

## Metabolic features of adiposity and glucose homeostasis among school-aged Inuit children from Nunavik (Northern Quebec, Canada)

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### ABSTRACT

In contrast to most Indigenous people in Canada, Inuit appeared until recently to have been protected from type 2 diabetes (T2D) related to obesity. We assessed the associations of metabolites (amino acids, acylcarnitines) with adiposity and biomarkers of T2D in school-aged Inuit children of Nunavik (Canada). Concentrations of metabolite were measured in plasma samples from a cross-sectional analysis of 248 children (mean age = 10.8 years). We assessed associations of plasma metabolites with adiposity measures (BMI, skinfold thicknesses) and T2D markers (insulin, glucose, adiponectin). Plasma concentrations of valine and tyrosine were higher in obese and overweight children compared to those of normal weight children ( $P < 0.05$ ). An increment of 1-SD in BMI ( $SD = 3.3 \text{ kg/m}^2$ ) was statistically associated with an increment of 0.21 (95% CI: 0.08, 0.33) for valine, 0.15 (95% CI: 0.02, 0.27) for isoleucine and 0.17 (95% CI: 0.04, 0.29) for tyrosine. Insulin concentration increased with concentrations of all amino acids ( $P < 0.05$ ) except methionine. None of the acylcarnitines measured were statistically significantly associated with adiposity or T2D biomarkers. A signature of metabolites, particularly higher levels of branched-chain amino acids, might allow for early detection of T2D among school-aged Inuit children.

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## Introduction

Childhood adiposity carries a significant burden of chronic diseases [1,2], psychological disorders [3] and health-care costs [4]. The most prevalent approach of determining obesity – a crude index of adiposity – is to compare the body mass index (BMI) to a reference-standard that takes into account child age and sex [5]. The Inuit population of Nunavik (Northern Quebec, Canada) has experienced a low prevalence of obesity until the 1970s, when transition towards a western-style diet and sedentarity started taking its toll. We recently reported that the International Obesity Task Force (IOTF) BMI reference-standard is the most specific for identifying obesity accompanied by higher insulin disturbances in school-aged Nunavik Inuit children [6].

Because T2D has a strong adiposity-dependent component, authors have suggested the use of the term “diabesity” when referring to the common factors of

diabetes and obesity [7]. The prevention of T2D, for which obesity is the major contributor, requires a better understanding of the pathophysiological changes that could lead to earlier diagnosis and intervention. Tabák et al. [8] reported that changes in current biomarkers of T2D (insulin-resistance, fasting and 2 h post-load plasma glucose) appear two to four years before its diagnosis. Data from the emerging field of metabolomics – the study of the metabolome, i.e. small molecule chemicals within a biological sample – indicate that circulating concentrations of certain metabolites, in particular amino acids (AA) and acylcarnitines (AC), allow for early detection of diet-induced linking obesity and T2D [9,10].

Metabolomic research has identified branched-chain AA (BCAA: valine, isoleucine, leucine), aromatic AA (AAA: phenylalanine and tyrosine), and short-chain AC (C3 and C5) as the most promising metabolites distinguishing individuals with insulin-

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resistance or T2D, independently of BMI [9,11,12]. In addition, these metabolites allow differentiating between normal weight and obese individuals [9] and predicting insulin-resistance improvement, regardless of the amount of weight lost [13]. Increased plasma concentrations of BCAA and AAA are also predictive of T2D incidence [14]. Studies among children observed that BCAA are elevated in obese subjects and that this increase is associated with insulin resistance in longitudinal analyses, independently of initial BMI [15–20].

Alongside being the constituents of proteins, AA can serve as energy fuel and give rise to several metabolites; circulating AA levels vary according to different physiological states [21] and at high concentrations are known to modulate insulin resistance [9]. ACs are generated through fatty acid oxidation and AA catabolism that are used as complementary sources of energy in cardiac and skeletal muscle mitochondria [22]. AC profiles can help identify mitochondrial dysfunction [23], which is increasingly being linked to insulin resistance [24].

In Canada, obesity and T2D prevalence are higher in non-Inuit Indigenous compared to non-Indigenous people [25,26]. The Nunavik Inuit Health Survey, *Qanuippitaa?* 2004, revealed high proportions of obesity (W = 31.8%, M = 25.8%) [27,28] higher than the rest of Canada (2004: W = 23.2%, M = 22.9%) [29]. The prevalence of diabetes among Nunavimmiut (4.7%) [28] is similar to that of the Canadian adult population (2004: 4.8%) [30], despite the younger age structure of the Nunavik population. Among circumpolar Inuit populations, although a low prevalence was reported in studies conducted before the 1980s compared with western populations, results from studies conducted over the last two decades indicate that the prevalence of diabetes is rising [31]. Until recently, no studies had assessed the metabolic profiles of Indigenous children. A recent study conducted in an American Indigenous community revealed that plasma concentrations of several AA are altered in adolescents with obesity and are associated with insulin sensitivity [32]. The present study aims at assessing if Inuit children with either adiposity or insulin resistance can be discriminated using plasma levels of AA and AC previously associated with obesity-related T2D. We access data from the Nunavik Child Development Study (NCDS) to determine the associations between AA and AC in relation with adiposity measures and biomarkers of T2D in school-aged Inuit children.

## Materials and methods

### Ethics statement

Written informed consent was obtained from the mother of each participating child, and oral consent was obtained from the child. Consent and assent forms were approved by the Nunavik Nutrition and Health Committee and the research ethics review board of Laval and Wayne State Universities.

### Study design and participants

The cross-sectional analysis of the NCDS study included 294 school-aged Inuit children from the 14 villages of Nunavik, a region north of the 55th parallel in Arctic Quebec (Canada), who were recruited between September 2005 and February 2010. The design and recruitment have been described in detail elsewhere [33]. Briefly, inclusion criteria were age between 8.5 and 14.5 years, birth weight  $\geq 2.5$  kg, gestation duration  $\geq 35$  weeks, no major birth defects, and no major neurological or chronic health problems affecting growth.

### Anthropometric parameters

Anthropometric parameters were measured by research nurses trained to use standard measurement procedures. Weight was quantified on a digital balance, and height was recorded using a stadiometer. Two measurements were taken for each parameter, and a third was obtained if a discrepancy was noted between the initial measurements for weight ( $>500$  g) and height ( $>0.5$  cm). Final growth parameter values were based on the average of the two closest measurements. BMI was calculated as the ratio of weight (kg) to squared height ( $m^2$ ). The body weight status (normal weight, overweight or obese) of each participant was determined using the IOTF BMI reference-standard [34]. The IOTF system (widely used internationally) is based on data from six large, nationally-representative, cross-sectional surveys on child growth – in Brazil, Great Britain, Hong Kong, the Netherlands, Singapore, and the USA. The cut-off values (for age and sex) are extrapolations of adult BMI cut-off points for overweight ( $25 \text{ kg}/m^2$ ) and obesity ( $30 \text{ kg}/m^2$ ).

Triceps skinfold thickness (TST, right arm) and subscapular skinfold thickness (SST) were measured in mm with a skinfold calliper by a research nurse. TST was measured on relaxed right arm, at mid-distance between the hood and the tip of the elbow, and SST from the back at about 1 inch below the lower border

of the shoulder blade. Two measurements were performed for each skinfold thickness parameter, and a third was obtained in case of a discrepancy >3 mm between the two initial measurements. The final values were the average of the two closest measurements.

### **Blood sampling**

A non-fasting venous blood sample (20 mL) was collected from each child. Following centrifugation, the plasma was transferred in plastic tubes and kept frozen at  $-80^{\circ}\text{C}$  until time of analysis. Samples were shipped on dry ice to the *Centre de Toxicologie du Québec* (CTQ) for AA and AC analysis and to the *Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec* for T2D biomarkers measurements.

### **Laboratory analyses**

Plasma concentrations of insulin and adiponectin were determined using Milliplex kits (Millipore, Billerica, MA) and a Luminex reader (Bio-Rad Lab, Hercules, CA). Glucose concentration was measured with the Amplex-Red Glucose assay kit accordingly to the manufacturer's instructions (Life Technologies).

Plasma concentrations of AA [arginine (Arg), glutamic acid (Glu), isoleucine (Ile), leucine (Leu), methionine (Met), phenylalanine (Phe), tyrosine (Tyr), valine (Val)] and AC [carnitine (C0), acetyl- (C2), propionyl- (C3), butyryl- (C4), isobutyryl- (IsoC4), glutaryl- (C5-DC), hexanoyl- (C6) and octanoylcarnitine (C8)] were determined by isotope-dilution liquid chromatography/hybrid quadrupole-time-of-flight (QToF) mass spectrometry using the method described by Roy et al. [35]. Inter-day coefficients of variation ranged from 5.2% to 10.9% for AA and 5.0% to 9.8% for AC. Accuracy ranged from  $-7.3\%$  to  $12.8\%$  for amino acids as assessed against SRM 1950 certified values (National Institute of Standards and Technology, Gaithersburg, MD, USA). We substituted plasma metabolite concentrations below the limit of detection (LOD) by a value equal to half the LOD.

### **Statistical analysis**

Characteristics of children according to IOTF weight status were compared using chi-square test for categorical variable (sex) and compared using analysis of variance (ANOVA) for continuous variables. Firstly, arithmetic means ( $\pm$ SEM) of metabolites concentrations according to IOTF weight status were compared using a one-way analysis of variance (ANCOVA) with Tukey's post-test for continuous variables and adjusted for age (continuous) and sex. Tests for trend across IOTF weight

status were assessed using the SAS software PROC GLM CONTRAST. Secondly, we performed adjusted linear regression analyses to assess the association of each metabolite (dependent variables) with different sets of independent variables: measurements of adiposity (BMI, triceps skinfold thickness and subscapular skinfold thickness) as well as biomarkers of obesity (adiponectin) and T2D (insulin, glucose). Age- and sex-adjusted standardised estimates (95% CI) of metabolite concentrations according to a 1-SD increase of each independent variable were plotted on a graph. In order to visually compare metabolite concentrations with independent variables, all plasma metabolite concentrations were standardised (mean = 0, SD = 1) and the standardised mean-difference with 95% confidence interval (CI) was calculated for each metabolite and plotted on a graph. All analyses were performed with SAS software version 9.1.3 (SAS Institute, Cary, NC). *P* values reported are 2-sided ( $P < 0.05$ ).

## **Results**

### **Samples for analysis**

Of the 294 available participants, 4 had missing data on height and were excluded. Metabolite concentrations were quantified in plasma samples of 248 participants, due to missing or insufficient volume sample. Therefore, the present cross-sectional analysis comprised 248 children aged 8 to 14 years.

### **Characteristics of the study participants**

Sample characteristics are summarised in Table 1. Of the 248 children, 75 (30.3%) were classified as either overweight (22.6%) or obese (7.7%) according to the IOTF criteria. Normal weight, overweight and obese children did not differ significantly in terms of sex, age, non-fasting plasma glucose and adiponectin concentrations. In contrast, height, weight, BMI, BMI z-score, triceps skinfold thickness, and subscapular skinfold thickness as well as non-fasting plasma insulin concentrations were significantly higher in children exhibiting obesity compared to those with overweight, who themselves had higher values than their normal weight peers (all  $p_{\text{trend}}$ -values  $< 0.001$ ).

### **AA and AC concentrations according to body weight status**

AA and AC concentrations according to IOTF BMI status are provided in Table 2. None of the AC measured were statistically significantly associated with IOTF BMI status.

**Table 1.** Baseline characteristics of Inuit children according to their IOTF weight status.

	All (n = 248)	IOTF weight status			P <sub>trend</sub>
		Normal weight (n = 173)	Overweight (n = 56)	Obese (n = 19)	
Age, yr	10.8 ± 0.8	10.8 ± 0.8	10.7 ± 0.9	10.8 ± 0.9	0.65
Girls, n (%)	123 (49.6)	86 (49.7)	25 (44.6)	12 (63.2)	0.25
<b>Anthropometric data</b>					
Height, cm	142 ± 7.6	140 ± 6.5 <sup>1</sup>	145 ± 7.8 <sup>2</sup>	151 ± 7.4 <sup>3</sup>	<0.001
Weight, kg	40.8 ± 10	36.1 ± 5.1 <sup>1</sup>	47.0 ± 6.9 <sup>2</sup>	64.6 ± 12 <sup>3</sup>	<0.001
BMI, kg/m <sup>2</sup>	20.0 ± 3.3	18.4 ± 1.4 <sup>1</sup>	22.3 ± 1.6 <sup>2</sup>	28.1 ± 4.0 <sup>3</sup>	<0.001
BMI z-score	0.68 ± 0.7	0.31 ± 0.47 <sup>1</sup>	1.37 ± 0.3 <sup>2</sup>	2.05 ± 0.3 <sup>3</sup>	<0.001
TST, mm	11.5 ± 5.8	8.98 ± 3.0 <sup>1</sup>	15.4 ± 4.7 <sup>2</sup>	23.4 ± 7.3 <sup>3</sup>	<0.001
SST, mm	8.74 ± 6.5	6.01 ± 2.6 <sup>1</sup>	12.1 ± 5.4 <sup>2</sup>	23.5 ± 9.1 <sup>3</sup>	<0.001
<b>Metabolic biomarkers</b>					
Non-fasting plasma insulin, pmol/L	136 ± 143	109 ± 106 <sup>1</sup>	159 ± 140 <sup>2</sup>	312 ± 268 <sup>3</sup>	<0.001
Non-fasting plasma glucose, mmol/L	6.31 ± 1.6	6.20 ± 1.7	6.50 ± 1.4	6.71 ± 1.5	0.21
Adiponectin, µg/ml	11.4 ± 5.7	11.7 ± 5.8	10.9 ± 5.9	9.87 ± 3.9	0.19

Values are presented as arithmetic mean ± SD unless indicated otherwise.

Characteristics of children according to IOTF weight status were compared using chi-square test for categorical variable (sex) and compared using analysis of variance (ANOVA) for continuous variables. Values with different superscript numbers are statistically different ( $P < 0.05$ ).

Abbreviations: BMI, Body Mass Index; SST, Subscapular skinfold thickness; TST, Triceps skinfold thickness.

**Table 2.** Adjusted<sup>a</sup> means (±SEM) of metabolite concentrations according to IOTF weight status\*.

	IOTF weight status			P <sub>trend</sub>
	Lean (n = 173)	Overweight (n = 56)	Obese (n = 19)	
<b>BCAA (mg/L)</b>				
Valine (Val)	21.1 ± 0.4 <sup>1</sup>	23.0 ± 0.7 <sup>2</sup>	24.3 ± 1.2 <sup>2</sup>	0.01
Isoleucine (Ile)	7.5 ± 0.2	7.7 ± 0.3	8.2 ± 0.5	0.22
Leucine (Leu)	13.0 ± 0.3	13.0 ± 0.6	13.8 ± 1.0	0.42
<b>AAA (mg/L)</b>				
Phenylalanine (Phe)	9.5 ± 0.1	9.6 ± 0.3	10.0 ± 0.4	0.31
Tyrosine (Tyr)	12.9 ± 0.2 <sup>1</sup>	13.7 ± 0.4 <sup>1,2</sup>	14.7 ± 0.8 <sup>2</sup>	0.03
<b>Other amino acids (mg/L)</b>				
Glutamic acid (Glu)	9.2 ± 0.7	8.9 ± 1.3	10.9 ± 2.2	0.47
Arginine (Arg)	16.3 ± 0.4	16.0 ± 0.6	15.8 ± 1.1	0.65
Methionine (Met)	4.6 ± 0.1	4.6 ± 0.2	4.8 ± 0.4	0.53
<b>Acylcarnitines (µg/L)</b>				
C0 (Carnitine)	10,531 ± 182	10,777 ± 321	10,558 ± 551	0.96
C2 (Acetyl)	1307 ± 51	1409 ± 90	1365 ± 155	0.72
C3 (Propionyl)	97.4 ± 2.9	89.7 ± 5.2	87.0 ± 8.9	0.27
C4 (Butyryl)	32.5 ± 1.1	34.2 ± 1.9	35.4 ± 3.2	0.39
IsoC4 (Isobutyryl)	25.0 ± 0.7	24.7 ± 1.2	21.4 ± 2.1	0.11
C5-DC (Glutaryl)	4.9 ± 0.3	5.3 ± 0.5	4.5 ± 0.8	0.70
C6 (Hexanoyl)	7.9 ± 0.2	7.7 ± 0.4	8.7 ± 0.6	0.24
C8 (Octanoyl)	13.1 ± 0.7	13.0 ± 1.2	13.4 ± 2.1	0.89

<sup>a</sup>Adjusted for age (continuous) and sex. Arithmetic means (±SEM) of metabolites concentrations according to IOTF weight status were compared using a one-way analysis of variance (ANCOVA) with Tukey's post-test for continuous variables. Values with different superscript numbers are statistically different ( $P < 0.05$ ).

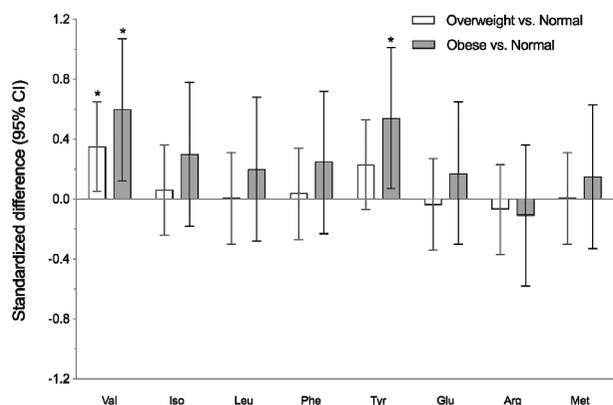
Abbreviations: BCAA, Branched-chain amino acids; AAA, Aromatic amino acids.

Among children with obesity and overweight, plasma valine ( $P = 0.01$ ) and tyrosine ( $P = 0.03$ ) concentrations were higher than those of their normal weight counterparts. Standardised mean (95% CIs) differences in plasma AA (Figure 1) and AC (supplemental Figure 1S) concentrations were also calculated and presented for obese or overweight children and their normal weight counterparts. Standardised mean differences were statistically significant for valine (overweight vs. normal weight: 0.35, 95% CI = 0.05 to 0.65; obese vs. normal weight: 0.60, 95% CI = 0.12 to 1.07) and tyrosine (obese vs. normal weight: 0.54, 95% CI = 0.07 to 1.01). No other

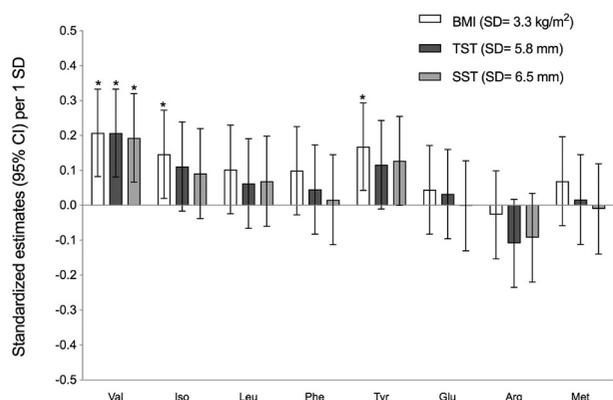
significant differences were observed between weight status categories.

### AA and AC concentrations in relation to adiposity and metabolic markers

Plasma AC levels did not vary according to adiposity or T2D biomarkers (see supplemental figures). Figure 2 depicts the age- and sex-adjusted standardised estimates of plasma AA concentrations (95% CI) according to 1-SD increment in markers of adiposity. An increment of 1 SD in BMI (SD = 3.3 kg/m<sup>2</sup>) was statistically



**Figure 1.** Standardised differences (95% CI) of amino acid concentrations between overweight and normal weight, obese and normal weight Inuit children. The weight status was determined according to the IOTF cut-offs for age and sex.

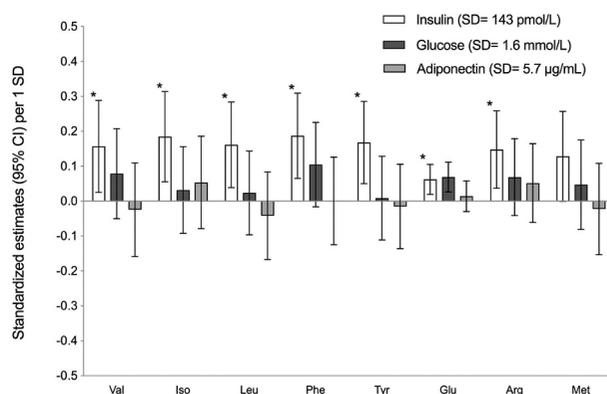


**Figure 2.** Adjusted standardised estimates<sup>a</sup> (95% CI) of amino acid concentrations per increment in adiposity measurements (BMI, subscapular skinfold thickness, Triceps skinfold thickness) among Inuit children.

<sup>a</sup> Estimates were obtained by multivariate linear regression adjusted for child age and sex. Abbreviations: BMI, body mass index; SST, subscapular skinfold thickness; TST, triceps skinfold thickness.

associated with an increment of 0.21 (95% CI: 0.08, 0.33) for Val, 0.15 (95% CI: 0.02, 0.27) for Ile and 0.17 (95% CI: 0.04, 0.29) for Tyr. Increments of 1 SD (SD = 5.8 mm) in triceps skinfold thickness and 1 SD (SD = 6.5 mm) in subscapular skinfold thickness were statistically associated with an increment for Val of 0.21 (95% CI: 0.08, 0.33) and 0.19 (95% CI: 0.07, 0.32), respectively.

Figure 3 depicts the age- and sex-adjusted standardised estimates of plasma AA concentrations (95% CI) according to 1-SD increment in biomarkers. All AA except Met were significantly associated with non-fasting insulin concentration. An increment of 1 SD for insulin concentration (SD = 143 pmol/L) was accompanied by an increase of AA varying between 0.06 and 0.19. In contrast, an augmentation of 1 SD for insulin was associated with a decrease of -0.14 (95% CI: -0.28,



**Figure 3.** Adjusted standardised estimates<sup>a</sup> (95% CI) of amino acid concentrations per increment in biomarkers (glucose, insulin, adiponectin) among Inuit children. Biomarkers were collected in non-fasting state.

<sup>a</sup> Estimates were obtained by multivariate linear regression adjusted for child age and sex.

-0.01) of octanoyl carnitine (C8). An increase of 1 SD (SD = 3.9 mmol/L) of plasma glucose was only significantly associated with an increase of 0.07 (95% CI: 0.03, 0.11) Glu, whereas adiponectin was not associated with any metabolite concentrations. All data related to crude metabolite concentrations are presented in Tables S1, S2 and S3 of Appendix (Supplementary materials).

## Discussion

We used of a metabolite profiling approach covering BCAA, AAA, and AC to gain insight on obesity-related T2D in Inuit children from Nunavik, an Indigenous population that have adapted to severe cold and a diet comprising predominantly marine foods. Our results suggest that a metabolite signature is associated with obesity and biomarkers of T2D in children from this population. Plasma concentrations of Val, Ile and Tyr increased with BMI, whereas an augmentation of non-fasting plasma insulin level was associated with higher concentrations of all AA except Met.

Some metabolic features noted in these school-aged children are similar to those observed in children experiencing obesity or overweight from other populations. In a cross-sectional study involving 69 US children aged 8 to 18 years, McCormack et al. [15] reported that obesity was significantly associated with an elevation in fasting concentrations of circulating BCAA (Val, Ile, Leu). In their 18-month longitudinal analyses of a subsample of children (n = 17), the authors noted that baseline BCAA concentrations were associated with a higher insulin resistance, independently of the initial BMI. Using an untargeted metabolomic approach and

a principal component analysis (PCA), Perng et al. [19] observed among 262 US children (aged 6–10) that a factor dominated by BCAA and short-chain AC was associated with adiposity and elevated cardiometabolic biomarkers (HOMA-IR, insulin, hs-CRP, IL-6, and leptin), but was not linked to adiponectin levels [19]. In a follow-up study in which the authors characterise metabolomic profiles of four overweight/obesity and metabolic risk phenotypes among 524 adolescents aged approximately 13 years, a principal component analysis revealed that a factor representing mainly BCAA (Val, C3 and C5 AC) was highest in participants belonging to the overweight/obese and high metabolic risk group [36]. A serum amino acid signature including BCAA and AAA was found to be associated with obesity and serum triglycerides among 6–12 years Mexican children [37]. In a longitudinal cohort followed by the same researchers, the amino acid signature was associated with serum triglycerides and with the risk of hypertriglyceridaemia after 2 years of follow-up. In the only study on this topic conducted among Indigenous children, Short et al. [32] examined if differences in plasma concentrations of several amino acids and their metabolites were present between 11–17 year-old boys and girls with obesity ( $n = 58$ ) and those of normal weight ( $n = 36$ ). Compared with the normal weight group, participants in the obese group had significantly higher plasma levels of 17 AA including BCAA (+110% to 16%) and AAA (+115% to 32%). Concentrations of all AA were positively correlated with body fat and negatively correlated with insulin sensitivity [32].

In contrast, Mihalik et al. [38] observed no evidence of alteration in AA and AC metabolism in obese ( $BMI \geq 95$ th for age and sex) or obese with T2DM adolescents compared to their normal-weight counterparts [17]. Similarly, Michaliszyn et al. [16] reported lower fasting plasma concentrations of several AA (Leu/Ile, Phe, Met and Arg) in obese adolescents with and without dysglycemia compared to normal-weight adolescents [16]. They also observed that an increment in the glycaemic status was associated with a reduction of circulating AA levels. Unfortunately, because school-aged of children did not fast prior to blood collection in the present study, we could not categorise them according to their glycaemic status.

The reasons behind such inconsistency in the literature are unclear. Both Mihalik et al. [17] and Michaliszyn et al. [16] suggested that obese adolescents might exhibit different metabolic adaptations than adults, for whom the disease progression is spread over a longer period of time. They also argue that plasticity and growth during childhood and adolescence might have

a major impact on metabolism (increased anabolism or decreased catabolism), which makes comparisons difficult with adult metabolomic profiles.

The differences observed between obese and normal weight school-aged of Inuit children concerning plasma concentrations of Val and Tyr might represent an early manifestation of the adverse metabolic consequences of nutritional transition [39]. This Westernisation of diet (regimen rich in refined sugars, saturated animal fats, and engineered foods, but low in fruits, vegetables and fibre) [40] has been proposed to explain the obesity/T2D epidemic in Indigenous populations [41,42] whose traditional diet was based on local food gathered from fishing, hunting, wild plant- and fruit-picking [43–48]. An effect of diet through an alteration of gut microbiota is also likely. Ridaura et al. [49] observed in obese mice that the gut exhibits a higher level of expression of microbial genes involved in the biosynthesis of essential AA (Phe, Lys, Val, Leu and Ile) and nonessential AA (Arg, Cys and Tyr), which contributes to the elevation of their circulating concentrations. Alternatively, the increase in circulating Val and Tyr levels in obese compared with normal weight children could be due to a decrease in the expression of the large neutral AA transporter (LAT1) in obese children [50].

Of mention is the lack of an association between plasma AC levels and biomarkers of adiposity and glucose homeostasis in the present study. This is in contrast to the above-mentioned study of Perng et al. which found that the highest levels of a BCAA-related factor (Val, C3 and C5) were present in adolescents belonging to the overweight/obese and high metabolic risk group [36]. Hence, although Val is increased in obese Inuit children, it is not accompanied by a similar increase in propionylcarnitine (C3), a product of BCAA catabolism, in particular valine and isoleucine catabolism [9]. Higher levels of C3 (and C5) have been noted in metabolically unhealthy individuals, independently of body mass index [11], suggesting that these AC may represent promising biomarkers for discriminating metabolic wellness among obese individuals. Hence, obesity among Inuit children may not exhibit the hallmarks of the unhealthy metabolic phenotype that may lead to obesity-related T2D in the long term. Using criteria developed for other populations, obesity has been on the rise in circumpolar Inuit populations over the past decades, especially among women [51,52]. While the impact of obesity on metabolic indicators has been lower than in other populations, better quality epidemiological data are required and community based-health interventions urgently needed to prevent future consequences of the emerging obesity epidemics [52].

Our study has limitations and therefore results should thus be interpreted with caution. Firstly, because of limitations inherent to cross-sectional investigations, no causal relationship can be ascertained. Secondly, our participants were Inuit children aged between 8.5 and 14.5 years, and therefore generalisation of our results to other populations and other age groups is limited. Thirdly, our participants provided a non-fasting blood sample for laboratory analysis. This non-fasting condition is an obstacle to the use of the homeostasis model assessment (HOMA) to assess insulin resistance (HOMA-IR) and beta-cell function (HOMA- $\beta$ ) [53] or associations of metabolites with other established cardiometabolic risk biomarkers. Furthermore, metabolite concentrations are influenced by dietary exposure [54] and are therefore more variable than they would have been under fasting conditions. It is therefore remarkable that significant associations were noted between amino acids and adiposity as well as metabolic biomarkers, despite the non-fasting condition of participants. Fourthly, we did not collect information pertaining to the stage of puberty. Increased secretion of growth hormone (GH) and changes in protein metabolism during puberty can interfere with the dynamics of BCAA at the muscle cell level. As discussed above, in contrast to adults, associations between plasma concentrations of some metabolites and obesity in youth are not well established and studies showed controversial results. A systemic review indicated that several potential factors could interfere with these associations, including age and sex in relation to the hormonal and metabolic changes associated with puberty and rapid growth during this phase of life [55].

Finally, we measured traditional markers of adiposity that may not be optimal in this population. Wells et al. observed that adiposity tends to be greater in populations inhabiting colder environments [56]. Therefore, inclusion of surrogate markers of fatness localisation such as waist circumference or visceral fat might provide more insight regarding the associations between obesity and T2D, and metabolite concentrations. Of interest, a recent report identified a BCAA-based metabolic score that can predict steatosis grade in a group of young patients aged 9 to 19 years ( $n = 68$ ) with severe obesity [57]. In the future, this score may provide a feasible alternative to expensive imaging tests or biopsy to identify obese children and adolescents with severe steatosis, a key metabolic event in the pathogenic sequence leading to T2D.

In conclusion, adiposity or insulin-resistance among children can be discriminated by using metabolites, especially those related to amino acid metabolism.

Data from the present study indicate that metabolites such as Val, Tyr and likely isoleucine might have the ability to metabolically discriminate excess adiposity among children. Although overweight/obesity increases the risk of T2D, levels of metabolites such as BCAA in individuals with excessive weight might be clinically useful to discern which children are at higher risk for T2D. However, do higher levels of these metabolites allow us to better predict and identify future risks of T2D for overweight/obese children? Unfortunately, cross-sectional studies cannot adequately and accurately answer this question. Longitudinal studies among children are warranted to investigate if increased plasma levels of these putative metabolites allow for early identification and better predict individuals at higher risk of developing T2D.

## Abbreviations

AAA: aromatic amino acid; BCAA: branched-chain amino acid; IOTF: International Obesity Task Force; T2D: type 2 diabetes

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## Authorship

G.M. participated in the data collection; T.C.M.M., P.A., M. L. designed the analysis; T.C.M.M., C.R., P.Y.T., P.A., M.L. contributed to the data analysis; T.C.M.M., P.A., M.L. conducted the analysis and interpretation, and wrote the first draft of the manuscript; T.C.M.M., C.R., P.Y.T., A.S.J., S.M., G.M., P.A., M. L. revised critically the manuscript for important intellectual content; P.A. and M.L. had primary responsibility for the manuscript final content. All authors read and approved the final version of the manuscript.

## Ethical standards disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Nunavik Nutrition and Health Committee and the research ethics review board of Laval and Wayne State Universities. Written informed consent was obtained from the mother of each participating child, and oral consent was obtained from the child.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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