnaire may be a potential screening tool to identify children who would benefit from interventions to promote cardiometabolic health in young children.

There is some evidence that leptin may play a role in eating-related behaviors. Leptin has been demonstrated to signal satiety by suppressing a stimulator of food intake, Neuropeptide Y, from the hypothalamic region of the brain resulting in reduced food intake [14, 37]. Leptin-deficient children have shown increased hunger and food intake during meals and decreased post-meal satiety [38, 39]. Leptin does not solely act in the brain to reduce food intake; it is also involved in the reward pathways of food by reducing the motivation to acquire the rewarding stimuli via modulation of the dopaminergic neurons [14]. Functional magnetic resonance imaging (fMRI) demonstrated that in leptin-deficient individuals, images of food were associated with a higher activation in areas of the brain that are associated with pleasure and reward compared to the images of non-food items that were shown [39].

Although the main role of leptin is to signal satiety and regulate food intake by transmitting signals to the brain, it has also been associated with a number of cardiometabolic disturbances [12]. Serum leptin concentrations appear to increase with the number of components of metabolic syndrome in adults [34]. Dose-dependent effects of leptin on sympathetic nerve activity have also been demonstrated, potentially leading to hypertension [40] and contributing to metabolic syndrome [41].

Interestingly, several studies have demonstrated that leptin concentrations can be used to predict future obesity [18, 42]. Infants with serum leptin concentrations less than 2.7 ng/mL had a significantly higher BMI when assessed in follow-up at a median age of 8.8 years. Similar results were found in another study that demonstrated baseline levels of leptin were predictive of metabolic syndrome score after a 2-year follow up in 18-year old men [17]. There is evidence that the leptin pathway may be dysregulated and may lead to central resistance at the level of the hypothalamus [14, 25, 26], which may promote increased food intake, weight gain and cardiometabolic disturbances [25, 26]. The increased food seeking-behavior in

leptin-deficient children has been shown to continue into later adulthood [43], suggesting the potential of intervening in early childhood to prevent future cardiometabolic disorders. Interventions can improve healthy eating and cardiometabolic outcomes [3, 6, 44–46].

We did not find an association between the total NutriSTEP® score and insulin concentrations. Although both leptin and insulin are normally involved in the long-term regulation of body weight and energy homeostasis [16, 26, 43], insulin is a regulator of short-term food intake [26, 43]. The concentration of circulating insulin has been shown to be proportional to recent carbohydrate and protein intake [26]. There are rapid rises following carbohydrate intake in contrast to leptin, which does not have similar dramatic shifts. It was not feasible to collect fasting insulin levels in this longitudinal cohort of young children and it is therefore possible that an association between leptin and fasting insulin may exist. We did however adjust for the time since the child's last meal. As indicated in Table 4, the time since the child's last meal was significantly associated with their insulin concentrations ($p < 0.001$); this finding suggests that measuring fasting blood insulin concentrations may demonstrate a stronger association with the total NutriSTEP® score than random insulin levels.

Similar to leptin, high insulin concentrations in the context of normal glucose (i.e. insulin resistance) has been linked to the development of metabolic syndrome and may actually contribute to weight gain [41, 47, 48]. It has been demonstrated that serum leptin concentration is correlated with insulin concentration [34, 49, 50] and in turn, leptin and insulin concentrations rise in parallel in obesity. Importantly, it has been demonstrated that at any given BMI, higher leptin concentrations are associated with greater insulin resistance [11, 19, 34, 35, 51].

This study has several limitations. Due to the cross-sectional nature, causality between nutritional risk and leptin cannot be determined. It is not clear if the dysregulation of leptin promotes unhealthy eating behaviors or conversely, unhealthy eating behaviors promote leptin dysregulation. However, other studies in infants and in adolescents suggest leptin dysregulation may precede cardiometabolic disturbances [17, 18, 40, 42]. Additionally, the lab results were non-fasting because children were assessed during routine visits and the identified relationships may have been different with fasting lab results. This is especially relevant for the insulin measurements, that are dependent on the elapsed time since the carbohydrate intake and the type of carbohydrate consumed (i.e. of slow or fast absorption). The NutriSTEP questionnaire however does not provide information on the type of carbohydrate consumption and thus, we did not account for the effect of the type of carbohydrate on insulin measurements. Furthermore, it will be important to determine if leptin predicts future nutritional risk or nutritionally related health outcomes such as obesity and cardiometabolic risk in this cohort, in a longitudinal follow-up. Due to funding constraints, leptin was not measured on all children, and the sample size is therefore smaller. This is still the largest study of leptin levels in preschool-aged children to our knowledge. Children who were included in the study were demographically similar to those for whom laboratory data were not available (Table 1).

Strengths of our study include a relatively large study population taken from an age group of children that is understudied with respect to cardiometabolic markers. The recruitment of participants was from primary care practices in Toronto who may not be representative of children in other settings. Over 40% of the children in this study were from higher risk ethnic groups for cardiometabolic disturbances, reflective of Toronto's multicultural diversity and relevant for studies of cardiometabolic risk. The NutriSTEP® questionnaire is valid and reliable in determining nutritional risk in preschoolers [1] but has not been validated against similar cardiometabolic laboratory measures.

The NutriSTEP® nutritional risk score was positively associated with leptin concentrations in pre-school aged children. This may indicate nutritional risk can lead to very early dysregulation of leptin before cardiometabolic disorder consequences manifest. We plan longitudinal follow-up to determine whether these relationships persist and if they translate to development of future cardiometabolic risk. Studies to determine which children are at greatest risk of cardiometabolic disturbances may help identify those to target for intervention. The NutriSTEP® may be considered an important screening tool to identify children at most risk of cardiometabolic disturbances who may benefit from early intervention.

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