Apolipoprotein B48: a novel marker of metabolic risk in overweight children?

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Abstract

Clinical studies in adults indicate there is a positive and significant association between insulin resistance, dyslipidaemia, fasting intestinally derived lipoproteins (via apoB48 (apolipoprotein B48)) and visceral fat. All of these factors contribute to increased risk of CVD (cardiovascular disease). Since little is known about postprandial dyslipidaemia in overweight children, we sought to compare fasting levels of apoB48 with the HOMA-IR (homeostasis model assessment of insulin resistance) score, classic lipid profile and VAT (visceral adipose tissue). Pre-pubertal, overweight boys and girls were recruited from the wider-Edmonton area (Alberta). Body composition was determined using both dual-energy X-ray absorptiometry and MRI (magnetic resonance imaging). Fasting apoB48 was quantified in plasma using an adapted SDS/PAGE immunoblotting technique, and insulin, glucose, TC (total cholesterol), TAG (triacylglycerol), LDL (low-density lipoprotein) and HDL (high-density lipoprotein) were determined by calorimetric assay. In this overweight sample, we observed elevated fasting apoB48 concentrations, greater than the normal adult range. In addition, apoB48 was significantly related to HOMA-IR and TAG levels. Although apoB48 was positively correlated with TC and LDL and negatively associated with HDL, these relationships did not achieve significance. Our ongoing MRI analysis reveals a positive relationship between apoB48 and VAT volume. To our knowledge, this is the first study to report apoB48 concentrations in overweight pre-pubertal children. Thus this article will provide a brief rationale for our study and its methodology.

Introduction

In the last 25 years, the percentage of overweight children (6–11 years old) within the Western world has increased substantially. For example, within Canada, the proportion of obese pre-pubertal children has jumped from 1% to 8% [1]. Clearly, this trend is a cause for concern due to the intimate association of childhood obesity with the adult development of T2DM (Type 2 diabetes mellitus), CVD (cardiovascular disease), hypertension, myocardial infarction, kidney disease and cancer [2]. It is believed that insulin resistance, dyslipidaemia and metabolic perturbations linked to obesity are key risk factors for T2DM and CVD [3]. Furthermore, clinical studies indicate there is a positive and significant association between obesity, dyslipidaemia and novel lipid sub-fractions including, intestinally derived chylomicrons (via apoB48 (apolipoprotein B48)) [4]. In adults, elevated concentrations of plasma chylomicrons (via apoB48) are associated with the development of atherosclerosis and cluster with other known risk factors such as excess weight and insulin resistance [5]. However, to date, there have been no reports regarding the contribution of chylomicrons to CVD risk in young children. As well, the impact of insulin resistance and obesity, specifically, VAT (visceral adipose tissue), is unclear. Our ongoing research, as part of a larger study at the University of Alberta, is attempting to provide a significant contribution to the field, in that we are (i) determining fasting plasma apoB48 associated chylomicron concentrations, (ii) measuring VAT volume, and (iii) evaluating the relationship between apoB48 and VAT in a sample of overweight pre-pubertal children.

ApoB48

Chylomicron particles are secreted from the intestine and function to transport dietary fat in the circulation. Inherently, the corresponding concentration of chylomicron particles in plasma depends upon the prandial state of the individual [6]. During the fasting state, native chylomicrons are both produced in and secreted by the intestine at a basal rate [6]. Increased secretion of chylomicrons becomes stimulated upon ingestion of a fat-containing meal. Furthermore, there is now emerging evidence that both the production and secretion of chylomicron particles may be up-regulated during disease states [7]. The concentration of chylomicron particles during the postprandial phase typically peaks between 3 and 5 h. Under normal conditions, native chylomicrons are rapidly hydrolysed within the circulation by lipoprotein lipase, releasing TAG (triacylglycerol), free (non-esterified) fatty acids and free cholesterol, becoming

Key words: apolipoprotein B48 (apoB48), cardiovascular disease, chylomicron, diabetes, dyslipidaemia, obesity.

Abbreviations used: apoB48, apolipoprotein B48; CVD, cardiovascular disease; MRI, magnetic resonance imaging; T2DM, Type 2 diabetes mellitus; TAG, triacylglycerol; VAT, visceral adipose tissue.

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smaller dense chylomicron-remnants. For some individuals (particularly those with a risk of CVD, insulin resistance and/or high visceral fat volumes), chylomicrons are poorly hydrolysed and their clearance via hepatic receptor-mediated pathways can be delayed [8]. Clinically, this has become pertinent for individuals who have concentrations of LDL (low-density lipoprotein) cholesterol within the normal range, yet have persistent and substantial impairment in the metabolism and clearance of chylomicrons and their remnants [9]. The etiological significance of delayed clearance of chylomicron remnants is that these particles have been shown to become entrapped and accumulate within in the intima of arterial vessels [10]. Moreover, we have shown that chylomicron remnants can be preferentially retained relative to other lipoprotein fractions and can contribute substantially to the intimal deposition of cholesterol [11]. Consequently, in the context of childhood obesity, chylomicron concentration (via apoB48 detection) offers a unique opportunity to better understand the potential risk of CVD.

In humans, apoB48 is associated exclusively with intestinal chylomicron particles and is reflective of chylomicron concentration in the plasma. Over the years there have been several methods used to separate and quantify plasma apoB48 concentration. The 1.063 g/ml plasma density fraction is often used and apoB48 is typically isolated via SDS/PAGE and measured via classic densitometric quantification. An ELISA method has also been employed for determining apoB48 concentrations [12]. More recently, investigators from Australia have designed a unique breath test that can conveniently estimate postprandial apoB48 concentrations [13]. Along with others, our own group has relied on accurate determinations of apoB48 directly from plasma, using an adapted SDS/PAGE immunoblotting technique [14]. We have found this technique to be sensitive and accurate, ensuring complete determination of total apoB48 (as opposed to partial density fraction estimations). Moreover, we have recently validated this methodology to be applicable for both human and animal apoB48 detection.

**Visceral adipose tissue**

Indicators of general body fatness measures such as BMI (body mass index) are positively correlated with total and regional body fat. However, more specific measures of body fat are useful when examining the relationship between adiposity and metabolic risk factors for T2DM and CVD. VAT has emerged as a considerably stronger predictor of morbidity and mortality compared with BMI [15]. VAT refers to adipose tissue found in three body cavities: thoracic, intra-abdominal and intrapelvic [16]. It is a highly complex endocrine organ, secreting a wide variety of adipocytokines, involved in satiety, energy expenditure, lipid metabolism and inflammation [17]. The metabolic role of adipose tissue is poorly understood, particularly our understanding of how it may affect intestinal regulation. We know that elevated levels of abdominal visceral fat are linked to insulin resistance and dyslipidaemia in children [18]. Our general approach is to better appreciate the intestinal–cytokine–adipose axis in the regulation and development of early obesity.

Our understanding of adipose tissue and its role in regulating metabolism has changed dramatically over the last few years, similarly, the methodology used to measure adipose tissue has (and continues to) evolve rapidly. While adipose tissue can be indirectly assessed using waist circumference, a direct measure requires either computer tomography or MRI (magnetic resonance imaging). Typically, paediatric studies analyse a single cross-sectional slice or a subsection of the abdomen to represent VAT. Although this methodology correlates reasonably well with whole body measures of adiposity [19], single cross-sectional images of the abdomen may not truly reflect total VAT volume [20]. Few published reports have quantified VAT volume because it is technically challenging, labour and time intensive. In our study, we have elected to use MRI because it is a safe and accurate modality for quantifying VAT. Moreover, we examined contiguous slices from T11 (thoracic vertebra 11) to the top of the femoral heads, a range that encompasses the intra-abdominal and intrapelvic adipose tissue. We believe that measurement of total VAT volume will provide a comprehensive assessment of the fat depot and will allow us to examine the specific role of VAT in relation to insulin resistance and dyslipidaemia.

**Conclusion**

Within a sample of overweight, pre-pubertal children, we have observed elevated fasting apoB48 concentrations that exceed the normal values for adults. In our sample, apoB48 is significantly and positively correlated with TAG and the HOMA-IR (homeostasis model assessment of insulin resistance) score. Moreover, our MRI analysis has revealed a positive relationship between apoB48 and VAT volume, particularly in overweight boys. These preliminary findings suggest that apoB48 may be an important clinical indicator of CVD risk in overweight, pre-pubertal populations. We hope to expand our database and broaden our investigations to include both overweight and non-overweight boys and girls. This in turn will enable us to better understand the relationship between VAT volume and apoB48 and the role of the intestinal–cytokine–adipose axis in CVD during early life.

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