Adiponectin: distribution, and associations with age, sex, adiposity, lifestyle factors, family history and insulin resistance in children and adolescents

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1. ABSTRACT

Adiponectin is inversely related to obesity, insulin resistance and progression to type 2 diabetes in adults. However its distribution, determinants and its association with insulin resistance (IR) are less well studied in the pediatric population. The objectives of this study were to describe, in youth, the age- and sex-specific distribution of adiponectin concentrations, and its association with demographic, anthropometric, and lifestyle factors, parental diabetes, and markers of IR. This study was a secondary analysis of a sample of 1632 French Canadian youth aged 9, 13 and 16 years who participated in the Quebec Child and Adolescent Health and Social Survey, a province-wide school-based survey conducted in 1999. Boys had lower adiponectin concentrations than girls by 17% ($p<0.0001$). Adiponectin concentrations decreased by age-group to a greater extent in boys than girls over the age range studied (27.7% vs. 13.3%, $p_{interaction}=0.009$). Mean adiponectin decreased by 8.1% in boys and 11.2% in girls ($p<0.0001$) for every unit increase in BMI Z-score. Growth-related change in BMI explained half the age effect in boys and all the age effect in girls. Self-reported pubertal status, physical activity, smoking and parental diabetes were not independently associated with adiponectin. Fasting insulin and HOMA-IR were not associated with adiponectin concentration after adjusting for BMI. However, an interaction term for adiponectin and BMI Z-score was significant in a multiple linear regression model with fasting insulin as the dependent variable. In conclusion, male sex and changes in body fat may be major determinants of decreasing adiponectin concentrations in growing youth. There was no association between adiponectin and markers of IR. The relationship between adiposity and markers of IR is attenuated in those with higher adiponectin concentrations, making adiponectin a potential intervention target or risk stratification marker for which the normative data presented herein may be useful.

RÉSUMÉ

L'adiponectine est inversement reliée à l'obésité, à l'insulinorésistance et à l'évolution vers le diabète de type 2 chez les adultes. Cependant, sa distribution, ses déterminants et son lien avec l'insulinorésistance (IR) sont moins bien étudiés dans la population pédiatrique. Les objectifs de cette étude étaient de déterminer, chez les jeunes, la distribution des concentrations d'adiponectine selon l'âge et le sexe et son association avec des facteurs démographiques, anthropométriques et reliés aux habitudes de vie, ainsi qu'avec les antécédents parentaux de diabète et les indicateurs d'IR. Cette étude était une analyse secondaire de données provenant d'un échantillon de 1632 jeunes Canadiens français âgés 9, 13 et 16 ans qui ont participé à L'Enquête Sociale et de Santé auprès des Enfants et des Adolescents Québécois, une enquête en milieu scolaire en 1999 à l'échelle de la province. Le taux d'adiponectine était 17% ($p<0.0001$) moins élevé chez les garçons que chez les filles. Le taux d'adiponectine diminuait avec l'âge dans une plus forte mesure chez les garçons que chez les filles dans la tranche d'âge étudiée (27,7% vs 13,3%, $p_{interaction}=0,009$). Le taux moyen d'adiponectine diminuait de 8,1% chez les garçons et de 11,2% chez les filles ($p<0.0001$) pour chaque augmentation d'une unité d'écart type d'IMC. Le fait que l'IMC varie avec la croissance expliquait la moitié de l'effet de l'âge chez les garçons et tout l'effet de l'âge chez les filles. Le stade de puberté, l'activité physique, l'usage du tabac et les antécédents parentaux de diabète autodéclaré n'étaient pas associés avec l'adiponectine. L'insuline à jeun et le modèle HOMA d'insulinorésistance n'étaient pas associés avec l'adiponectine après l'ajustement des données pour l'IMC. Toutefois, une interaction significative entre l'adiponectine et le score Z d'IMC était présente dans un modèle de régression linéaire multiple avec l'insuline à jeun comme variable dépendante. En conclusion, le sexe masculin et les changements au niveau de la graisse corporelle peuvent être des déterminants majeurs de la diminution d'adiponectine chez les jeunes en période de croissance. Cette diminution est accompagnée par l'absence d'association entre l'adiponectine et l'IR. L'association entre l'adiposité et les indicateurs d'IR est réduite chez ceux ayant un plus haut taux d'adiponectine, faisant de celle-ci une cible d'intervention potentielle ou un indicateur de stratification du risque pour lequel les données normatives présentées peuvent être utiles.

2. INTRODUCTION

Adipokines have become the subject of intense research due to the recognition that fat is a secretory tissue, and to the obesity pandemic, which is now well documented in both youth and adults. In the US, the National Health and Nutrition Examination Surveys (NHANES) show that the prevalence of obesity in adolescents increased from 6.1% to 16.5% over three decades (1;2). In a recent report based on the National Longitudinal Survey of Children and Youth, in Canada the prevalence of overweight and obesity among 2 to 17 year-old boys and girls was estimated at 27% and 25%, respectively. Corresponding figures for obesity were 9% and 7% (3). Many obesity-related health concerns are due to its association with the insulin resistance syndrome and its long term consequences which include type 2 diabetes and atherosclerosis. The link between obesity and metabolic abnormalities are still not fully understood, but the role of secretory products of fat tissue such as fatty acids, inflammatory cytokines and energy-regulating adipokines are being studied.

Adiponectin is an adipokine secreted exclusively by adipose tissue. Its concentration is inversely related to adiposity and the development of insulin resistance, type 2 diabetes and cardiovascular disease in adults (4). There are several biochemical and physiologic reasons to believe that adiponectin may be a mediator of obesity-related insulin resistance. First, it has structural homology to the inflammatory cytokine tumor necrosis factor-alpha (TNF-α) (5). Second, its expression is regulated by peroxisome proliferator activated receptor-gamma (PPARγ) (6) which is a known mediator of insulin sensitivity. Third, adiponectin receptors are highly expressed in liver and muscle (7), two insulin sensitive tissues.

Several longitudinal studies in adults suggest that low adiponectin is a risk factor for type 2 diabetes. For example, adiponectin concentrations are 19-22% lower in adults who subsequently develop diabetes than in those who do not (8-10). In addition, the negative association between adiponectin and insulin resistance appears to be independent of adiposity (11;12).

Although the distribution of adiponectin has been reported in adults (13;14), there is no information about its distribution in representative samples of youth. Limited data

suggest that adiponectin is 33% lower in obese children with diabetes than in obese children without diabetes (15). Twelve pediatric studies have shown a negative association between adiponectin and insulin resistance (15-26), but the association was independent of adiposity in only six of these reports (15-20). In youth, puberty leads to changes in body fat, contributes to insulin resistance (27), and may modify the association between adiponectin and insulin resistance.

The purpose of the analyses reported in this thesis are 1) to describe the distribution of adiponectin concentrations in a large, representative sample of children and adolescents, 2) to examine variation in adiponectin with age, pubertal status, adiposity, and lifestyle factors, and 3) to describe its association with insulin resistance, and to determine if adiponectin modifies the effects of other risk factors on insulin resistance.

3. LITERATURE REVIEW

3.1 Population distribution of adiponectin concentration

Mean adiponectin concentrations have been reported for select subgroups of the population, including obese or lean, and diabetic or nondiabetic adults. In general, values vary between 2.8 and 19.9 mg/L (9-13;15-17;19;21-23;25;26;28-33). There are only a few studies of samples that estimate mean adiponectin concentrations in representative, population-based samples (see Table 1). The Funagata study in Japan reported adiponectin levels of 9.72 ± 2.12 in men and 7.91 ± 2.43 mg/L in women (8). The ARIC study in the USA reported a median adiponectin level of 8.84 mg/L (interquartile range 6.24-11.7) (34).

The distribution of adiponectin levels were described in more detail in the CARDIA study which recruited 5115 black and white men and women aged 18-30 years from 4 clinical centers in the United States in 1985-6. Adiponectin levels were measured in 2975 participants with normal fasting glucose who participated in the 15 year follow-up assessment. Geometric means ranged from 5.9 mg/L for Black men to 13.6 mg/L for White women (14). The values at selected percentiles were also reported and are shown in Table 1.

Only one pediatric population-based sample reported age- and sex-specific means (±SD) of adiponectin among Taiwanese school children aged 6-10, 11-14 and 15-18 years (24). The values ranged from 5.9±3.2 to 7.3±3.0 mg/L. Although adiponectin distribution is known to be right-skewed, percentile values were not reported in this study. There are no other reports that describe the distribution of adiponectin concentrations in general populations of children or adults.

Defining a cut-off value for abnormal levels of a biological parameter may be easier in adults, because abnormality can be defined according to disease-related criteria and because the range of "normal" values is considered stable across a large age range. However, in youth, reference ranges for many biological variables including adiponectin concentrations, change with age. Therefore, age- and sex-specific distributions of adiponectin with values at selected percentiles may be useful for determining diagnostic

or prognostic cut-offs when and if adiponectin becomes an accepted clinical marker for disease risk.

3.2 Sex: Relationship with adiponectin

Adiponectin levels are higher in females than males. This has been described in most adult studies (see Table 2) in a wide variety of settings. One exception is a study of 144 obese Caucasian and Pima Indian participants, which found no sex-difference in adiponectin levels. Higher adiponectin levels in women than men have been reported in representative samples of adults and in diabetic patients, and in multiple ethnic groups including Japanese, Korean, German and US Whites and Blacks. Interestingly, this sex-difference among adults extends into the older age group that includes postmenopausal women. Physiologically, the hormonal and body composition differences between men and women diminish after the menopause. This suggests that there are nonhormonal reasons for differences in adiponectin levels between males and females.

In addition to a direct association between adiponectin level and sex, one cross-sectional study (29) and one nested case control study (10) have shown that sex is an effect modifier such that, compared to men, women with low adiponectin levels have a greater risk of having or developing type 2 diabetes.

Studies in youth draw different conclusions. Few studies in children and adolescents have formally examined sex differences in adiponectin levels. Studies that have assessed children less than 10 years old (23) or pooled children ranging in age from 3 to 18 years (21) have found no sex differences in adiponectin levels. However, one study that evaluated Black US youth aged 12.2 – 21.5 years found that girls had higher adiponectin levels than boys (22). Reinehr studied pre-pubertal and pubertal children aged 6 – 15 years and, consistent with other studies, did not find differences in adiponectin levels between pre-pubertal boys and girls, but pubertal girls had higher adiponectin levels than pubertal boys (26). Unlike studies of older adults, these findings suggest that sex hormones may have a role in determining adiponectin levels in youth. On balance, females have higher adiponectin levels than males starting at the time of puberty and the difference persists into young and older adulthood.

3.3 Age: Relationship with adiponectin

In adults, evidence of an association between adiponectin levels and age is conflicting. Unadjusted analyses from two cross-sectional studies suggest that adiponectin levels increase with age (8;14). However, three other studies (12;29;33), one of which adjusted for changes in BMI (33), found no age effect over a wide age range. (See Table 2). Failure to adjust for BMI in the majority of these studies may confound a true relationship. BMI increases with age in adults, except in the very elderly, and BMI is inversely associated with adiponectin.

In youth, some investigators have detected an association between age and adiponectin levels, but others have not (see Table 2). Degawa-Yamauchi (22) found no association over the age range 12.2 to 21.5 years in a pooled analysis of boys and girls in a convenience sample. Cruz (15) reported decreased adiponectin levels with increasing age in obese Mexican youth aged 10-16 years with or without diabetes. Two studies of German youth spanning the prepubertal and pubertal age range showed adiponectin was lower at older ages (21;26). One of these studies reported that adiponectin decreased with age in boys but not in girls (21), suggesting that the effects of growth and development on adiponectin are different for boys and girls.

Overall, during the pubertal years, limited data suggest that adiponectin levels decrease with increasing age, more so in boys than girls. In adulthood, adiponectin levels may increase with age.

An important limitation of studies evaluating the relationship between age or pubertal status and adiponectin levels is that almost all are cross-sectional. Although these data are valuable in describing populations, they are inadequate for following change over time within individuals. If the eventual aim is to use adiponectin concentration as a predictor of later-onset metabolic abnormalities, longitudinal studies that follow trajectories are necessary.

3.4 Body fat: Relationship with adiponectin

3.4.1 Measurement of adiposity

Many measures of body fat have been used in research. The most direct measures estimate fat area or volume, or percent body fat using imaging technology including computed tomography (CT) scans, magnetic resonance imaging (MRI) and dual energy X-ray absorptiometry (DEXA). Although these newer technologies are accurate and precise, they are expensive and not feasible in large epidemiological studies. Body mass index (BMI) calculated from height and weight information provides a valid and reliable alternative to direct measures (35), and is relatively simple to measure and inexpensive. Furthermore, it is the method recommended for clinical use by the Canadian Pediatric Society (36) and the American Academy of Pediatrics (37). An alternative is estimating percent body fat using measurement of skinfold thickness in specific areas such as over the triceps or below the scapula.

3.4.2 Relationship with adiponectin

The inverse association of adiposity and adiponectin levels is well established. This association was first reported in a convenience sample of 87 non-obese and 57 obese adults (33). There was a significant negative correlation between BMI and adiponectin levels in men ($r = -0.71$) and women ($r = -0.51$). These findings have been reproduced in adults and youth across a variety of ethnic groups, in various study sample types including convenience samples, clinic-based cohorts and population-based cohorts, as well as in groups along the spectrum from non-obese to obese and to type 2 diabetic individuals, using a variety of measures of adiposity (see table 2). A few studies failed to identify such an association, including a population-based longitudinal study of Japanese adults which found no association between adiponectin levels and BMI after adjusting for age and waist-to-hip ratio, a measure of central adiposity (8). Another study of obese and non-obese White and Pima Indian adults found no association with percent body fat by DEXA measurement, but did find a relationship with waist-to-thigh ratio (11). One study

of obese German youth found a relationship with percent body fat by skinfold thickness, but not BMI Z-score. The first two of these examples, suggest that the amount and distribution of body fat are important determinants of adiponectin levels. In these two studies abdominal fat appeared to be a better correlate of adiponectin than total fat. The third study evaluated only obese youth and the restricted range of BMI Z-scores may have limited their ability to demonstrate an association. The overwhelming evidence is for a robust inverse association between adiposity and adiponectin levels.

3.5 Insulin resistance: Relationship with adiponectin

3.5.1 Measurement of insulin resistance

Direct measures of the rate at which the body disposes of glucose in response to insulin include the hyperinsulinemic, euglycemic clamp (clamp) and the frequently sampled intravenous glucose tolerance test (FSIVGTT). These tests are considered reference standard methods. They are labour-intensive and expensive, and are ideally suited to small studies that investigate physiology. Other methods of estimating insulin resistance from single blood samples are widely accepted in large epidemiological studies. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and fasting insulin concentrations have been validated as measures of insulin resistance against clamp or FSIVGTT. If diabetic participants are excluded, correlation coefficients vary from -0.35 (38) to -0.54 among adults (39). In one study of prepubertal children, correlations of insulin sensitivity with HOMA-IR and fasting insulin were both r= -0.4 (40). Two independent validation studies of prepubertal and pubertal, lean and obese youth found fasting insulin and HOMA-IR correlated with directly-measured insulin sensitivity with r= -0.86 to –0.92 (41;42). Among these studies, correlations with directly-measured insulin sensitivity were generally stronger for fasting insulin than for HOMA-IR.

3.5.2 Independent relationship with adiponectin

Insulin resistance is a biological phenomenon of decreased cellular sensitivity to insulin action, especially found in muscle cells, hepatocytes and adipocytes. From an epidemiologic perspective, insulin resistance is important because of its association with the metabolic syndrome of obesity, dyslipidemia, inflammation and hypertension, and with morbid conditions such as diabetes and cardiovascular disease. The search for independent predictors of insulin resistance may lead to preventive strategies and treatments.

Obesity is strongly associated with insulin resistance. In addition, as mentioned above, obesity is inversely associated with adiponectin concentrations. Therefore, it is not unexpected that adiponectin concentrations are inversely associated with insulin resistance. The important question is "Is there an association between adiponectin concentrations and measures of insulin resistance independent of adiposity?"

In adults, many studies have demonstrated an independent association between adiponectin and measures of insulin resistance. One small study of overweight and obese US Whites and Pima Indians found adiponectin concentration was associated with insulin resistance measured by clamp independent of percent body fat measured by DEXA. Cross-sectional studies of population-based cohorts of Japanese teachers (12), Japanese clinic attendees (8) and healthy US adults (14) have found associations between adiponectin levels and HOMA-IR independent of BMI or waist circumference. In adults, there is consistent evidence for an independent association between adiponectin and insulin resistance.

In youth, several studies have examined the question (see table 2). All thirteen of the pediatric studies listed in table 2 show a univariate association between adiponectin levels and insulin resistance. Six of these studies found an independent association between adiponectin levels and insulin resistance, and six did not. Several methodological differences may explain the discrepant findings. First, the studies that showed an independent association between adiponectin and insulin resistance studied smaller sample sizes. Studies with small sample sizes may lack statistical power, but published studies with small sample sizes tend to overestimate effect size (43). Second,

there were differences in the weight status of the study samples – the studies that showed an independent association had an overrepresentation of obese individuals. There may be a stronger independent association between adiponectin and insulin resistance in obese individuals than in lean individuals. Third, the studies that showed an independent association tended to use more precise measures of both adiposity and insulin resistance than those that did not. Use of more precise measures improves the ability to find small effects. Overall, because of the heterogeneity of study methodology and heterogeneity of results, there is still uncertainty about the relationship between adiponectin concentration and insulin resistance among youth.

3.6 Adiponectin as a risk factor for type 2 diabetes

There are many adult studies showing a cross-sectional association between adiponectin concentration and type 2 diabetes. Stronger evidence for a causal role for adiponectin in the development of diabetes comes from four longitudinal studies of individuals who were not diabetic at baseline but developed diabetes over the course of the study. The first was a population-based cohort study of 3706 adults from a rural community in Japan of whom 1792 (48%) were followed up after 5 years. Those who were not diabetic and were in the lowest quartile of adiponectin concentration at baseline (<5.3 mg/L) had a significantly increased risk of developing diabetes compared to the highest quartile (>11.7 mg/L) (OR 3.5 [95%CI 1.1-10.9]) (8). Another report of a longitudinal cohort of Pima Indians used incidence density sampling in a nested case-control study. Seventy individuals who developed diabetes over a mean period of 4.6 years (mean adiponectin 4.3 mg/L) were compared to age, sex, BMI matched controls (mean adiponectin 5.3 mg/L) (9). The incidence rate ratio was 0.63 (95%CI 0.43-0.92) per mg/L increment of adiponectin concentration. Another nested case-control study in Germany identified cases who developed diabetes over a 2-3 year period (mean adiponectin 5.3 mg/L) and controls (mean adiponectin 6.9 mg/L) (10). Higher adiponectin was associated with a lower risk of diabetes (OR 0.90 [95%CI 0.84-0.97] per mg/L). A case-cohort study of 15792 US adults identified 581 incident cases of diabetes over 3 years and 572 controls (34). In an adjusted model, high adiponectin concentrations

were protective against diabetes (Hazard Ratio 0.50 [95%CI 0.30-0.83] per mg/L). These studies strongly support that adiponectin concentration is a predictor of type 2 diabetes in adults.

There are no longitudinal studies of adiponectin and diabetes in youth. However, one cross-sectional study in Mexican children with (n=40) and without (n=73) type 2 diabetes found that high adiponectin concentration was associated with a lower prevalence of diabetes (OR 0.86) after adjusting for age, sex and BMI (15) suggesting that adiponectin may be a risk factor for diabetes in youth.

3.7 Biological properties of adiponectin

3.7.1 Secretion and regulation

Adiponectin is a protein secreted solely by fat cells (44). Studies in animals and in humans show that the amount of adiponectin in circulation is inversely related to body fat content, regardless of the measure used (14;19;33). The distribution of body fat has an additional effect. Compared to subcutaneous fat, intraabdominal fat secretes more adiponectin and is more susceptible to decreased secretion with increased fat mass (28;45). The mechanism of this apparent paradoxical decrease in adiponectin secretion is not known, but the increase in other adipokines, particularly the inflammatory cytokine TNF-α is believed to downregulate adiponectin production (46).

Contrary to downregulation by TNF-α, adiponectin secretion is upregulated by PPARγ (6). PPARγ is a known insulin sensitizer, and drugs that act as PPARγ agonists are efficacious anti-diabetes medications. Individuals who take PPARγ agonists have higher adiponectin levels (6;47;48).

3.7.2 Structure

Adiponectin is a hormone. Structurally, it is composed of a collagen-like domain and a globular domain. The globular domain seems to be the biologically active part of the molecule, but there is no evidence that it circulates in isolation. Adiponectin

circulates in various molecular weight forms or multimers. Some studies have shown that the larger multimers are more biologically active in terms of insulin sensitivity. These multimers are structurally similar to TNF-α (5). The significance of this similarity is unknown but it fuels the hypothesis that the metabolic consequences of obesity constitute an inflammatory condition.

3.7.3 Function

We have some insight into the biological activity of adiponectin from studies of mice and rats that are genetically engineered to have no adiponectin. These rodents are not obese at baseline, but are unable to process dietary fat, with consequent fat infiltration in the liver and resistance to insulin. Giving these mice adiponectin reverses the abnormality. Mice given excess adiponectin lose weight and have increased energy expenditure (46). Experiments in humans show that those with lower adiponectin levels have inappropriate fat accumulation in muscle tissue and less activation of insulin receptor phosphorylation (19;49).

These controlled animal experiments indeed show that adiponectin has biological activities that would improve insulin sensitivity. However, the net effect of these and other interacting biological systems is what is clinically important at an individual or a population level. Therefore one must ask what determines adiponectin levels in humans, particularly during growth and development, and whether adiponectin has a clinically relevant role in the development of human obesity-related insulin resistance and diabetes.

3.8 Measurement of adiponectin

Variability in the measurement of adiponectin relates to biological variability, and analytic variability which is composed of random measurement error, and measurement bias.

3.8.1 Biological variability

The biological variability of adiponectin can be accounted for by at least two phenomena. There is evidence in several reports that circulating adiponectin has a diurnal rhythm with concentrations that rise in the late morning and decrease in the late evening (50;51). Other studies suggest that there is no diurnal rhythm, especially in obese individuals (29;51;52). Adiponectin also exhibits ultradian pulsatile secretion with multiple pulses during the day, so concentrations can fluctuate by up to 160% from trough to peak. Although the diurnal variation can be controlled by consistent timing of blood procurement (i.e., early morning), pulsatility remains a source of variability.

3.8.2 Measurement error

Measurement error is often a function of the assay used. There are currently two commercially available assays for adiponectin. The radioimmunoassay (RIA) (Linco Research Inc.) can measure concentrations as low as 1 mg/L and has a linear range from 0.78 mg/L to 200 mg/L. The intra-assay coefficient of variation (CV) of the RIA is reported as 4% and the inter-assay CV is 8%. The enzyme linked immunosorbent assay system (ELISA) (R&D Systems) has a lower limit of detection of 0.079 mg/L, but a linear assay range of 3.9 mg/L to 250 mg/L. The intra-assay CV is 3.5% and the inter-assay CV is 6.5%) (53). Although the precision of the ELISA may be superior, the RIA may be preferable since the former is not linear below 3.9 μg/L, which is within the range of values expected for human populations.

3.8.3 Measurement bias

Measurement bias or systematic error is impossible to measure in the case of adiponectin determination since there is no reference standard method for assaying adiponectin. However, pre-analytical variables may introduce bias. For example, samples can be degraded if they are not handled correctly. This would lead to systematic underestimation of adiponectin concentrations in all individuals and would lead to

nondifferential misclassification. Measures to avoid degradation include immediate centrifugation and placing samples on ice. Of note, repetitive cycles of freezing and thawing do not seem to degrade samples (54). Further, the nature of the molecule assayed may itself introduce bias. Adiponectin is known to circulate in homomultimers of two distinct molecular weight forms. The distribution of these forms is known to differ between females and males (55), and possibly with other variables. The higher molecular weight form is known to be more biologically active (55). Whether or not the currently available assays of total adiponectin concentration detect these two molecular weight forms differentially is not known. Since this potential systematic difference in detection could be correlated with other variables of interest such as sex, the resulting differential misclassification could have an impact on biological or clinical conclusions.

3.9 Conclusion

Adiponectin is an adipokine that is clearly inversely associated with obesity and insulin resistance in adults. The literature with respect to the association between adiponectin and insulin resistance in youth is contradictory. Of particular concern is the lack of evidence for this association from large population-based studies of youth. Furthermore, despite the abundance of evidence from Asia and Germany and the US, there are no data on Canadian youth.

In addition to the relationships between adiponectin, obesity, and insulin resistance, among youth there are other relationships that emerge. The association between adiponectin and sex and the relationship between adiponectin and age seem to develop through the pubertal years making it important to study girls and boys separately and further, to take into account pubertal status.

Adiponectin is emerging as a risk factor for health problems such as diabetes, hypertension and heart disease, yet there is very limited information on the distribution of this hormone in populations. There are no reported population norms for youth. Having normative data for Canadian youth would be helpful to document secular trends and to determine levels of risk once more is known about the role of adiponectin as a predictor of health outcomes.

Table 1. Distribution of adiponectin concentration in studies of population-based samples

Study	Population	Subgroups	Adiponectin concentration distribution (mg/L)												
Adult Studies			**Mean ± SD or Median (interquartile range)**												
Daimon (8)	Population-based Japanese	Diabetes	8.01 ± 2.55												
		IGT	8.60 ± 2.43												
		NGT	9.06 ± 2.41												
Duncan (34)	Population-based US, no DM		8.84 (6.24–11.70)												
			Males							**Females**					
			Mean ± SD or percentile							**Mean ± SD or percentile**					
			3	**5**	**25**	**50**	**75**	**95**	**97**	**3**	**5**	**25**	**50**	**75**	**95**	**97**
Schaffler (13)	Clinic-based German	Type 1 DM	2.0		5.6		13.1		24.5	2.5		7.4		19.4		39.3
		Type 2 DM	2.0		4.2		9.4		21.4	2.4		5.5		14.3		26.6
		Normal Controls	2.0		4.1		10.5		21.7	2.0		4.9		12.2		20.6
Steffes (14)	Clinic-based US, nondiabetic	Black		2.0	3.6	6.0	9.0	15.6			3.6	6.0	9.0	13.0	21.6	
		White		3.6	6.0	8.4	12.0	18.0			6.0	10.0	14.0	19.0	27.6	
Yamamoto (12)	Japanese teachers, no CVD		7.2 ± 4.6							13.5 ± 7.9						
Choi (30)	Population-based Korean >60 y/o		10.9 ± 1.9							14.4 ± 1.7						
Pediatric Studies			**Males** **Mean ± SD**							**Females** **Mean ± SD**						
Bottner (21)	Population-based nonobese German 8-18 y/o		6.72 ± 0.22							7.30 ± 0.20						
Tsou (24)	Population-based Korean	6-10 y/o	7.0 ± 2.8							6.9 ± 2.7						
		11-14 y/o	5.9 ± 3.2							6.8 ± 2.9						
		15-18 y/o	7.3 ± 3.0							7.1 ± 3.3						

Table 2. Summary of studies evaluating the association of adiponectin with sex, age, obesity and insulin resistance

Adult Studies										
Study	**Study Characteristics**					**Effects**				
	n	**Study Sample**	**Age Studied**	**Adiposity Measure**	**IR Measure**	**Sex**	**Age**	**Obesity**	**IR**	**IR (indep.)**
Choi (30)	1737	• Pop'n-based cohort • Korean	>60			Y				
Spranger (10)	563	• Pop'n-based nested CCS • German	35-65			Y Eff mod (adip'n vs DM stronger in F)				
Hotta (29)	265	• Diabetic & nondiabetic	Adult			Eff mod (adip'n vs DM stronger in F)	N			
Duncan (34)	1153	• Pop'n-based case-cohort • US	45-64	BMI		Y		Y		
Schaffler (13)	892	• DM1, DM2 & controls • German		BMI		Y		Y		
Arita (33)	144	• Obese & nonobese	30-60	BMI		Y	N (after adj for BMI)	Y		
Hulver (31)	85	• Obese & nonobese • Black & White US	Mean 45	BMI	FPI			Y(Black) N(White)	Y(Black) N(White)	
Steffes (14)	3355	• Healthy clinic cohort • US	33-45	WC	HOMA	Y	Y	Y	Y	Y
Daimon (8)	1792	• Pop'n-based cohort • Japanese	50-70	BMI	HOMA	Y	Y	N	Y	Y(NGT,IGT) N(DM)
Yamamoto (12)	967	• Pop'n-based cohort • Japanese teachers	30-65	BMI	HOMA	Y	N	Y	Y	Y
Weyer (11)	144	• Overweight & obese • White & Pima US	18-50	DEXA %BF, W:T	Clamp	N		N(DEXA) Y(W:T)	Y	Y

Table 2 Cont'd. Summary of studies evaluating the association of adiponectin with sex, age, obesity and insulin resistance

Pediatric Studies										
Study	**Study Characteristics**					**Effects**				
	n	**Study Sample**	**Age Studied**	**Adiposity Measure**	**IR Measure**	**Sex**	**Age**	**Obesity**	**IR**	**IR (indep.)**
Reinehr (26)	42	• Obese • German	6-15	BMI Z-score, Skinfold %BF	HOMA	Y(pubertal) N(pre-pubertal)	Y	N(BMI) Y(%BF)	Y	
Tsou (24)	500	• Population-based • Taiwanese	6-18	BMI, Relative BW				Y	Y	N
Bottner (21)	335	• Cohort normal weight & obese • German	3.4-17.9	BMI Z-score	HOMA	N Eff mod age-adip	Y (M) N (F)	Y	Y	N
Degawa (22)	86	• Convenience • Black & White US	12.2-21.5	BMI-Z score	HOMA	Y(Blacks) N(Whites)	N	Y	Y	N
Stefan (23)	83	• Cohort • Pima	5, 10	DEXA %BF, BMI	FPI	N		Y	Y	N
Vikram (25)	62	• Population-based • Indian boys	14-18	BMI, Skinfold %BF	HOMA			Y	Y	N
Weiss (20)	490	• Obese nondiabetic • US	4-20	BMI Z-score	HOMA			Y	Y	Y
Cruz (15)	113	• DM & nonDM obese • Mexican	10-16	BMI, WHR	FPI, HOMA		Y	Y	Y	Y
Asayama (16)	60	• Obese and nonobese • Japanese	6-14	CT Abdo fat, CT %BF	FPI	N		Y	Y	Y
Bush (18)	150	• Obese & nonobese • Black & White US	5-16	DEXA%BF, CT Abdo fat	IVGTT	N	N	Y	Y	Y
Bacha (17)	49	• Obese & Nonobese • US	12-14	DEXA %BF	Clamp	Y (obese) N (nonobese)		Y	Y	Y
Weiss (19)	22	• Obese & nonobese • US	13-14	MRI Abdo fat	Clamp			Y	Y	Y

IR - insulin resistance; IR (indep.) - association with insulin resistance independent of adiposity; Y - yes; N - no; DM1 - type 1 diabetes; DM2 - type 2 diabetes; DM - type 2 diabetes; CCS – case-control; BMI - body mass index; DEXA %BF - percent body fat determined by Dual energy X-ray absorptiometry; W:T - ratio of waist circumference to thigh circumference; Relative BW - body weight relative to ideal; Skinfold %BF - percent body fat calculated based on skinfold thickness; CT Abdo fat - visceral fat area measured by computed tomography scan; CT %BF - percent body fat calculated by CT abdomen; MRI Abdo fat - visceral fat area measured by magnetic resonance imaging; FPI – fasting plasma insulin; HOMA – homeostasis model assessment of insulin resistance; Clamp – insulin resistance measured by hyperinsulinemic euglycemic clamp; IVGTT – insulin resistance by intravenous glucose tolerance test.

4. OBJECTIVES

The objectives of this study were:

1) to describe the distribution of adiponectin concentrations in a large, representative sample of children and adolescents,
2) to examine the variation of adiponectin with age, pubertal status, adiposity, and lifestyle factors, and
3) to describe its association with insulin resistance, and to determine if adiponectin modifies the effects of other risk factors on insulin resistance.

5. MANUSCRIPT

Adiponectin, adiposity and insulin resistance in children and adolescents

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5.1 Abstract

Context: Determinants of adiponectin and its association with insulin resistance (IR) are less well studied in youth than in adults.

Objectives: To describe, in youth, the age- and sex-specific distribution of adiponectin concentrations, and the association with demographic, anthropometric, and lifestyle factors, parental diabetes, and markers of IR.

Design, Setting, Participants: We studied 1632 French Canadian youth aged 9, 13 and 16 years who participated in the Quebec Child and Adolescent Health and Social Survey, a province-wide school-based survey conducted in 1999.

Results: Boys had lower adiponectin concentrations than girls by 17% ($p<0.0001$). At age 16, mean adiponectin concentrations were 27.7% (boys, $p<0.0001$) and 13.3% (girls, $p<0.0001$) lower than at age 9 ($p_{interaction}=0.009$). Mean adiponectin decreased for every unit increase in BMI Z-score by 8.1% in boys and 11.2% in girls ($p<0.0001$). Growth-related change in BMI explained half the age effect in boys and all the age effect in girls. Self-reported pubertal status, physical activity, smoking and parental diabetes were not independently associated with adiponectin. Fasting insulin and HOMA-IR were not associated with adiponectin concentration. However, the interaction of adiponectin and BMI Z-score was significant in a multiple regression model of fasting insulin.

Conclusions: Male sex and changes in body fat may be major determinants of the decreasing adiponectin concentrations of growing youth, which are accompanied by a dissociation of adiponectin and markers of IR. The relationship between adiposity and markers of IR is attenuated in those with higher adiponectin concentrations, making adiponectin a potential intervention target or risk marker.

5.2 Introduction

Adiponectin is an adipokine that is secreted solely by adipose tissue and its concentration is inversely related to the degree of adiposity. It circulates in high concentrations as a multimer. Adiponectin has structural homology to TNF-α (5), its expression is regulated by PPARγ (6) and its receptors are highly expressed in liver and muscle (7). These features make it a candidate mediator in the development of obesity-related insulin resistance (IR). Longitudinal studies in adults suggest that low adiponectin is a risk factor for type 2 diabetes - adiponectin concentrations are 22% lower in adults who subsequently develop diabetes than in those who do not (10). In addition, the negative association between adiponectin and IR appears to be independent of adiposity (11;12).

Although the distribution of adiponectin concentrations has been reported in adults (14), there is no information about its distribution in representative samples of youth. Limited data suggest that adiponectin is 33% lower in obese children with diabetes than those without diabetes (15). Nine pediatric studies have shown a negative association between adiponectin and various measures of IR (15-19;22-25)(8-16), but the association was independent of adiposity in only five of these reports (15-19). In youth, puberty leads to changes in body fat, contributes to IR (27), and may modify the association between adiponectin and IR.

The objectives of this report are 1) to describe the age- and sex-specific distribution of adiponectin concentrations in a large, representative sample of children and adolescents, 2) to examine the relationship of adiponectin with age, pubertal status, adiposity, and lifestyle factors, and 3) to describe the association between adiponectin and surrogate measures of IR.

5.3 Methods

Subjects

The study population included a sample of French Canadian children and adolescents who participated in the Quebec Child and Adolescent Health and Social

Survey (QCAHS), a school-based survey conducted between January and May 1999 in the province of Quebec, Canada. The survey design and methods, reported previously (56), are only summarized here. The QCAHS used a cluster sampling design to draw three provincially representative, independent samples of youth aged 9, 13 or 16 years (one sample per age).

Among eligible youth, questionnaires and anthropometry were completed by 82% (1243/1520), 78% (1161/1498) and 76% (1133/1495) of 9-, 13- and 16-year-olds, respectively. The analyses described here were restricted to French Canadian participants because some of the data was collected only in this subgroup as part of a QCAHS substudy of genetic variables. French Canadians comprised 80% (999/1243), 78% (908/1161) and 82% (923/1133) of all 9-, 13-, and 16-year-old QCAHS participants respectively. A fasting blood sample was provided by 63% (628/999), 69% (629/908) and 75% (695/923) of 9-, 13- and 16-year-old French Canadians, respectively. Twelve percent (235/1952) of participants were excluded because parents refused analyses other than glucose and lipids, or because blood samples were thawed or of insufficient quantity. Three individuals were excluded because they had diabetes. Those missing pubertal status (n=59), physical activity scores (n=6), and 13- and 16-year olds missing data on tobacco use (n=21) were excluded. Those who provided blood samples did not differ significantly from those who did not, by sex, BMI Z-score, parental income or education, or parental history of diabetes. The study was approved by the Ethics Review Board of Ste-Justine Hospital [see Appendix 2]. Informed assent and consent were obtained from participants and their legal guardians.

Clinical variables

Height was measured to the nearest 0.1 cm at maximal inspiration using a standard measuring tape and a triangular level. Weight was measured in light indoor clothing with shoes removed. Skinfold thickness was measured to the nearest 0.1 cm with a Lange caliper (Beta technology, USA). BMI was calculated as weight divided by the square of height (kg/m^2). Participants and their parents both completed questionnaires [see Appendix 3]. Pubertal status was assessed using a modification of a validated self-report scale (57), by indicating for each of the following body changes if they had not

started, barely started, definitely started, or stopped changing: pubic hair, facial hair and voice change for boys, or pubic hair and breast development for girls. Menstruation was categorized as not started or started. Responses were used to categorize participants as prepubertal (all responses 'not started'), postpubertal (all responses 'stopped changing' (menstruation 'started')), or intrapubertal (any other combination of responses). The physical activity score was based on a one-week recall modified from Sallis et al. (58). Participants were asked to indicate which of 18 activities they performed for at least 15 minutes on each of the previous 7 days. Their responses were summed to create the physical activity score (maximum possible score = 126). Smoking was determined by asking 13- and 16- year olds the number of cigarettes usually smoked per day in the last 30 days. Nine-year olds were asked if they had ever smoked a whole cigarette. For multivariable analyses, all 9-year-olds were categorized as smoking 0 cigarettes per day, since only 2.1% reported ever having smoked a whole cigarette. Parental history of diabetes was obtained from the parent questionnaire.

Biochemical variables

After an overnight fast, venous blood was collected between 0800h and 1000h in a 1 g/L EDTA collection tube, and placed on ice. Samples were centrifuged on site within 45 minutes of collection, transported on dry ice and stored at –80°C. Adequacy of the fasting period was checked by nurses before blood was collected. Plasma glucose was measured with the Beckman Coulter CX®7 analyzer using the glucose oxidase method. Interassay coefficients of variation (CVs) were 3.8%, 1.3% and 1.4% at 2.17, 6.38 and 13.69 mmol/L, respectively (n=24) (Triad® Link levels 1, 2 and 3; Beckman Coulter, Inc). Plasma insulin was measured with the Ultrasensitive Insulin assay on the Access® immunoassay system (Beckman Coulter). Interassay CVs were 4.1% and 5.0% at 92 (n=24) and 285 pmol/L (n=23), respectively (Lyphochek® Immunoassay Plus Control, levels 1 and 2; Bio-Rad). Plasma adiponectin was measured by radioimmunoassay (LINCO Research, Inc, St. Charles, MO). Interassay CVs were 10.5% and 12.2% at 3.4 and 37.7 mg/L, respectively (n=24). Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated as insulin (mU/L) x glucose (mmol/L) / 22.5 (59).

Statistical analysis

Sample quantiles of adiponectin were used to estimate the population percentiles. Nonparametric 95% CIs for the percentiles were constructed by the method of Hutson (60). Percentiles were considered significantly different if their 95% CIs did not overlap.

Variables with skewed distributions (BMI, subscapular and triceps skinfolds, adiponectin, insulin, HOMA-IR) were $\log_e$-transformed. Age- and sex-specific Z-scores were estimated for each participant for BMI, subscapular and triceps skinfolds, and adiponectin from the weighted distributions of the study sample. Z-scores were computed as (value – mean) / SD. Hierarchical maximum likelihood linear regression was used to estimate regression coefficients for univariate and multivariate associations [see Appendix 4]. Explanatory variables were treated as fixed effects, and clustering between subjects in the same school was treated as a random effect. Regression coefficients for models of $100\text{Log}_e\text{Adiponectin}$ or $100\text{Log}_e\text{Insulin}$ represent the percentage difference in adiponectin or insulin concentration, respectively for one unit increment in the explanatory variable. Statistical significance was assessed using F-tests. Least squares linear regression was used to estimate the proportion of variance (R^2) explained by the models. Because of the complex survey design, sampling weights and clustering effects were estimated and incorporated into all computations except correlations and regression models. Statistical analyses were performed with SAS software (SAS Institute, Inc. Cary NC) and SUDAAN (Research Triangle Institute).

5.4 Results

Participant characteristics are shown in Table 3. Parental history of diabetes was reported for 7% (46/639) of boys and 5% (31/668) of girls (among those whose parents responded to the questions about diabetes).

Table 3. Characteristics of participants

Characteristic		Boys n=804	Girls n=828
Age (y): n (%)	9	248 (31)	260 (32)
	13	269 (33)	235 (28)
	16	287 (36)	333 (40)
Pubertal status: n (%)	Pre-	109 (14)	118 (14)
	Intra-	685 (85)	581 (70)
	Post-	10 (1)	129 (16)
BMI (kg/m^2): Mean ± SD		20.2 ± 4.2	20.3 ± 4.5
BMI category*: n (%)	Overweight	106 (13)	113 (14)
	Obese	80 (10)	66 (8)
Fasting insulin (pmol/L): Mean ± SD		41.1 ± 34.2	45.8 ± 25.4
Fasting glucose (mmol/L): Mean ± SD		5.3 ± 0.4	5.1 ± 0.4
Physical activity score: Mean ± SD		9.6 ± 7.7	7.7 ± 6.4
Smoking (cigarettes/day)†: n (%)	0	421 (76)	375 (66)
	1-5	81 (14)	123 (22)
	>5	54 (10)	70 (12)

*defined according to the 2000 CDC growth charts (61), overweight: ≥85th and <95th percentile, obese: ≥95th percentile;
†13- and 16-year olds only; boys n=556, girls n=568.
Physical activity score was the sum of the number of activities from a list of 18 that were performed for at least 15 minutes on each of the previous 7 days (maximum score 126).

Adiponectin distribution

Age- and sex-specific distributions of adiponectin concentrations are shown in Table 4. Distributions of adiponectin concentrations were skewed positively. Girls had higher adiponectin concentrations than boys, except at the 5th and 10th percentiles for 9 year-olds. Overall, adiponectin concentrations decreased with age. However differences were smaller between 13 and 16 year-olds than between 9 and 13 year-olds. Age-related differences were less marked in girls than in boys, and were not present at the lower percentiles.

Table 4. Distribution of adiponectin concentrations (mg/L) by sex and age

	Boys			Girls		
	9 years (n=248)	13 years (n=269)	16 years (n=287)	9 years (n=260)	13 years (n=235)	16 years (n=333)
%ile (95%CI)						
5th	5.8 (5.2-6.4)	4.4 (4.1-4.7)	4.0 (3.6-4.6)	5.8 (5.3-6.6)	5.4 (5.1-6.1)	5.2 (4.6-6.2)
10th	6.6 (6.1-6.8)	5.0 (4.6-5.6)	4.8 (4.4-5.1)	6.9 (6.3-7.5)	6.6 (6.0-7.0)	6.4 (5.8-7.1)
25th	8.4 (7.8-9.0)	6.6 (6.2-6.9)	6.1 (5.8-6.7)	9.3 (9.0-9.9)	8.1 (7.6-8.5)	8.0 (7.5-8.3)
50th	10.6 (10.1-11.4)	8.6 (8.1-9.1)	7.9 (7.4-8.3)	11.9 (11.2-12.6)	10.4 (9.8-11.1)	9.9 (9.3-10.3)
75th	13.8 (13.0-14.5)	11.0 (10.2-11.5)	10.0 (9.5-10.5)	15.2 (13.9-15.9)	13.4 (13.0-14.0)	12.3 (11.6-13.0)
90th	16.6 (15.5-17.9)	14.2 (12.7-14.8)	12.4 (11.6-13.0)	18.5 (17.5-20.6)	16.3 (15.1-18.0)	14.8 (14.1-15.9)
95th	18.4 (17.0-19.0)	15.3 (14.4-15.9)	13.5 (12.8-15.4)	21.3 (19.6-23.6)	18.4 (16.8-18.6)	16.7 (15.5-17.9)
Mean (95%CI)	11.2 (10.7-11.7)	9.1 (8.6-9.5)	8.2 (7.8-8.5)	12.5 (11.9-13.0)	10.9 (10.4-11.4)	10.3 (9.9-10.6)

Associations with adiponectin

In univariate linear regression, sex explained 5% of the variation in adiponectin concentrations. Adiponectin concentrations in boys were 17% (95%CI 14-21%) lower than in girls. Due to a significant age sex interaction (described below), all subsequent analyses were conducted separately for boys and girls. Table 5 shows the sex-specific univariate effect sizes of age, pubertal status, anthropometric, and lifestyle factors on adiponectin concentration. Increased age and advanced pubertal status showed substantial

negative associations with adiponectin concentration. All measures of adiposity were negatively associated with adiponectin. Neither physical activity nor smoking was significantly associated with adiponectin concentrations. Parental history of diabetes was not associated with adiponectin concentrations among participants whose parents responded (p=0.7 for boys and girls) (data not shown) [see Appendix 5].

Table 5. Univariate associations between selected variables and adiponectin concentration

	Boys n=804			Girls n=828		
Explanatory variable	**β, %* (SE)**		**p**	**β, %* (SE)**		**P**
Age: 9 y	Ref.			Ref.		
13 y	-22.1	(3.2)	<0.0001	-12.7	(3.5)	0.0004
16 y	-31.3	(3.2)	<0.0001	-18.1	(3.4)	<0.0001
Pubertal status: pre-	Ref.			Ref.		
intra-	-20.1	(4.0)	<0.0001	-13.9	(3.8)	0.0003
post-	-33.5	(12.5)	0.008	-23.5	(4.8)	<0.0001
BMI, kg/m^2	-3.1	(0.3)	<0.0001	-3.0	(0.3)	<0.0001
BMI Z-score, 1 SD	-8.8	(1.3)	<0.0001	-11.2	(1.2)	<0.0001
Subscapular skinfold Z-score, 1SD	-6.5	(1.3)	<0.0001	-10.9	(1.2)	<0.0001
Triceps skinfold Z-score, 1SD	-2.7	(1.4)	0.05	-8.0	(1.3)	<0.0001
Physical activity score	-0.2	(0.2)	0.2	0.1	(0.2)	0.5
Smoking†: 0 cigarettes/d	Ref.			Ref.		
1-5 cigarettes/d	-5.7	(4.6)	0.2	-3.6	(3.7)	0.3
>5 cigarettes/d	-3.5	(5.5)	0.5	-6.0	(4.6)	0.2

*β refers to the percent difference of mean adiponectin concentration for one unit increment of the explanatory variable;
† smoking data for 13 and 16 year-olds only (boys n=556, girls n=568).

BMI Z-score, and scapular and triceps skinfold thickness Z-scores were highly correlated (Pearson r ranged from 0.74 to 0.82, p<0.0001) and collinear, so we included

only the BMI Z-score in multivariable analyses, since it is the most clinically applicable measure [see Appendix 6]. Parental history of diabetes was not included in multivariable analyses because of the low response rate.

Only age and BMI-Z score were significantly associated with adiponectin in a multivariable model that included age, pubertal status, BMI Z-score, physical activity, and smoking (Table 6 Model A). The effect of pubertal status was eliminated after adjusting for age ($p = 0.7$ and 0.6 for boys and girls, respectively)(data not shown) [see Appendix 7]. Boys aged 13 and 16 had adiponectin concentrations 19.5% and 27.7% lower than 9-year-olds, respectively; a 1 SD increase in BMI Z-score was associated with an 8.1% decrease in mean adiponectin concentration. Girls aged 13 and 16 had adiponectin concentrations 8.8% and 13.3% lower than 9-year-olds, respectively; a 1 SD increase in BMI Z-score was associated with an 11.2% decrease in mean adiponectin concentration. However, these variables explained only 16 and 15% of the variation in adiponectin concentrations in boys and girls, respectively.

Adjusting for BMI instead of BMI Z-score markedly reduced the effect size of age in boys and completely eliminated it in girls (Table 6 Model B). BMI is a surrogate measure of total body fat. BMI Z-score is a standardized measure that indicates the relative degree of fatness of an individual compared to sex- and age-matched peers. Conceptually, the difference between BMI and BMI Z-score is the fat mass that is associated with growth and development. Comparison of the age parameters in models A and B suggests that almost half the age effect in boys and all of the age effect in girls is accounted for by growth-related change in BMI. This finding emphasizes the importance of body fat as a determinant of adiponectin concentration.

Table 6. Multivariate models of adiponectin concentration

		Boys n=804			Girls n=828		
	Explanatory variable	**β, %***	**(SE)**	**p**	**β, %***	**(SE)**	**p**
Model A	Age†: 9 y	Ref.			Ref.		
	13 y	-19.5	(3.7)	<0.0001	-8.8	(3.9)	0.02
	16 y	-27.7	(3.9)	<0.0001	-13.3	(4.2)	0.001
	BMI Z-score‡, 1 SD	-8.1	(1.3)	<0.0001	-11.2	(1.2)	<0.0001
			$R^2 = 0.16$			$R^2 = 0.15$	
Model B	Age†: 9 y	Ref.			Ref.		
	13 y	-12.9	(3.9)	0.0008	0.3	(4.0)	0.9
	16 y	-16.7	(4.3)	<0.0001	-0.1	(4.3)	0.9
	BMI, kg/m^2	-2.3	(0.4)	<0.0001	-2.8	(0.3)	<0.0001
			$R^2 = 0.16$			$R^2 = 0.15$	

All models are adjusted for pubertal status, physical activity score and smoking.
* β refers to the percent difference of mean adiponectin concentration for one unit increment of the explanatory variable;
† interaction between sex and age p=0.009;
‡ interaction between sex and BMI Z-score p=0.10.

There was a significant age-sex interaction whereby adiponectin concentration decreased to a greater extent with increased age in boys than in girls (p=0.009). There was also a significant interaction between BMI Z-score and age in boys, with 13- and 16-year old boys showing a greater decrease in adiponectin with increased fatness than 9-year old boys (p=0.007). This was not observed in girls (p=0.5). Sex did not modify the relationship between BMI Z-score and adiponectin (p=0.1).

Adiponectin and markers of insulin resistance

Fasting insulin was used as a surrogate measure of IR. Table 7 shows the effect of

adiponectin on insulin concentration. Insulin concentration was 6.8% (95%CI 3.3-10.3) lower in boys and 9.3% (95%CI 5.8-12.8) lower in girls for each 1 SD increase in adiponectin, in univariate analysis. Adiponectin explained only 1.6-2.9% of the variation in insulin concentrations. After adjusting for BMI Z-score, adiponectin had no independent effect on insulin concentration. However higher concentrations of adiponectin did attenuate the detrimental effect of BMI Z-score on insulin concentrations (for interaction term adiponectin Z-score x BMI Z-score, p = 0.02 and p = 0.0007 for boys and girls, respectively) (Table 7 Model 4 and Figure).

Table 7. Models showing the relationship between adiponectin and fasting insulin concentrations

		Boys n=804				Girls n=828			
	Explanatory variable	β,% * (SE)		p	Model R^2	β,% * (SE)		P	Model R^2
1†	adiponectin, 1SD	-6.8	(1.8)	0.0002	0.016	-9.3	(1.8)	<0.0001	0.029
2‡	adiponectin, 1SD	-7.1	(1.8)	<0.0001	0.18	-9.4	(1.6)	<0.0001	0.26
3‡	adiponectin, 1SD	-1.7	(1.6)	0.3	0.37	-2.6	(1.5)	0.1	0.41
	BMI, 1SD	25.4	(1.6)	<0.0001		21.8	(1.6)	<0.0001	
4‡	adiponectin, 1SD	-1.8	(1.6)	0.3	0.38	-2.5	(1.5)	0.1	0.41
	BMI, 1SD	24.7	(1.6)	<0.0001		20.2	(1.6)	<0.0001	
	adiponectin x BMI	-3.5	(1.5)	0.02		-4.9	(1.4)	0.0007	

†, Model 1 – unadjusted.
‡, Models 2, 3 and 4 – adjusted for age, pubertal status, physical activity score and smoking.
* β refers to the percent difference of mean insulin concentration for an increase of one (1) age- and sex-specific SD of adiponectin, or BMI. For the interaction term in model 4, β may be interpreted as the incremental change in $\beta_{\text{BMI Z-score}}$ for every 1 SD increase in adiponectin or vice versa.

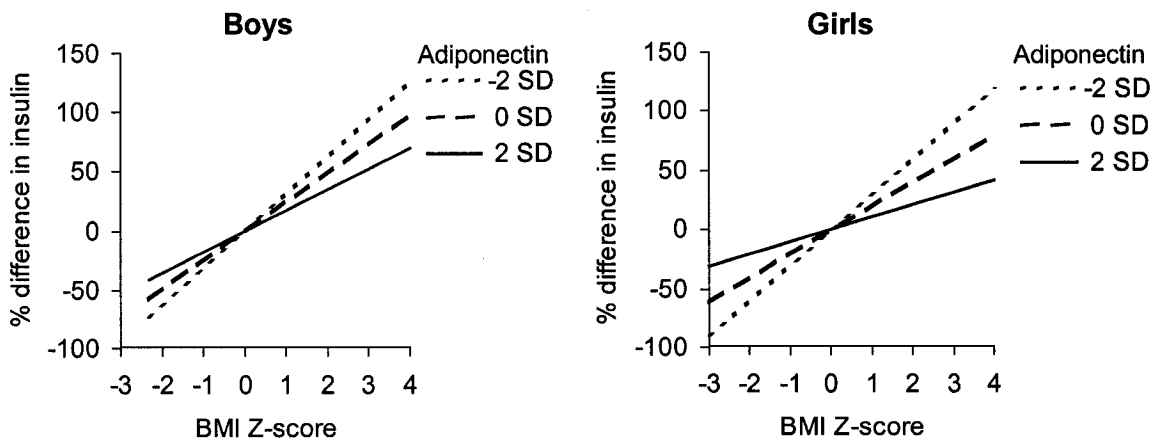

Figure. Association between BMI Z-score and insulin concentration at selected adiponectin Z-scores after adjustment for age, pubertal status, physical activity score and smoking.

The same analyses repeated with HOMA-IR as the surrogate measure of insulin resistance did not change any of the conclusions. Parameter estimates for the effect of adiponectin on HOMA-IR were within 0.5 absolute percentage points of those for its effect on fasting insulin concentration (data not shown).

5.5 Discussion

To our knowledge, this is the first report of the distribution of adiponectin concentrations in a representative sample of Caucasian peripubertal youth. It is also the largest study to date addressing the association of adiponectin with age, sex, adiposity, lifestyle factors and markers of IR in youth. Variations in adiponectin by sex, age and adiposity were of the same magnitude as those that predict diabetes in adults (10) suggesting that the differences in adiponectin apparent in this group of healthy children may be relevant to future disease risk.

Sex differences in adiponectin concentrations

Both the age- and sex-specific distributions and regression analyses showed a clear sex difference in adiponectin concentrations. Girls had higher mean adiponectin concentrations than boys at all ages. With our large population-based sample, we were able to show this difference even in the youngest age group (most of whom were prepubertal), in whom previous studies had failed to demonstrate sex differences (16;21;23;24;26). In addition, the progressive decline in adiponectin concentrations with age was more than twice as great in boys as in girls. Bottner *et al.* (21) showed similar patterns in German boys, but in girls, they found no change in adiponectin concentrations through puberty, possibly because of a small sample size or the exclusion of overweight children. Furthermore, in our study, boys and girls had different age-specific relationships between adiposity and adiponectin concentrations. Older boys had a steeper decrease in adiponectin with increasing BMI Z-score than younger boys. In girls, age did not modify the association between BMI Z-score and adiponectin concentrations.

Prepubertal sexual dimorphism has also been reported for body composition, leptin, IGF-1 concentrations (62) and HOMA-IR (63), suggesting that unknown mechanisms other than sex hormones contribute to sex-related differences in metabolism and energy homeostasis even before puberty begins. However, testosterone does seem to influence adiponectin concentrations at older ages. Two studies of pubertal children reported that adiponectin concentrations were negatively correlated with androgen concentrations (particularly testosterone in boys) (21;24). These studies support the observed interaction between sex and age in our study. Additional evidence for testosterone suppressing adiponectin concentrations comes from studies in vitro and in eugonadal and castrated mice treated with testosterone (64), and in hypogonadal men treated with testosterone (65). Furthermore, our results suggest that during puberty, boys may develop an increased susceptibility to obesity-induced hypoadiponectinemia. This may be due to direct hormonal effects or sex-specific changes in fat distribution that develop during puberty (66). The tendency of men to accumulate visceral fat (67), and the relatively greater contribution of visceral fat than subcutaneous fat in determining adiponectin concentration (18) have been well documented.

Adiposity and adiponectin concentrations

The inverse association between several measures of fatness and adiponectin was consistent with previous reports in youth (15-19;21-26). In this study, we were able to further distinguish the effects of relative fatness and the normal accrual of fat with pubertal development. We used BMI Z-score as a measure of relative degree of fatness compared to sex- and age-matched peers. We used BMI as a measure of total fat. In growing school-age children, the BMI measure incorporates both relative fatness (BMI Z-score), and a unique fat mass that increases directly with age. This fat mass is not anatomically distinct, but defining it allowed us to determine how much of the association between age and adiponectin was due to normal age-related changes in body composition. In girls, all of the age effect on adiponectin could be explained by BMI. In boys, a large proportion of the age effect could be explained by BMI (presumably the remainder is the androgen effect discussed above). It seems that even among growing youth, total fat mass is the major determinant of adiponectin concentrations, and the age effect is largely a result of increased fat mass with increased age. One interpretation of this finding is that not all decreases in adiponectin are unfavorable. There may be an unidentified adaptive physiologic reason that normal accrual of body fat is associated with decreasing adiponectin during the pubertal years.

Adiponectin and markers of insulin resistance

Our results do not support an independent effect of adiponectin on markers of IR in youth, at a population level. In combination with the association between normal fat accrual and lower adiponectin during the pubertal years, this suggests that there may be mechanisms to reduce the effects of low adiponectin on IR in this age group. Previous reports support a role for adiponectin in the pathogenesis of IR (11;12) and type 2 diabetes (10) in adults. However, only 5 (15-19) of 9 pediatric studies (15-19;22-25) demonstrated an independent association between adiponectin and measures of IR. All three studies that used direct measures of IR showed an independent association (17-19). Studies using surrogate measures of IR that showed an independent association sampled a higher proportion of obese individuals. The existence of a stronger association between adiponectin and IR with increasing adiposity could partly explain the discrepant findings.

Our demonstration of an interaction between BMI Z-score and adiponectin concentration on insulin concentration supports this hypothesis.

In the QCAHS, adiponectin concentrations modified the relationship between BMI Z-score and fasting insulin concentration, suggesting that high adiponectin concentrations protect against obesity-induced IR. Since BMI is a rough index of body fat, such a hypothesis can only be tested with more rigorous measures of body composition. However, mouse models treated with adiponectin showed that adiponectin protects against IR to a greater degree in obese than in normal weight mice (68). The mechanism is not known, but TNF-α, which correlates with obesity and obesity-induced IR (69), may be involved. TNF-α expression in adipocytes is suppressed by adiponectin (46). This may explain how high adiponectin concentrations in overweight individuals protect against IR. PPARγ may also have a role. PPARγ agonists increase adiponectin concentrations in adults with IR (6) and type 2 diabetes (48). These drugs are known to improve insulin sensitivity while causing weight gain. This apparent paradox may be partly explained by the attenuating effect of high adiponectin concentrations on obesity-related IR, a hypothesis supported by our results. If this attenuating effect of higher adiponectin concentrations on obesity-induced IR is replicated in prospective studies, adiponectin may prove to be an intervention target. Interventions directed at this mechanism specifically may include PPARγ agonists.

Potential value of normative data

The possible role of adiponectin as an effect modifier of the adiposity-IR relationship also makes it a potential risk stratification tool. Although we do not yet fully understand the importance of absolute concentrations of adiponectin, the age- and sex-specific percentile norms we have reported may be useful in the future for identifying children at risk of morbidity.

The additional value of these percentile distributions will be in the evaluation of population-wide trends with the evolution of the obesity epidemic, and for comparison with groups of children with the metabolic syndrome.

Study limitations

After accounting for the effects of sex, age, puberty, anthropometry and lifestyle factors, 80% of the variation in adiponectin concentration remained unexplained. Genetic or other unknown metabolic or hormonal factors may be important determinants. However it may not be possible to explain much more of the variation in adiponectin concentrations because of biological variation; adiponectin shows random pulsatility in its secretion (52). Furthermore, measurement of total adiponectin may not be the appropriate biological measure to assess associations with the variables of interest. The lack of a statistically significant association between adiponectin concentration and measures of IR does not rule out the possibility that the distribution of multimeric complexes or intact versus globular domain adiponectin are important in determining insulin sensitivity among youth.

Further limitations to this study include the relatively low proportion (roughly 54%) of French Canadian youth sampled who had adiponectin measured, which may have introduced selection bias. However, those who provided blood samples were similar to those who did not. Pubertal status was not independently associated with adiponectin. The large proportion of intrapubertal participants may indicate misclassification of both prepubertal and postpubertal participants, which could have diluted a true effect [see Appendix 7]. We also found no associations between adiponectin and lifestyle factors or family history of diabetes. There is limited evidence of these associations in adults (70-72). Although there may truly be no associations in youth (particularly for tobacco use, given the low cumulative exposure), it is possible that our measures were unable to detect meaningful differences in these variables, or that misclassification may have diluted the effect (e.g. up to 2.1% of 9 year olds may have been incorrectly designated as nonsmokers). Finally, drawing conclusions beyond those of associations should be avoided in cross-sectional studies. Prospective studies in youth are needed to evaluate the role of adiponectin in the obesity-IR pathway.

Conclusions

Low adiponectin was associated with male sex, increased age and adiposity, but not with physical activity, smoking or parental history of diabetes. The association with

age was driven mainly by changes in BMI, especially in girls. This suggests that fat mass, no matter what its origin (developmental or energy excess), is a major determinant of adiponectin concentrations in youth. Although adiponectin was not independently associated with markers of IR, it did appear to attenuate the detrimental effect of adiposity. The role of adiponectin in the obesity-IR pathway is less clear in healthy youth than in adults. Further interventional or longitudinal studies are needed to determine whether adiponectin can protect against obesity-mediated IR among youth.

[End of manuscript.]

6. CONCLUSION

6.1 Summary of contributions

Obesity has emerged as a major health problem among youth in Canada (73) and worldwide (74). The relationship between obesity and insulin resistance and diabetes is well-established in adults and has been demonstrated more recently in youth. One putative mediator of this relationship is the adipokine adiponectin. Longitudinal studies in adults support the hypothesis that low adiponectin is a predictor of incident diabetes. In adults, several large cross-sectional studies of both select and population-based samples show that adiponectin levels are lower in men, in obese individuals, and in those with insulin resistance. In youth, studies of adiponectin generally have small samples sizes and have often been conducted in select samples. Few studies investigate the interaction between age or pubertal status and sex, and measures of adiposity. Despite the variety of groups studied, there are no studies of Canadian youth. No studies have evaluated health behaviours as correlates of adiponectin levels in youth. Furthermore, there are no descriptions of the population distribution of adiponectin levels in youth by age and sex. The QCAHS, school-based survey of health and social characteristics of youth in the province of Quebec provided the opportunity to describe population distributions of adiponectin concentration, to examine its potential mediators and to evaluate its association with insulin resistance.

Using this representative sample, population age- and sex-specific normative data for adiponectin levels for selected percentile values were generated. If adiponectin concentration is a risk factor of diabetes or cardiovascular disease, these data will be useful for describing secular trends and for identifying youth at risk for these diseases.

Potential determinants of adiponectin were also studied. Age, sex and obesity were significantly associated with adiponectin levels. Pubertal status, physical activity and smoking were not independently associated with adiponectin level. The lack of association with pubertal status may be due to its correlation with age and the imprecision of its measurement in this survey (see Appendix 7). Comparison of regression models revealed that the decline in adiponectin levels with increased age was explained to a large

extent by body fat accrual. Assessment of interactions showed that the association between obesity and adiponectin is stronger in older than younger boys, but did not vary by age group in girls. This observation is in accordance with other studies of peripubertal youth that suggest testosterone is an important determinant of low adiponectin levels in boys.

In this study, adiponectin was not independently associated with fasting insulin or HOMA-IR after adjusting for BMI Z-score. However, interaction analyses suggested there was a relationship between adiponectin and measures of insulin resistance at higher BMI Z-scores. This is consistent with other pediatric studies showing that in samples in which obese youth are overrepresented, an independent association between adiponectin concentrations and insulin resistance can be detected. Two interpretations of the interaction are possible: i) the obesity–insulin resistance relationship is stronger in individuals with lower adiponectin concentrations, or ii) the adiponectin–insulin resistance relationship is stronger in individuals with higher BMI. It is impossible to determine which may be the appropriate biological interpretation from a cross-sectional study. Either way, it indicates that obese individuals with low adiponectin are more likely to be insulin resistant than nonobese individuals or those with high adiponectin.

6.2 Limitations

The cross-sectional nature of this study is one of the primary limitations. Although associations can be determined, the main goal of such a study is to contribute to the inference of causality. This becomes particularly difficult when studying a relatively poorly understood biological variable like adiponectin. Noting associations between obesity and adiponectin and between adiponectin and insulin resistance does not help us understand where along the continuum adiponectin plays a role, or even if it has a role rather than being a parallel metabolic coincidence. The choice of a dependent variable in a regression model is based on a hypothesis of causality, but this assumption cannot be verified in a cross-sectional study. Furthermore, the association between adiponectin and age, pubertal status and age-related variations in BMI within groups cannot be mistaken for longitudinal changes associated with these variables within individuals.

Another study limitation relates to the nature of secondary data analysis. Using a database to answer questions for which it was not developed can lead to insufficient or inadequate data that cannot be corrected for by any analytical means. The original intent of the QCHAS was fairly broad, and its design as a school-based survey targeted at children limited the quantity and quality of data that could be collected, particularly by questionnaire. This has several consequences. First, some variables relevant to adiponectin, obesity and insulin resistance such as dietary habits and genetic variables, were unmeasured. After accounting for relevant variables available in the current study, 80% of the variation in adiponectin concentration remained unexplained. Unmeasured predictors of adiponectin may have played an important role in the models. Second, unmeasured confounders may have led to erroneous conclusions about relationships between the variables studied. This is a legitimate concern in an observational study. Third, the quality of data collected may have led to erroneous conclusions. For example, although there may be no association between lifestyle factors and adiponectin, it is possible that the questionnaire instruments used to measure physical activity or tobacco use lacked discriminant validity, leading to nondifferential misclassification that biased results towards the null hypothesis.

Selection bias was a potential limitation. The initial survey design was quite rigorous in developing a representative sample. However, those who consented to all procedures and were able to provide complete data amounted to a 54% response proportion of French Canadians sampled. Although those who provided blood samples did not differ significantly from those who did not, by sex, BMI Z-score, parental income or education, or parental history of diabetes, their similarity on non-measured variables cannot be assumed.

6.3 Future research directions

Analysis of these cross-sectional data raises several questions about the role of adiponectin in growth and development. A better understanding of dynamic changes may be possible by following trajectories of adiponectin. This life course epidemiology approach allows repeated measures of exposures and outcomes to inform researchers

about normative trajectories and how trajectories can be deflected by developmental changes such as puberty and by various health states such as the development of obesity.

These cross-sectional data also generate hypotheses about the role of adiponectin in the obesity-insulin resistance pathway. For example, low adiponectin may be a predictor of risk for insulin resistance in youth who develop obesity. Another hypothesis is that low adiponectin does not confer risk of insulin resistance until adulthood when the processes of growth and development are complete. Prospective studies are needed to determine if adiponectin is a predictor of insulin resistance in youth who develop obesity. Comparison of the effect of adiponectin concentrations on the development of insulin resistance in obese versus nonobese individuals requires longitudinal study in large samples. An alternative to observational studies may include large randomized trials to determine if weight loss affects the relationship between baseline adiponectin concentrations and development of insulin resistance in youth.

Although biological insight may be gained by studying insulin resistance, understanding the effect of adiponectin on clinical outcomes such as diabetes may have a greater impact on public health. The potential to identify and possibly modify diabetes risk may affect the long-term morbidity, shortened life expectancy and health-care costs associated with this disease and its complications. Three well-designed prospective studies demonstrate that adiponectin concentration predicts development of diabetes in adults (8-10). Currently, there are no such studies in youth. Determining whether low adiponectin in youth is a risk factor for diabetes later on is an important step since much of the variation in adiponectin during youth seems to be physiologic (ie. growth and development) rather than pathologic. The paucity of prospective longitudinal data in youth is in part due to the fact that both type 2 diabetes in youth and adiponectin are fairly new research fields. Now that initial cross-sectional data seems to support an association, longitudinal studies are likely forthcoming. One of the major limitations to longitudinal studies is that type 2 diabetes in youth is still quite rare, so prospective studies of adiponectin and diabetes in youth will require many person-years of follow-up and will be very resource intensive.

6.4 Summation

Findings from this study suggest that adiponectin concentrations in growing youth vary by sex and with both physiological age-related and pathological states of increased adiposity. The detrimental relationship between low adiponectin and markers of insulin resistance is seen only in individuals with a high BMI Z-score. These findings corroborate other studies and may in part explain discrepancies in the literature.

As obesity and type 2 diabetes among youth continue to increase it will be more important to evaluate longitudinal relationships between adiponectin, obesity, insulin resistance and type 2 diabetes to determine whether this adipokine will be a useful marker of disease risk or a therapeutic target.

7. REFERENCES

Reference List

(1) Jolliffe D. Extent of overweight among US children and adolescents from 1971 to 2000. Int J Obes Relat Metab Disord. 2004;28:4-9.

(2) Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. JAMA. 2004;291:2847-50.

(3) Shileds, M. Measured obesity: Overweight Canadian children and adolescents. #82-620-MWE2005001. 2005. Ottawa, ON, Statistics Canada Publication. Ref Type: Report

(4) Beltowski J. Adiponectin and resistin--new hormones of white adipose tissue. Med Sci Monit. 2003;9:RA55-RA61.

(5) Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab. 2002;13:84-89.

(6) Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes. 2001;50:2094-99.

(7) Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. 2003;423:762-69.

(8) Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H et al. Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese Population: the Funagata study. Diabetes Care. 2003;26:2015-20.

(9) Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet. 2002;360:57-58.

(10) Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H et al. Adiponectin and protection against type 2 diabetes mellitus. Lancet. 2003;361:226-28.

(11) Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2001;86:1930-1935.

(12) Yamamoto Y, Hirose H, Saito I, Tomita M, Taniyama M, Matsubara K et al. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. Clin Sci (Lond). 2002;103:137-42.

(13) Schaffler A, Herfarth H, Paul G, Ehling A, Muller-Ladner U, Scholmerich J et al. Identification of influencing variables on adiponectin serum levels in diabetes mellitus type 1 and type 2. Exp Clin Endocrinol Diabetes. 2004;112:383-89.

(14) Steffes MW, Gross MD, Schreiner PJ, Yu X, Hilner JE, Gingerich R et al. Serum adiponectin in young adults--interactions with central adiposity, circulating levels of glucose, and insulin resistance: the CARDIA study. Ann Epidemiol. 2004;14:492-98.

(15) Cruz M, Garcia-Macedo R, Garcia-Valerio Y, Gutierrez M, Medina-Navarro R, Duran G et al. Low adiponectin levels predict type 2 diabetes in Mexican children. Diabetes Care. 2004;27:1451-53.

(16) Asayama K, Hayashibe H, Dobashi K, Uchida N, Nakane T, Kodera K et al. Decrease in serum adiponectin level due to obesity and visceral fat accumulation in children. Obes Res. 2003;11:1072-79.

(17) Bacha F, Saad R, Gungor N, Arslanian SA. Adiponectin in youth: relationship to visceral adiposity, insulin sensitivity, and beta-cell function. Diabetes Care. 2004;27:547-52.

(18) Bush NC, Darnell BE, Oster RA, Goran MI, Gower BA. Adiponectin is lower among African Americans and is independently related to insulin sensitivity in children and adolescents. Diabetes. 2005;54:2772-78.

(19) Weiss R, Dufour S, Groszmann A, Petersen K, Dziura J, Taksali SE et al. Low adiponectin levels in adolescent obesity: a marker of increased intramyocellular lipid accumulation. J Clin Endocrinol Metab. 2003;88:2014-18.

(20) Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med. 2004;350:2362-74.

(21) Bottner A, Kratzsch J, Muller G, Kapellen TM, Bluher S, Keller E et al. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. J Clin Endocrinol Metab. 2004;89:4053-61.

(22) Dagawa-Yamauchi M, Dilts JR, Bovenkerk JE, Saha C, Pratt JH, Considine RV. Lower serum adiponectin levels in African-American boys. Obes Res. 2003;11:1384-90.

(23) Stefan N, Bunt JC, Salbe AD, Funahashi T, Matsuzawa Y, Tataranni PA. Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. J Clin Endocrinol Metab. 2002;87:4652-56.

(24) Tsou PL, Jiang YD, Chang CC, Wei JN, Sung FC, Lin CC et al. Sex-related differences between adiponectin and insulin resistance in schoolchildren. Diabetes Care. 2004;27:308-13.

(25) Vikram NK, Misra A, Pandey RM, Dwivedi M, Luthra K. Adiponectin, insulin resistance, and C-reactive protein in postpubertal Asian Indian adolescents. Metabolism. 2004;53:1336-41.

(26) Reinehr T, Roth C, Menke T, Andler W. Adiponectin before and after weight loss in obese children. J Clin Endocrinol Metab. 2004;89:3790-3794.

(27) Goran MI, Gower BA. Longitudinal study on pubertal insulin resistance. Diabetes. 2001;50:2444-50.

(28) Park KG, Park KS, Kim MJ, Kim HS, Suh YS, Ahn JD et al. Relationship between serum adiponectin and leptin concentrations and body fat distribution. Diabetes Res Clin Pract. 2004;63:135-42.

(29) Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol. 2000;20:1595-99.

(30) Choi KM, Lee J, Lee KW, Seo JA, Oh JH, Kim SG et al. Serum adiponectin concentrations predict the developments of type 2 diabetes and the metabolic syndrome in elderly Koreans. Clin Endocrinol (Oxf). 2004;61:75-80.

(31) Hulver MW, Saleh O, MacDonald KG, Pories WJ, Barakat HA. Ethnic differences in adiponectin levels. Metabolism. 2004;53:1-3.

(32) Nemet D, Wang P, Funahashi T, Matsuzawa Y, Tanaka S, Engelman L et al. Adipocytokines, body composition, and fitness in children. Pediatr Res. 2003;53:148-52.

(33) Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257:79-83.

(34) Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM et al. Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes. 2004;53:2473-78.

(35) Dietz WH, Robinson TN. Use of the body mass index (BMI) as a measure of overweight in children and adolescents. J Pediatr. 1998;132:191-93.

(36) The use of growth charts for assessing and monitoring growth in Canadian infants and children. Can J Diet Pract Res. 2004;65:22-32.

(37) Krebs NF, Jacobson MS. Prevention of pediatric overweight and obesity. Pediatrics. 2003;112:424-30.

(38) Cheng C, Campbell KL, Kushner H, Falkner BE. Correlation of oral glucose tolerance test-derived estimates of insulin sensitivity with insulin clamp measurements in an African-American cohort. Metabolism. 2004;53:1107-12.

(39) Vaccaro O, Masulli M, Cuomo V, Rivellese AA, Uusitupa M, Vessby B et al. Comparative evaluation of simple indices of insulin resistance. Metabolism. 2004;53:1522-26.

(40) Cutfield WS, Jefferies CA, Jackson WE, Robinson EM, Hofman PL. Evaluation of HOMA and QUICKI as measures of insulin sensitivity in prepubertal children. Pediatr Diabetes. 2003;4:119-25.

(41) Conwell LS, Trost SG, Brown WJ, Batch JA. Indexes of insulin resistance and secretion in obese children and adolescents: a validation study. Diabetes Care. 2004;27:314-19.

(42) Gungor N, Saad R, Janosky J, Arslanian S. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. J Pediatr. 2004;144:47-55.

(43) Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629-34.

(44) Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC et al. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes. 2001;50:1126-33.

(45) Motoshima H, Wu X, Sinha MK, Hardy VE, Rosato EL, Barbot DJ et al. Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: effects of insulin and rosiglitazone. J Clin Endocrinol Metab. 2002;87:5662-67.

(46) Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med. 2002;8:731-37.

(47) Hirose H, Kawai T, Yamamoto Y, Taniyama M, Tomita M, Matsubara K et al. Effects of pioglitazone on metabolic parameters, body fat distribution, and serum adiponectin levels in Japanese male patients with type 2 diabetes. Metabolism. 2002;51:314-17.

(48) Yang WS, Jeng CY, Wu TJ, Tanaka S, Funahashi T, Matsuzawa Y et al. Synthetic peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. Diabetes Care. 2002;25:376-80.

(49) Stefan N, Vozarova B, Funahashi T, Matsuzawa Y, Weyer C, Lindsay RS et al. Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. Diabetes. 2002;51:1884-88.

(50) Gavrila A, Peng CK, Chan JL, Mietus JE, Goldberger AL, Mantzoros CS. Diurnal and ultradian dynamics of serum adiponectin in healthy men: comparison with leptin, circulating soluble leptin receptor, and cortisol patterns. J Clin Endocrinol Metab. 2003;88:2838-43.

(51) Calvani M, Scarfone A, Granato L, Mora EV, Nanni G, Castagneto M et al. Restoration of adiponectin pulsatility in severely obese subjects after weight loss. Diabetes. 2004;53:939-47.

(52) Yildiz BO, Suchard MA, Wong ML, McCann SM, Licinio J. Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. Proc Natl Acad Sci U S A. 2004;101:10434-39.

(53) Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem. 2004;50:1511-25.

(54) Suominen P. Evaluation of an enzyme immunometric assay to measure serum adiponectin concentrations. Clin Chem. 2004;50:219-21.

(55) Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T et al. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. J Biol Chem. 2003;278:9073-85.

(56) Paradis G, Lambert M, O'Loughlin J, Lavallee C, Aubin J, Berthiaume P et al. The Quebec Child and Adolescent Health and Social Survey: design and methods of a cardiovascular risk factor survey for youth. Can J Cardiol. 2003;19:523-31.

(57) Carskadon MA, Acebo C. A self-administered rating scale for pubertal development. J Adolesc Health. 1993;14:190-195.

(58) Sallis JF, Condon SA, Goggin KJ, Roby JJ, Kolody B, Alcaraz JE. The development of self-administered physical activity surveys for 4th grade students. Res Q Exerc Sport. 1993;64:25-31.

(59) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from

fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-19.

(60) Hutson AD. Calculating nonparametric confidence intervals for quantiles using fractional order statistics. J Appl Stat. 1999;26:343-53.

(61) Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z et al. 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat. 2002;11:1-190.

(62) Garnett SP, Hogler W, Blades B, Baur LA, Peat J, Lee J et al. Relation between hormones and body composition, including bone, in prepubertal children. Am J Clin Nutr. 2004;80:966-72.

(63) Murphy MJ, Metcalf BS, Voss LD, Jeffery AN, Kirkby J, Mallam KM et al. Girls at five are intrinsically more insulin resistant than boys: The Programming Hypotheses Revisited--The EarlyBird Study (EarlyBird 6). Pediatrics. 2004;113:82-86.

(64) Nishizawa H, Shimomura I, Kishida K, Maeda N, Kuriyama H, Nagaretani H et al. Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. Diabetes. 2002;51:2734-41.

(65) Lanfranco F, Zitzmann M, Simoni M, Nieschlag E. Serum adiponectin levels in hypogonadal males: influence of testosterone replacement therapy. Clin Endocrinol (Oxf). 2004;60:500-507.

(66) He Q, Horlick M, Thornton J, Wang J, Pierson RN, Jr., Heshka S et al. Sex-specific fat distribution is not linear across pubertal groups in a multiethnic study. Obes Res. 2004;12:725-33.

(67) Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev. 2000;21:697-738.

(68) Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med. 2001;7:941-46.

(69) Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest. 1995;95:2409-15.

(70) Kriketos AD, Gan SK, Poynten AM, Furler SM, Chisholm DJ, Campbell LV. Exercise increases adiponectin levels and insulin sensitivity in humans. Diabetes Care. 2004;27:629-30.

(71) Miyazaki T, Shimada K, Mokuno H, Daida H. Adipocyte derived plasma protein, adiponectin, is associated with smoking status in patients with coronary artery disease. Heart. 2003;89:663.

(72) Pellme F, Smith U, Funahashi T, Matsuzawa Y, Brekke H, Wiklund O et al. Circulating adiponectin levels are reduced in nonobese but insulin-resistant first-degree relatives of type 2 diabetic patients. Diabetes. 2003;52:1182-86.

(73) Tremblay MS, Willms JD. Secular trends in the body mass index of Canadian children. CMAJ. 2000;163:1429-33.

(74) Popkin BM, Gordon-Larsen P. The nutrition transition: worldwide obesity dynamics and their determinants. Int J Obes Relat Metab Disord. 2004;28 Suppl 3:S2-S9.

(75) Moreno LA, Fleta J, Mur L, Feja C, Sarria A, Bueno M. Indices of body fat distribution in Spanish children aged 4.0 to 14.9 years. J Pediatr Gastroenterol Nutr. 1997;25:175-81.

(76) Mueller WH, Kaplowitz HJ. The precision of anthropometric assessment of body fat distribution in children. Ann Hum Biol. 1994;21:267-74.

(77) Desmangles JC, Lappe JM, Lipaczewski G, Haynatzki G. Accuracy of pubertal Tanner staging self-reporting. J Pediatr Endocrinol Metab. 2006;19:213-21.

(78) Schlossberger NM, Turner RA, Irwin CE, Jr. Validity of self-report of pubertal maturation in early adolescents. J Adolesc Health. 1992;13:109-13.

(79) Bonat S, Pathomvanich A, Keil MF, Field AE, Yanovski JA. Self-assessment of pubertal stage in overweight children. Pediatrics. 2002;110:743-47.

(80) Canadian Diabetes Association. Definition, Classification and Diagnosis of Diabetes and Other Dysglycemic Categories. Canadian Journal of Diabetes. 2003;27:S7-S9.

Appendix 3

QCAHS questionnaire items – pubertal status, physical activity, smoking, family history

This appendix includes items from questionnaires given to children (age 9 years), adolescents (age 13 or 16 years), and their parents. The questions from the child and adolescent questionnaire were used to categorize youth as pre-, intra- or post-pubertal, to calculate their physical activity scores, to categorize them as smoking 0, upto 5 or greater than 5 cigarettes per day. Questions from the parent questionnaire were used to identify one or both parents as having diabetes, hypertension, hypercholesterolemia or cardiovascular disease.

SANTÉ QUÉBEC

Questionnaire aux enfants

parle-nous de ta santé

ENQUÊTE SOCIALE ET DE SANTÉ

auprès des enfants
et des adolescents québécois

QE

SANTÉ QUÉBEC
1200, avenue McGill College, bureau 700
Montréal (Québec) H3B 4J8
(514) 873-4749

N° de dossier :
N° de l'intervieweur :
ADM ___ LA 1
Date de réception : jr ma an

Les changements de ton corps

En vieillissant, ton corps change. Ces changements ont une influence sur la santé. C'est pourquoi nous te posons les questions suivantes.

23. As-tu des poils en-dessous des bras ou autour de ton sexe ?

1 • Je n'en ai pas encore
2 • Je commence tout juste à en avoir
3 • J'en ai déjà pas mal
4 • Je pense que mes poils ont fini de pousser

Si tu es un garçon • Va à la question 26

24. Tes seins ont-ils commencé à grossir ?

1 • Ils n'ont pas encore commencé à grossir
2 • Ils commencent tout juste à grossir
3 • Ils ont déjà pas mal grossi
4 • Je pense qu'ils ont fini de grossir

25. As-tu commencé à être menstruée, à avoir tes règles ?

1 • Oui
2 • Non

Si tu es une fille • Va à la question 28

26. Ta voix est-elle devenue plus grave ?

1 • Elle n'a pas encore commencé à changer
2 • Elle commence tout juste à changer
3 • Elle a déjà bien changé
4 • Je pense qu'elle a fini de changer

27. As-tu des poils au visage ?

1 • Je n'en ai pas encore
2 • Je commence tout juste à en avoir
3 • J'en ai déjà pas mal
4 • Je pense qu'ils ont fini de pousser

L'activité physique

32. Au cours de la semaine dernière, du lundi au dimanche, coche la ou les journées où tu as fait les activités suivantes *durant au moins 15 minutes d'affilée.*

	Je n'en ai pas fait	Lundi	Mardi	Mercredi	Jeudi	Vendredi	Samedi	Dimanche
a. Cours d'éducation physique à l'école	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
b. Vélo	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
c. Corde à danser, jouer à l'élastique	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
d. Ballon chasseur, balle au mur, lancer la balle, kick-ball	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
e. Course, course à relais, jouer à la tag	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
f. Patin à roues alignées (rollerblades), rouli-roulant (skateboard)	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
g. Natation	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
h. Badminton, tennis	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
i. Ballet (jazz ou classique)	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
j. Gymnastique (au sol, aux appareils)	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
k. Basketball	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
l. Volleyball	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
m. Soccer	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
n. Hockey sur glace ou hockey bottine	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
o. Patinage sur glace	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
p. Glissade	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
q. Planche à neige (snowboard), ski alpin	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
r. Ski de randonnée, ski de fond	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
s. Autres • *Nomme-les :*								
• _____	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
• _____	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •
• _____	0 •	1 •	2 •	3 •	4 •	5 •	6 •	7 •

La cigarette et l'alcool

40. As-tu déjà essayé de fumer une cigarette, même si c'est juste quelques *puffs* ?

1 • Oui
2 • Non • *Va à la question 42*

41. As-tu déjà fumé une cigarette *au complet* ?

1 • Oui
2 • Non

Ta puberté et ta sexualité

Les changements biologiques de ton corps durant la puberté ont une influence sur divers aspects de ta santé. C'est pourquoi nous te posons les questions suivantes.

43. As-tu des poils en-dessous des bras ou autour de ton sexe ?

- Je n'en ai pas encore 1
- Je commence tout juste à en avoir 2
- J'en ai déjà pas mal 3
- Je pense que mes poils ont fini de pousser 4

Si tu es un garçon • Passe à la question 59

44. Tes seins ont-ils commencé à grossir ?

- Ils n'ont pas encore commencé à grossir 1
- Ils commencent tout juste à grossir 2
- Ils ont déjà pas mal grossi 3
- Je pense qu'ils ont fini de grossir 4

45. As-tu commencé à être menstruée, à avoir tes règles ?

- Oui 1
- Non 2

Si tu es une fille • Passe à la question 74

59. Ta voix est-elle devenue plus grave ?

- Elle n'a pas encore commencé à changer 1
- Elle commence tout juste à changer 2
- Elle a déjà bien changé 3
- Je pense qu'elle a fini de changer 4

60. As-tu des poils au visage ?

- Je n'en ai pas encore 1
- Je commence tout juste à en avoir 2
- J'en ai déjà pas mal 3
- Je pense qu'ils ont fini de pousser 4

Activité physique

74. *Au cours de la semaine dernière*, du lundi au dimanche, indique la ou les journées où tu as pratiqué les activités suivantes durant au moins 15 minutes d'affilée. Indique « Je n'en ai pas fait », si c'est le cas.

- *Les activités physiques pratiquées durant un cours à l'école doivent être indiquées en « A » seulement.*

	Je n'en ai pas fait	Lundi	Mardi	Mercredi	Jeudi	Vendredi	Samedi	Dimanche
A. Cours d'éducation physique à l'école	0	1	2	3	4	5	6	7
B. Vélo (pour aller à l'école, faire des courses, te balader ...)	0	1	2	3	4	5	6	7
C. Patin à roues alignées (rollerblades), rouli-roulant (skateboard)	0	1	2	3	4	5	6	7
D. Course ou jogging	0	1	2	3	4	5	6	7
E. Conditionnement physique ou musculation	0	1	2	3	4	5	6	7
F. Danse aérobique	0	1	2	3	4	5	6	7
G. Natation	0	1	2	3	4	5	6	7
H. Badminton, tennis	0	1	2	3	4	5	6	7
I. Karaté ou judo	0	1	2	3	4	5	6	7
J. Ballet, jazz ou classique	0	1	2	3	4	5	6	7
K. Danse libre	0	1	2	3	4	5	6	7
L. Gymnastique (au sol, aux appareils)	0	1	2	3	4	5	6	7
M. Basketball	0	1	2	3	4	5	6	7
N. Volleyball	0	1	2	3	4	5	6	7
O. Soccer	0	1	2	3	4	5	6	7
P. Hockey sur glace ou hockey bottine	0	1	2	3	4	5	6	7
Q. Planche à neige (*snowboard*), ski alpin	0	1	2	3	4	5	6	7
R. Ski de randonnée (ski de fond)	0	1	2	3	4	5	6	7
S. Autres, *nomme-les* : 1. _____ 2. _____ 3. _____		1 1 1	2 2 2	3 3 3	4 4 4	5 5 5	6 6 6	7 7 7

Ton expérience de la cigarette

100. As-tu déjà essayé de fumer une cigarette, même si c'est juste quelques *puffs* ?

Oui 1
Non . . . . . 2 • *Passe à la question 106*

101. As-tu déjà fumé une cigarette *au complet* ?

Oui 1
Non . . . . . 2 • *Passe à la question 106*

104. *Les jours où tu as fumé*, combien de cigarettes as-tu fumées habituellement ?

• *Choisis une seule réponse.*

Je n'ai pas fumé au cours des 30 derniers jours 1
Moins d'une cigarette par jour (quelques *puffs* par jour) . . 2
1 à 2 cigarettes par jour(2) 3
3 à 5 cigarettes par jour 4
6 à 10 cigarettes par jour 5
11 à 20 cigarettes par jour 6
Plus de 20 cigarettes par jour 7

Santé des parents biologiques

Cette enquête s'intéresse à la santé de votre enfant. Cependant, certains aspects de la santé des parents ou de leur mode de vie ont une influence sur celle des enfants.

C'est pourquoi nous posons les questions suivantes.

- *Veuillez répondre pour les deux parents biologiques.*

Si vous n'êtes pas l'un d'eux, répondez aux questions suivantes au meilleur de votre connaissance.

57. Est-ce qu'un médecin, une infirmière ou un autre professionnel de la santé a déjà dit que vous (père et mère biologiques)...

		Mère biologique	Père biologique
A. faisiez de l'hypertension artérielle (haute pression) ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8
B. aviez un taux de cholestérol élevé ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8

58. Est-ce qu'un médecin a déjà dit que vous faisiez (mère et père biologiques) ...

		Mère biologique	Père biologique
A. du diabète ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8
B. de l'asthme ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8
C. du rhume des foins ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8

59. Avez-vous déjà fait (mère et père biologiques) ...

		Mère biologique	Père biologique
A. une crise cardiaque ou de l'angine ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8
B. un accident cérébrovasculaire, une maladie cérébrovasculaire ou une maladie des vaisseaux périphériques ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8

60. Prenez-vous (mère et père biologiques) des médicaments ...

		Mère biologique	Père biologique
A. pour abaisser le cholestérol sanguin ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8
B. pour abaisser la tension artérielle ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8
C. pour le coeur ?	Oui	1	1
	Non	2	2
	Ne sais pas	8	8

Appendix 4

SAS macro for linear regression analysis

Regression parameters were estimated using PROC MIXED with school of origin as the random variable.

The R^2 for the models was estimated from a weighted least squares linear regression using PROC GLM. This method will overestimate the proportion of variance explained by the model from a cluster sample, but provides a rough guide, especially when comparing models.

```
*FULL MODEL WITH GLM R-SQUARE AND MIXED MODEL PARAMETERS;

*variable definitions:;
num = model number; date = date of analysis; byvar = group-specific
analysis (eg. by sex); where = restricted subgroup; classvar =
categorical variables; depvar = depend*ent variable; depname = name of
dependent variable; indvar = independent variables in the model;
indname = names of independent variables;

%macro mwglm (num, date, byvar, where, classvar, depvar, depname,
indvar, indname);
 %do; %if &byvar=%str() %then %let stratify=%str();
      %else %let stratify= Stratified by &byvar;
  %end;
 title1 "QCAHS 1999.       Date: &date";
 title2 "Model &num            Weighted (sangefsx) Least Squares
         Regression R-square";
 title3 "Dependent variable: &depname     &stratify";
 title4 "Independent variables: &indname";
 %if &byvar =%str() %then %goto skipsort;
 proc sort data=zubin.zpdata;
 by &byvar;
 %skipsort:
 ods select fitstatistics;

* LEAST SQUARES LINEAR REGRESSION BY PROC GLM FOR R2;
 proc glm data=zubin.zpdata;
  class &classvar;
  by &byvar;
  model &depvar = &indvar;
  weight sangefsx; /* sangefsx is the sample weights and design effects
                  for means analyzed by sex */
  %if &where = all %then %goto all;
  %if &where = parents %then %goto parents;
```

```
  %all:
    title5 "Excluded: diabetic or missing anthrop., adiponectin,
            puberty, smoking, physical";
    where dm_ins ne 1 and pub_cat3 ne . and smok_mo3 ne . and physsum2
      ne . and adpn ne . and bmi ne . and tric ne . and scap ne .;
    %goto theend;
  %parents:
    title5 "Excluded: Diabetes/Insulin and No Adiponectin and No Parent
           Info";
    where pardm ne . and parhibp ne . and parhicho ne . and parcvd ne .
          and dm_ins ne 1 and pub_cat3 ne . and smok_mo3 ne . and
          physsum2 ne . and adpn ne . and bmi ne . and tric ne . and
          scap ne .;
    %goto theend;
  %theend:
run;quit;

* HIERARCHICAL LINEAR REGRESSION BY PROC MIXED;
  %do; %if &byvar=%str() %then %let stratify=%str();
      %else %let stratify= Stratified by &byvar;
  %end;
 title2 "Model &num           Mixed model parameters";
 title3 "Dependent variable: &depname     &stratify";
 title4 "Independent variables: &indname";
ods listing exclude ClassLevels Fitstatistics;
proc mixed data=zubin.zpdata covtest;
  class en0 &classvar;
  by &byvar;
  model &depvar = &indvar / solution /*cl*/;
  random en0;
  %if &where = all %then %goto all2;
  %if &where = parents %then %goto parents2;
  %all2:
    title5 "Excluded: diabetic or missing anthrop., adiponectin,
           puberty, smoking, physical";
    where dm_ins ne 1 and pub_cat3 ne . and smok_mo3 ne . and physsum2
           ne . and adpn ne . and bmi ne . and tric ne . and scap ne .;
    %goto theend2;
  %parents2:
    title5 "Excluded: Diabetes/Insulin and No Adiponectin and No Parent
           Info";
    where pardm ne . and parhibp ne . and parhicho ne . and parcvd ne .
          and dm_ins ne 1 and pub_cat3 ne . and smok_mo3 ne . and
          physsum2 ne . and adpn ne . and bmi ne . and tric ne . and
          scap ne .;
    %goto theend2;
  %theend2:
run;quit;
%mend mwglm;
```

Appendix 5

Association of adiponectin concentration and parental history of diabetes or cardiac risk factors

Adiponectin concentration may be determined in part by genetic influences and familial environmental influences. Since adiponectin concentrations are associated with diabetes, heart disease and cardiovascular risk factors within adult individuals, we aimed to determine if adiponectin concentrations in youth were associated with parental diabetes, hypertension, hypercholesterolemia or cardiovascular disease as reported by parents. The proportion of participants in the current study whose parents reported the above conditions is shown in table 8 (See Appendix 3 for questionnaire items). This data is significantly limited by missing information. The proportion of missing responses to the parental history questions ranged from 19 to 27%. If the likelihood of responding was associated with presence or absence of parental disease and with adiponectin concentration in their children, the results may be biased. This bias is plausible since the parents knew it was a health survey and may have been influenced by the presence or absence of factors in their children associated with adiponectin concentration. Unfortunately, there is no way to measure this bias. Therefore, the main analyses did not evaluate family history in detail. However, keeping in mind the potential biases, the associations of adiponectin with parental diabetes, hypertension, hypercholesterolemia and cardiovascular disease with and without adjustment for age, pubertal status, adiposity, smoking and physical activity are presented in table 9 of this appendix. Even after adjusting for adiposity and lifestyle factors there is an association between adiponectin concentration and parental hypercholesterolemia and parental cardiovascular disease only in girls. This result must be interpreted cautiously given the incomplete data. It seems that whatever genetic or environmental factors predispose an individual to high cholesterol or to cardiovascular disease also predispose their female offspring to low adiponectin concentrations. However, these factors are independent of the child's adiposity and their physical activity and smoking behaviours. Further study of the shared family environment would shed light on the environmental component of familial causes

of low adiponectin. More rigorous studies of families including extended family, or direct genetic studies will further our understanding of the genetic causes of low adiponectin.

Table 8. Response rates and parental history of CVD and risk factors among parents of eligible youth*

n (%)	Boys		Girls	
Parental Disease	**Responders**	**Responders with disease**	**Responders**	**Responders with disease**
Diabetes	639 (79)	46 (7)	668 (81)	31 (5)
Hypertension	617 (77)	134 (22)	650 (79)	125 (19)
Hypercholesterolemia	593 (74)	174 (29)	601 (73)	154 (26)
Cardiovascular disease	627 (78)	47 (8)	651 (79)	43 (7)

* Eligible youth included 804 boys and 828 girls studied

Table 9. Association of adiponectin concentration in youth with parental CVD risk factors

Parental Disease Variable		Boys n=804 β, %*	(SE)	p	Girls n=828 β, %*	(SE)	p
[a]Diabetes	No	Ref.			Ref.		
	Yes	-0.2	(6.6)	0.97	-3.4	(7.9)	0.67
[a]Hypertension	No	Ref.			Ref.		
	Yes	-2.5	(4.0)	0.52	-2.3	(4.2)	0.59
[a]Hyper-cholesterolemia	No	Ref.			Ref.		
	Yes	0.4	(3.6)	0.92	-8.6	(3.6)	0.02
[a]Cardiovascular disease	No	Ref.			Ref.		
	Yes	-2.6	(6.5)	0.67	-15.5	(6.8)	0.02
[b]Diabetes	No	Ref.			Ref.		
	Yes	-6.3	(6.5)	0.33	-1.0	(7.7)	0.89
[b]Hypertension	No	Ref.			Ref.		
	Yes	0.2	(3.9)	0.966	-0.01	(4.1)	0.99
[b]Hyper-cholesterolemia	No	Ref.			Ref.		
	Yes	0.6	(3.5)	0.87	-8.3	(3.4)	0.02
[b]Cardiovascular disease	No	Ref.			Ref.		
	Yes	1.9	(6.4)	0.76	-13.7	(6.6)	0.04

* β refers to the percent difference of adiponectin concentration when each of the parental conditions is present

[a] Adjusted for age and pubertal status; [b] Adjusted for age, pubertal status, BMI Z-score, physical activity and smoking.

Appendix 6

Collinearity among measures of adiposity

Three measures of adiposity were available in this study: BMI, subscapular skinfold thickness (SSKF) and triceps skinfold thickness (TSKF). BMI is considered to be a measure of total adiposity, whereas SSKF is considered to represent central fat, and TSKF represents peripheral fat (75). The age- and sex specific Z-scores of the natural logarithm of the three measures of adiposity were strongly correlated with each other (see Table 10). Each was also significantly associated with adiponectin in univariate linear regression (see Table 11). Collinearity statistics were generated using PROC REG based on least squares regression models in boys and girls (see Table 12). Only one variance inflation factor was greater than 4.0 which is the standard cutoff above which to suspect multicollinearity. Two of the Eigenvalues in each model were close to zero, suggesting the possibility of multicollinearity, but condition indices did not exceed 15. Thus collinearity statistics were equivocal regarding multicollinearity among the three measures of adiposity.

However, observing the change in sign of the TSKF parameter in the multivariate model and the increase in standard error of all the parameters compared to univariate analysis in Table 11, it was decided that this was evidence of multicollinearity among the three measures of adiposity.

As a result of this finding, only one measure of adiposity was used in the main analyses. BMI Z-score was chosen for several reasons. First, BMI is a simple measure requiring commonly available tools (ie. measuring tape and weighing scale) whereas the skinfold measurements require calipers which are not available to most clinicians. Second, BMI is easy to measure and reliably so, whereas skinfold thickness measurement is notoriously imprecise (76). Third, BMI is a commonly used measure that has well described normative data in a North American population (61), making it a more easily understood measure of adiposity for readers.

Table 10. Pearson Correlation between measures of adiposity

Pearson r p value	SSKF Z-score Boys	 Girls	TSKF Z-score Boys	 Girls
BMI Z-score	0.81 <0.0001	0.79 <0.0001	0.77 <0.0001	0.72 <0.0001
SSKF Z-score		-	0.82 <0.0001	0.82 <0.0001

Table 11. Linear regression of adiponectin – association with adiposity measures individually and combined

	Boys n=804				Girls n=828			
Variable	**β, % * (SE)**		**p**	**Model R^2**	**β, % * (SE)**		**p**	**Model R^2**
1 BMI, 1 SD	-8.8	(1.3)	<0.0001	0.050	-11.2	(1.2)	<0.0001	0.092
2 SSKF, 1 SD	-6.5	(1.3)	<0.0001	0.028	-10.9	(1.2)	<0.0001	0.077
3 TSKF, 1 SD	-2.7	(1.4)	0.05	0.004	-8.0	(1.3)	<0.0001	0.046
4 BMI, 1 SD	-12.7	(2.5)	<0.0001	0.075	-9.6	(2.1)	<0.0001	0.101
SSKF, 1 SD	-4.0	(2.8)	0.15		-8.9	(2.3)	0.0001	
TSKF, 1 SD	10.1	(2.4)	<0.0001		7.2	(2.3)	0.0016	

* β - percent difference in adiponectin concentration for an increase of one (1) age and sex-specific SD in the explanatory variable; BMI – body mass index Z-score; SSKF – subscapular skinfold thickness Z-score; TSKF – triceps skinfold thickness Z-score; 1,2,3 univariate models; 4 multivariate model including all three adiposity measures.

Table 12. Association of Adiponectin and Measures of Adiposity with Multicollinearity Statistics

Boys					
Variable	**β**	**SE**	**p value**	**Variance Inflation**	
BMI Z-score	-12.9	2.5	<0.0001	3.35	
SSKF Z-score	-4.2	2.8	0.15	4.87	
TSKF Z-score	10.4	2.5	<0.0001	3.15	
Number	**Eigenvalue**	**Condition Index**	**Proportion of Variation**		
			BMI Z	**SSKF Z**	**TSKF Z**
1	2.59	1.00	0.04	0.03	0.04
2	0.27	3.08	0.51	<0.01	0.62
3	0.14	4.35	0.45	0.97	0.34
Girls					
Variable	**β**	**SE**	**p value**	**Variance Inflation**	
BMI Z-score	-9.8	2.1	<0.0001	3.09	
SSKF Z-score	-9.0	2.3	<0.0001	3.65	
TSKF Z-score	7.2	2.2	0.0011	3.34	
Number	**Eigenvalue**	**Condition Index**	**Proportion of Variation**		
			BMI Z	**SSKF Z**	**TSKF Z**
1	2.58	1.00	0.04	0.04	0.04
2	0.23	3.34	0.86	0.04	0.45
3	0.19	3.73	0.10	0.92	0.51

Appendix 7

Correlation of pubertal status and age group

Age is a measure of time lived, but in youth it is also a marker of physical and psychological developmental stages. One of the initial intentions of this study was to attempt to identify the independent association of adiponectin with age and pubertal status. Given the large sample size, it was reasonable to assume there would be enough random variation in the sample to allow independent associations to be studied. The distribution of pubertal status by age group in boys and girls demonstrated a strong relationship between age and pubertal status (see Table 13). However, table 13 also shows that a large proportion of youth in each age group were categorized as intrapubertal (85% of all boys and 70% of all girls). From a statistical point of view, this makes it more difficult to demonstrate a relationship with other variables in regression analysis. In the main analyses of this study, pubertal status was not independently associated with adiponectin after adjusting for age. There are several possible explanations. First, puberty may not be a relevant part of growth and development with respect to variation in adiponectin concentration. This is unlikely since others have shown a correlation with testosterone levels in boys (21;24) which rise during puberty. A second explanation is that age is a much more precise measure than the self-report pubertal status instrument used in this study. The pubertal status categories were constructed as described in the methods section of the manuscript using data from the questions shown in Appendix 3. Self-reporting of pubertal status is not reliable (kappa = 0.49-0.68 for Tanner stage agreement with a physician) (77), particularly when ascertained in a school setting (78) and may even be biased to overestimation in boys (79). It is very likely that there was significant misclassification of pubertal status. More objective assessment of pubertal status by trained observers is preferable but raises feasibility concerns in community-based epidemiological studies such as the QCAHS. Overall, these measurement issues along with the strong correlation between pubertal status and age group likely led to regression models suggesting that age and not pubertal

status is a strong predictor of adiponectin. However, this does not rule out puberty as a causal factor.

Table 13. Pubertal status by age group for boys and girls

n (%)	Boys				Girls			
Age (y)	Pre-	Intra-	Post-	Total	Pre-	Intra-	Post-	Total
9	104 (12.9)	143 (17.8)	1 (0.1)	248 (30.8)	117 (14.1)	143 (17.3)	0 (0)	260 (31.4)
13	5 (0.6)	262 (32.6)	2 (0.3)	269 (33.5)	1 (0.1)	227 (27.4)	7 (0.9)	235 (28.4)
16	0 (0)	280 (34.8)	7 (0.9)	287 (35.7)	0 (0)	211 (25.5)	122 (14.7)	333 (40.2)
Total	109 (13.6)	695 (85.2)	10 (1.2)	804 (100)	118 (14.2)	581 (70.2)	129 (15.6)	828 (100)

Appendix 8

Adiponectin concentration distribution by Center for Disease Control BMI category: Normal, overweight, obese

An interaction between adiponectin concentration and adiposity was shown as in the Figure. Therefore, adiponectin distributions by BMI category may be important in assessment of risk of insulin resistance or diabetes or other metabolic conditions. Therefore, the tables below show selected percentiles for adiponectin concentration by sex, age and Center for Disease Control BMI categories – normal weight (BMI < 85th percentile), overweight (BMI ≥85th percentile and < 95th percentile) and obese (≥95th percentile). Because of the small sample sizes for overweight and obese boys and girls, only the 25th, 50th and 75th percentiles are presented for these groups.

Table 14. Adiponectin distribution by sex and BMI category for 9 year-olds

%ile (95% CI)	Boys				Girls		
	Normal *n=150	Overweight *n=28.1	Obese *n=13.6		Normal *n=146.4	Overweight *n=22.2	Obese *n=15.7
5th	5.8 (4.6-8.0)				6.5 (3.5-9.3)		
10th	6.6 (4.7-8.8)				7.4 (5.1-10.1)		
25th	8.5 (6.2-10.9)	9.1 (6.4-11.3)	6.7 (5.2-8.7)		9.6 (6.9-12.2)	9.0 (5.4-11.1)	6.9 (4.9-9.8)
50th	10.9 (8.5-13.8)	11.4 (9.1-14.1)	8.7 (6.7-10.1)		12.3 (9.6-15.9)	11.7 (9.0-13.0)	9.8 (6.9-11.2)
75th	13.8 (10.9-17.9)	14.1 (11.4-16.8)	10.1 (9.1-15.6)		15.9 (12.3-21.0)	13.0 (11.7-14.8)	11.2 (9.8-14.3)
90th	16.9 (13.3-25.7)				19.6 (15.2-25.2)		
95th	18.4 (14.5-29.6)				21.8 (16.7-25.9)		

*n is the effective sample size after applying design effects and sampling weights

Table 15. Adiponectin distribution by sex and BMI category for 13 year-olds

%ile (95% CI)	Boys				Girls		
	Normal *n=159.4	Overweight *n=26.5	Obese *n=22.3		Normal *n=140	Overweight *n=23.2	Obese *n=17.9
5th	4.6 (2.8-6.5)				6.1 (4.2-8.3)		
10th	5.1 (3.1-7.3)				7.1 (4.4-9.5)		
25th	6.8 (4.7-8.9)	5.6 (4.1-7.6)	6.2 (4.4-7.6)		8.7 (6.7-11.3)	6.9 (5.4-8.0)	6.6 (4.6-8.3)
50th	8.9 (6.8-11.2)	7.6 (5.6-10.3)	7.6 (6.2-8.3)		11.5 (8.8-14.0)	8.0 (6.9-9.4)	8.3 (6.6-9.8)
75th	11.2 (9.0-15.2)	10.6 (7.6-12.2)	8.3 (7.6-12.7)		14.0 (11.5-18.4)	9.6 (8.0-12.5)	9.8 (8.3-15.4)
90th	14.4 (10.8-22.9)				17.1 (13.5-23.0)		
95th	15.6 (11.8-23.7)				18.4 (14.6-24.7)		

*n is the effective sample size after applying design effects and sampling weights

Table 16. Adiponectin distribution by sex and BMI category for 16 year-olds

%ile (95% CI)	Boys				Girls		
	Normal *n=193.7	Overweight *n=30.5	Obese *n=27.7		Normal *n=200.8	Overweight *n=38.6	Obese *n=13.4
5th	4.7 (2.7-6.0)				5.7 (2.2-8.0)		
10th	5.2 (2.8-6.9)				6.8 (3.2-8.7)		
25th	6.5 (5.0-8.3)	5.0 (3.7-7.6)	4.7 (3.6-6.1)		8.3 (6.3-10.2)	7.1 (5.5-8.5)	5.9 (5.0-7.3)
50th	8.3 (6.5-10.3)	7.6 (5.0-8.5)	6.1 (4.7-7.3)		10.3 (8.3-12.6)	8.6 (7.1-9.9)	7.3 (5.9-9.3)
75th	10.3 (8.3-13.0)	8.5 (7.6-12.9)	7.3 (6.1-11.2)		12.7 (10.4-16.0)	9.9 (8.6-13.9)	9.3 (7.3-9.8)
90th	12.4 (10.0-16.4)				15.1 (12.2-20.4)		
95th	13.7 (10.9-16.6)				16.9 (13.6-21.0)		

*n is the effective sample size after applying design effects and sampling weights

Appendix 9

Association between adiponectin level and fasting glucose

Adiponectin concentration is a predictor of incident type 2 diabetes in adults (8-10;34) and in one study has been associated with type 2 diabetes in obese children (15). This study excluded 3 participants who reported having diabetes or using insulin. Among youth, about 10 percent of those with diabetes have type 2 diabetes. Furthermore, it was not possible to determine from the questionnaire whether participants had type 1 or type 2 diabetes. Diabetes is diagnosed based on the concentration of plasma glucose either fasting or 2 hours after a 75 g oral glucose load. Fasting glucose concentrations were measured in this study. Only 2.2% of boys and 1.1% of girls had glucose values in the impaired fasting glucose range and none had undiagnosed possible diabetes according to cut-offs established by Canadian Diabetes Association (80) (See Table 17). Glucose values by age and sex are shown in Table 18. Rising glucose concentration is a precursor to development of diabetes. Although it was not possible to evaluate the association of adiponectin with diabetes, the association of adiponectin with fasting glucose concentration was assessed by hierarchical linear regression (see Table 19). Unadjusted and adjusted models showed no significant differences in mean glucose concentration for each standard deviation increase in adiponectin. Although BMI is associated with glucose, each standard deviation increment has a very small effect on glucose concentration (Model 3). None of the models explained more than 6% of variation in glucose concentration.

Adiponectin does not seem to be associated with fasting glucose concentrations in this population of youth. This may be a function of the healthy nature of this group and the narrow range of glucose values. In individuals with normally functioning pancreatic β cells, even in the setting of severe insulin resistance, glucose concentrations are maintained within a narrow range. The low R^2 values in Table 19 suggest that much of the variation of glucose in this population is random – either biologically random or due to measurement error.

Table 17. Categories of glycemia by sex

n (%)	Normoglycemic	Impaired Fasting Glucose*	Possible Diabetes*	Total
Boys	786 (97.8)	18 (2.2)	0 (0)	804 (100)
Girls	819 (98.9)	9 (1.1)	0 (0)	828 (100)

* Impaired fasting glucose is defined as fasting plasma glucose between 6.1 and 6.9 mmol/L, and possible diabetes is defined as fasting plasma glucose ≥ 7.0 mmol/L by the Canadian Diabetes Association (80).

Table 18. Glucose concentration distribution by sex and age

Sex	Age	n	Mean (mmol/L)	SD (mmol/L)	Minimum (mmol/L)	Maximum (mmol/L)
Boys	**9**	248	5.2	0.3	4.0	6.0
	13	269	5.3	0.4	3.7	6.9
	16	287	5.3	0.4	3.5	6.6
Girls	**9**	260	5.0	0.4	3.4	6.6
	13	235	5.2	0.3	4.3	6.4
	16	333	5.0	0.4	4.1	6.6

Table 19. Models showing the relationship between adiponectin and fasting glucose concentrations

	Boys n=804				Girls n=828			
Explanatory variable	**β, % * (SE)**		**p**	**Model R^2**	**β, % * (SE)**		**p**	**Model R^2**
1† **adiponectin, 1 SD**	-0.02	(0.01)	0.16	0.002	-0.02	(0.01)	0.07	0.004
2‡ **adiponectin, 1SD**	-0.02	(0.01)	0.21	0.041	-0.02	(0.01)	0.053	0.047
3‡ **adiponectin, 1SD**	0.02	(0.01)	0.053	0.053	-0.01	(0.01)	0.31	0.054
BMI, 1SD	0.04	(0.01)	0.001		0.04	(0.01)	0.007	
4‡ **adiponectin, 1SD**	0.02	(0.01)	0.047	0.055	-0.01	(0.01)	0.32	0.059
BMI, 1 SD	0.05	(0.01)	0.0003		0.05	(0.01)	0.04	
adiponectin x BMI	0.02	(0.01)	0.04		-0.02	(0.01)	0.07	

†, Model 1 – unadjusted.
‡, Models 2, 3 and 4 – adjusted for age, pubertal status, physical activity score and smoking.
* β refers to the absolute difference of mean glucose concentration (mmol/L) for an increase of one (1) age and sex-specific SD of adiponectin, or BMI. For the interaction term in model 4, β may be interpreted as the incremental change in $\beta_{\text{BMI Z-score}}$ for every 1 SD increase in adiponectin or vice versa.

Appendix 10

List of abbreviations

ARIC	Atherosclerosis Risk in Communities Study
BMI	Body mass index
CARDIA	Coronary Artery Risk Development in Young Adults Study
CDC	Center for Disease Control
CI	Confidence interval
CT	Computed tomography
CV	Coefficient of variation
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray absorptiometry
ELISA	Enzyme-linked immunosorbent assay
FSIVGTT	Frequently sampled intravenous glucose tolerance test
HOMA-IR	Homeostasis Model Assessment of insulin resistance
IGT	Impaired glucose tolerance
IR	Insulin resistance
MRI	Magnetic resonance imaging
NGT	Normal glucose tolerance
NHANES	National Health And Nutrition Examination Survey
OR	Odds ratio
PPAR-γ	Peroxisome proliferator activated receptor gamma
QCAHS	Quebec Child and Adolescent Health and Social Survey
RIA	Radioimmunoassay
SD	Standard deviation
SE	Standard error of the mean
SSKF	Subscapular skinfold thickness
TNF-α	Tumour necrosis factor alpha
TSKF	Triceps skinfold thickness
US	United States of America