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Predictors of postprandial glycaemia, insulinaemia and insulin resistance in adolescents

Ryan A. Williams, Karah J. Dring, Simon B. Cooper*, John G. Morris, Caroline

Sunderland, Mary E. Nevill

Exercise and Health Research Group; Sport, Health and Performance Enhancement (SHAPE) Research Centre; Department of Sport Science; Nottingham Trent University, Nottingham, UK

Corresponding Author: Dr Simon Cooper

Exercise and Health Research Group,
Sport, Health and Performance Enhancement (SHAPE) Research Centre,
Department of Sport Science,
Nottingham Trent University,
Nottingham,
NG11 8NS,
United Kingdom.
Telephone: +0044 (0)115 848 8059
Email: simon.cooper@ntu.ac.uk

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Abstract

Postprandial glycaemia and insulinaemia are important risk factors for type 2 diabetes. The prevalence of insulin resistance in adolescents is increasing, but it is unknown how adolescent participant characteristics such as BMI, waist circumference, fitness and maturity offset may explain responses to a standard meal. The aim of the present study was to examine how such participant characteristics affect the postprandial glycaemic and insulinaemic responses to an ecologically valid mixed meal. Data from the control trials of three separate randomised, crossover experiments were pooled, resulting in a total of 108 participants (52 boys, 56 girls; age: 12.5 ± 0.6 y; BMI: 19.05 ± 2.66 kg·m⁻²). A fasting blood sample was taken for the calculation of fasting insulin resistance, using the HOMA-IR model. Further capillary blood samples were taken before and 30-, 60- and 120-min after a standardised lunch, providing 1.5 g·kg⁻¹ body mass of carbohydrate, for the quantification of blood glucose and plasma insulin total area under the curve (tAUC). Hierarchical multiple linear regression demonstrated significant predictors for plasma insulin tAUC were waist circumference, physical fitness and HOMA-IR ($F_{(3, 98)}=36.78$, $p<.001$, Adj. $R^2=.515$). The variance in blood glucose tAUC was not significantly explained by the predictors used ($F_{(7, 94)}=1.44$, $p=.198$). Significant predictors for HOMA-IR were BMI and maturity offset ($F_{(2, 102)}=14.06$, $p<.001$, Adj. $R^2=.021$). In summary, the key findings of the study are that waist circumference, followed by physical fitness, best explained the insulinemic response to an ecologically valid standardised meal in adolescents. This has important behavioural consequences because these variables can be modified.

1. Introduction

Insulin resistance and reduced glucose tolerance are typically implicated in the aetiology of type 2 diabetes⁽¹⁾, with an increasing degree of insulin resistance in young people⁽²⁾. Furthermore, the development of insulin resistance and type 2 diabetes in children and adolescents is associated with an increased risk of a number of co-morbidities, such as cardiovascular disease, in later life^(3,4). Therefore, due to the potential concern for metabolic health across the lifespan, it is important to understand the factors that affect insulin resistance and glucose tolerance in young people. The postprandial response to an ecologically valid meal is an important marker of cardiometabolic health in young people and favoured over the more typically cited fasting markers⁽⁵⁻⁷⁾. However, the factors that affect the magnitude of the postprandial glycaemic and insulinaemic response in young people are not well understood.

There are many risk factors associated with the development of type 2 diabetes, some of which can be easily modified through lifestyle behaviour change⁽⁸⁾. One of the contributing factors to the stark increase in the prevalence of type 2 diabetes is weight status; particularly central adiposity. This can be assessed in various ways (such as, waist circumference, sum of skinfolds and BMI) and is considered an important risk factors for the development of insulin resistance and, subsequently, type 2 diabetes^(2,9,10). Sex and pubertal status are also other risk factors during childhood (up to 11 years old) and adolescence (11 – 18 years old), given that there is a degree of pubertal insulin resistance, which may be of greater magnitude in females⁽¹¹⁻¹³⁾; thus it is particularly important to understand the association between risk factors of insulin resistance during adolescence, which has not been explored to date. Low physical activity and physical fitness are risk factors for the development of type 2 diabetes⁽¹⁴⁾ in adults and are also linked with poor cardiometabolic health in children and adolescents⁽¹⁵⁾.

Traditionally, fasting glucose and insulin concentrations are commonly used in models of insulin resistance; the most common being the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)⁽²⁾. However, it has been argued that the use of such measures do not appropriately screen for related conditions, like type 2 diabetes⁽⁵⁻⁷⁾. Furthermore, HOMA-IR typically reflects hepatic insulin sensitivity and does not account for peripheral insulin sensitivity^(16,17). Instead, the use of a dynamic assessment of postprandial glycaemia and

insulinaemia have been suggested, as a more sensitive marker of cardiometabolic health given that young people spend most of awake time in the postprandial state.

One such method of assessing the postprandial glycaemic and insulinaemic response is the Oral Glucose Tolerance Test (OGTT)⁽¹⁷⁾, whereby glucose and insulin concentrations are determined at 0, 30, 36 and 120 min following a standard glucose load (75 g), which has been used in adolescents previously^(18,19). Recent work in adolescents has examined the responses to mixed-meals^(20–22), providing ecological insights about the responses to regularly consumed meals. Furthermore, assessment of postprandial insulinaemia is an applicable tool for identifying early insulin resistance in healthy, asymptomatic individuals⁽⁷⁾.

Adiposity is a well-known risk factor for the development of insulin resistance and type 2 diabetes^(2,9,10), but there is very little known about how adiposity affects postprandial responses in adolescents. A direct comparison of overweight/obese and normal weight adolescents, using BMI, found that those who were overweight/obese had a larger insulinaemic response to a standardised meal⁽²²⁾. This study, however, only considered BMI as a proxy of adiposity, and did not consider the measure of waist circumference which is the preferred measure of adiposity when considering cardiovascular disease risk⁽²³⁾. Future work should consider the discriminatory capabilities of multiple makers of adiposity and how these affect postprandial responses.

It has been suggested that physical fitness and fasting insulin resistance are inversely related in adolescents^(24–26). In addition, physical fitness is also inversely related to blood lipids and low-grade chronic inflammation in adolescents^(24,27), and metabolic syndrome incidence in adults⁽²⁸⁾. It has been reported in one study that higher physical fitness in young people (aged 7 to 15 y), assessed by time taken to complete a 1.6 km run, is inversely related to insulin resistance (assessed via HOMA-IR) in adulthood⁽²⁵⁾. It is worth noting, however, that this relationship was weaker when adjusting for childhood waist circumference, thus highlighting the importance of adiposity for metabolic health. However, no studies to date have examined whether physical fitness affects postprandial glycaemia and insulinaemia in adolescents, despite the importance of physical fitness for other risk factors for cardiometabolic health^(24,27).

Therefore, the aim of the present study is to explore the factors affecting the postprandial glycaemic and insulinaemic responses in adolescents, including an examination of the interaction between factors known to affect these responses, such as sex and adiposity. In

addition, the study will consider how physical fitness influences postprandial responses which is a completely a novel area of enquiry in adolescents.

2. Methods

2.1 Experimental Design

Data from three separate studies^(18,19, Williams et al., unpublished), with identical designs, were pooled to examine the postprandial responses to lunch. Each of the involved studies conformed to the Declaration of Helsinki guidelines and were approved by the Nottingham Trent University Human Ethics Committee. Participants were recruited from secondary schools in the East Midlands area of the UK. Written parental consent and participant assent were obtained during recruitment. A health screen was completed by a parent/guardian of the participant and checked by a lead investigator to ensure there were no medical conditions that would affect the child's participation. Participants were familiarised with all testing procedures at least 7 d in advance of the main experimental trial. Participants were instructed to refrain from eating or drinking from 9 pm the previous evening. Water was allowed *ad libitum*. Participants were also asked to refrain from physical activity in the 24 h preceding main trials. Participants reported to school at the beginning of the day (between 08:00 am and 08:30 am) and all procedures took place in a classroom at the school.

2.2. Participant Characteristics

2.2.1 Anthropometric Measurements

In total, the dataset comprised of 108 participants (52 boys) (Table 1). Participants underwent anthropometric measurements, consisting of stature (cm), body mass (kg) and sitting height (cm); which were used to calculate age at peak height velocity⁽²⁹⁾, which was subtracted from chronological age, in order to establish maturity offset. Height was measured with a Leicester Height Measure (Seca, Hamburg, Germany) accurate to 0.1 cm and body mass was measured using a Seca 770 digital scale (Seca, Hamburg, Germany) accurate to 0.1 kg. For descriptive purposes, participants are classified as normal weight, overweight or obese based on age- and sex-specific cut-points⁽³⁰⁾. Waist circumference was measured at the narrowest abdominal point, between the lower margin of the lowest palpable rib and the iliac crest, to the nearest 0.1 cm⁽²³⁾. Four skinfold sites were measured (triceps, subscapular, supraspinale and front thigh) as a surrogate of body composition. All measurements were repeated twice, on the right-hand side of the body, using the average of the two unless the

measured differed by 5% or more; in which case a third measure was taken and the median value used. The sum of the four skinfold thickness scores has been used as a marker of adiposity in previous research in this population^(20,21).

2.2.2 Assessment of Cardiorespiratory Fitness

In each study, assessment of physical fitness was assessed using the multi-stage fitness test⁽³¹⁾. Briefly, the test required participants to complete progressive 20 m shuttle runs until volitional exhaustion. The multi-stage fitness test begins at a speed of 8.0 km·h⁻¹ (level 1), increases to 9.0 km·h⁻¹ (level 2) and then by 0.5 km·h⁻¹ for every subsequent level completed. To ensure maximum effort from the participants, participants were 'paced' by a member of the research team and investigators provided verbal encouragement and maximum heart rate was monitored continuously (Firstbeat Technologies Ltd, Finland). Performance on the test was determined by the total distance covered (m) (Table 1).

****Table 1 here****

2.3 Experimental Procedures

2.3.1 Standardised Breakfast and Lunch

On the morning of the trials (~9.00 am), a standardised breakfast was provided; which provided 1.5 g.kg⁻¹ body mass of carbohydrate (cornflakes, milk, white toast and butter). The standardised lunch (the test meal) was provided 3 h post-breakfast (~12.00 noon) and contained 1.5 g.kg⁻¹ body mass of carbohydrate (chicken sandwich, baked crisps and an apple; with a cheese alternative for vegetarians (n = 2 participants had the cheese alternative)) (Table 2). Participants were given 15 min to consume breakfast and lunch. The postprandial period (2 h) started on the first mouthful of lunch⁽³²⁾.

****Table 2 Here****

2.3.2 Capillary Blood Samples

Capillary blood samples were preferred over venous samples due to ethical constraints in young people and have been used successfully previously in this population^(20,21). A fasting capillary blood sample was taken upon arrival at school. For the postprandial period, a baseline (pre-lunch) blood sample was taken at ~ 12 noon (always exactly 3 h post-breakfast), with additional blood samples at 30, 60 and 120 min post-lunch to represent the postprandial period.

In order to increase capillary blood flow, participants' hands were warmed via submersion in warm water prior to collection. A unistik single-use lancet (Unistik, Extra, 21G gauge, 2.0 mm depth, Owen Mumford Ltd, UK) was used and the blood collected into a 300 µl EDTA coated microvette (Sarstedt Ltd, UK). A single 25 µl whole blood sample was also collected using a pre-calibrated glass pipette (Hawksley Ltd, UK) and immediately deproteinised in 250 µl ice-cooled 2.5% perchloric acid, in 1.5 ml plastic vials. Both samples were then centrifuged at 1000 g for 4 min, at 4 °C (Eppendorph 5415C, Hamburg, Germany). Plasma was removed from the microvette and placed into 500 µl plastic vials for subsequent analysis. All samples were frozen immediately at -20 °C and transferred to -80 °C as soon as possible.

Blood glucose concentrations were measured in duplicate (GOD/PAP method, GL364, Randox, Ireland) and plasma insulin concentrations were measured in singular (ELISA; Mercodia Ltd, Sweden) were determined using commercially available methods and according to the manufacturer's instructions. The intra-assay coefficients of variation for the assays of blood glucose concentration and plasma insulin concentration were 2.3% and 3.2%, respectively. Blood glucose and plasma insulin total area under the curve (tAUC) following the standardised lunch was calculated (GraphPad Prism 7, GraphPad Software, USA), using methods described previously^(33,34). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as an index of insulin resistance⁽³⁵⁾. For descriptive purposes, participants were classed as "at risk" according to age and sex-specific cut-points⁽³⁶⁾.

2.4 Sample Size Justification

For multiple regression, it is recommended that sample size is a minimum of 10 participants per predictor variable⁽³⁷⁾. A maximum of 8 predictors were available, which would dictate a minimum sample size of 80 for sufficient power.

2.5 Statistical Analyses

All data were analysed using the open-source software RStudio v 1.2.1335 (RStudio Team., (2015), www.rstudio.com). A correlation matrix was created in order to evaluate multicollinearity between independent variables (sex, waist circumference, sum of skinfolds, body mass, body mass index, maturity offset, multi-stage fitness test performance and homeostatic model assessment of insulin resistance). Before analysis, waist circumference, sum of skinfolds, BMI and multi-stage fitness test performance were centred to the mean. Simple linear regression was initially conducted for each independent variable on each outcome variable (HOMA-IR, plasma insulin tAUC and blood glucose tAUC). Following

this, stepwise hierarchical multiple regression – backwards elimination – was used to develop models for each outcome variable, using the “lme4” package⁽³⁸⁾. At each stage, the independent variable that provided the lowest contribution to the model (through evaluation of SE and t-statistic) was removed and then the model was re-run.

3. Results

A total of 80 (74.1%) participants were considered normal weight, 18 (16.7%) overweight and 10 (9.3%) obese. Furthermore, 34 (31%) participants were considered “at risk” of insulin resistance, as calculated by HOMA-IR.

3.1 Multicollinearity between independent variables

Independent variables were assessed for multicollinearity prior to conducting the hierarchical multiple regression, the results of which are shown in Table 3. There was a strong correlation between BMI and body mass; which is not surprising given that body mass is used in the calculation of BMI. Therefore, these variables cannot be considered independent and thus, body mass was excluded from subsequent analyses. All other variables did not demonstrate strong correlations ($r < .90$) and were thus included in the models.

Table 3 Here

3.2 Plasma Insulin Total Area Under the Curve

3.2.1 Predictors Individually

Simple linear regression models for insulin tAUC, with each independent variable separately, can be seen in Table 4. Waist circumference was the strongest individual predictor, explaining 37.7% of the insulin tAUC variance ($p < .001$). BMI ($p < .001$, Adj. $R^2 = .330$), sum of skinfolds ($p < .001$, Adj. $R^2 = .287$), HOMA-IR ($p < .001$, Adj. $R^2 = .292$) and multi-stage fitness test performance ($p < .001$, Adj. $R^2 = .139$) were all significant individual predictors of plasma insulin tAUC. Sex ($p = .707$, Adj. $R^2 = -.008$) and maturity offset ($p = .079$, Adj. $R^2 = .020$) did not affect plasma insulin tAUC.

Table 4 Here

3.2.2 Final Model Development

The hierarchical regression (stepwise, backwards elimination) step-by-step process can be seen in Table 5. The final model (step 5) contained waist circumference, multi-stage fitness

test performance and HOMA-IR as predictors, explaining 51.5% of the variance in plasma insulin tAUC ($F_{(3, 98)} = 36.78$, $p < .001$, Adj. $R^2 = .515$). The model suggests that: for a 1 cm increase in waist circumference, insulin tAUC would increase by $921 \text{ pmol}\cdot\text{L}^{-1} \times 120 \text{ min}$ (95% CI; 564, 1278); for a 20 m increase in distance ran during the multi-stage fitness test, insulin tAUC would decrease by $6 \text{ pmol}\cdot\text{L}^{-1} \times 120 \text{ min}$ (95% CI; -12, -1); and for a 1 AU increase in HOMA-IR, the model suggests that insulin tAUC would increase by $6046 \text{ pmol}\cdot\text{L}^{-1} \times 120 \text{ min}$ (95% CI; 3595, 8497).

Table 5 Here

3.3 Blood Glucose Total Area Under the Curve

3.3.1 Predictors Individually

None of the available predictors provided a significant contribution to explaining the variance in blood glucose tAUC, individually (Table 6).

Table 6 Here

3.3.2 Final Model Development

The initial model (step 1) including all predictors did not provide sufficient explanation for the variance (3%) in blood glucose tAUC ($F_{(7, 94)} = 1.44$, $p = .198$). As no predictors significantly explained any variance in blood glucose tAUC individually, or in the hierarchical model, the backwards elimination process was terminated at step 1.

3.4 HOMA-IR

3.4.1 Predictors Individually

Simple linear regression models for HOMA-IR, with each independent variable separately, can be seen in Table 7. BMI was the strongest predictor for HOMA-IR; explaining 17.5% of the variance ($p < .001$). Waist circumference ($p < .001$, Adj. $R^2 = .153$), sum of skinfolds ($p = .008$, Adj. $R^2 = .057$), multi-stage fitness test performance ($p = .033$, Adj. $R^2 = .035$) and maturity offset ($p = .004$, Adj. $R^2 = .068$) also provided a significant contribution to the variance in HOMA-IR. Sex did not significantly explain variance in HOMA-IR ($p = .284$, Adj. $R^2 = .002$).

Table 7 Here

3.4.2 Final Model Development

The hierarchical regression (stepwise, backwards elimination) step-by-step process can be seen in Table 8. The final model containing BMI and maturity offset as independent variables (step 5; $F_{(2, 102)} = 14.06$, $p < .001$, $\text{Adj } R^2 = .201$) explaining 20.1% of the variance in HOMA-IR. Specifically, the model suggests that for each additional $1 \text{ kg}\cdot\text{m}^{-2}$ increase in BMI, HOMA-IR would increase by 0.14 AU; for each 1 y increase in maturity offset, HOMA-IR would increase by 0.17 AU.

Table 8 Here

4. Discussion

The main findings of the present study are that in adolescents: i) the combination of waist circumference, performance on the multi-stage fitness test and HOMA-IR collectively explained 51.5% of variance in the postprandial insulinaemic response to a standardised mixed-meal; ii) none of the independent variables (BMI, body mass, waist circumference, MSFT, sum of skinfolds, sex, maturity offset and HOMA-IR) explained the variance in the postprandial glycaemic response; iii) BMI and maturity offset collectively explained 20.1% of the variation in HOMA-IR. These findings highlight the importance of body composition, particularly central adiposity, in explaining the insulinaemic response to a standardised mixed meal in adolescents. Furthermore, the present study also highlights that physical fitness is an important explanatory variable when considering the postprandial insulinaemic response in adolescents.

The findings of the present study are novel because no study to date has investigated the factors affecting the postprandial glycaemic and insulinaemic responses in adolescents, which are recognised as important risk factors for cardio-metabolic disease⁽⁵⁻⁷⁾. Furthermore, most waking hours are spent in a postprandial state, therefore it seems logical to examine postprandial responses when evaluating an individual's metabolic function. Although glycaemia has potential clinical use for screening of disease prevalence and risk, there have been some arguments that more attention should be focused on postprandial insulinaemia^(5,7). Furthermore, we hypothesise that the changes in postprandial insulinaemic responses manifest earlier in the progression of cardiometabolic diseases than the postprandial glycaemic responses and should therefore be examined in young people. The present study provides novel evidence that waist circumference, physical fitness and HOMA-IR are key predictors of this postprandial insulinaemic response in adolescents. These novel findings

provide further evidence that more consideration should be given to the assessment of postprandial insulinaemia, alongside glycaemia, as a risk factor for metabolic health⁽⁵⁻⁷⁾, which highlights the utility of this marker for future research.

Out of all the explanatory variables, waist circumference provided the strongest individual explanation of the variance in the postprandial insulinaemic response and was also a strong predictor in the final model. These data are supported by a group comparison of postprandial insulinaemia whereby overweight/obese adolescents (aged 14-15 y) had a greater insulin AUC compared to normal weight adolescents⁽²²⁾, as well as supporting the relationship between adiposity and insulin sensitivity over a 2 y period in children (aged 9 - 11 years)⁽³⁹⁾. Whilst previous research has identified differences in postprandial insulinaemia between young people considered overweight and normal weight, the present study offers novel insights into the relationship of adiposity on postprandial responses in adolescents. Furthermore, waist circumference was superior compared to BMI and sum of skinfolds, which are also measures of body composition, therefore highlighting the importance and utility of this particular measure. Whilst central adiposity is of great importance for cardiometabolic disease risk, the direct measurement, via dual-energy x-ray absorptiometry for example, requires expensive and specialist radiological imaging equipment⁽⁴⁰⁾. However, waist circumference is strongly advocated as a surrogate measure of central adiposity and has been associated with cardiometabolic disease risk^(23,40). This has important practical implications, given the low-cost and non-invasive nature of such a measuring waist circumference. Collectively, these results demonstrate the importance of adiposity – particularly central adiposity (as measured by waist circumference) – for cardiometabolic health in youth; which is pertinent given that central adiposity is linked to the development of insulin resistance^(2,9,10).

Another novel finding of the present study was that physical fitness (assessed by distance covered on the multi-stage fitness test) was inversely related to plasma insulin tAUC. Physical fitness is known to be beneficial for many facets of cardiometabolic health⁽²⁷⁾. However, to the authors' knowledge, no other studies have examined the relationship between physical fitness and postprandial insulinaemia. The closest comparison comes from evidence in children (aged 6-8 y) where physical fitness was inversely related to fasting insulin resistance⁽²⁴⁾. Furthermore, there is evidence of improved beta-cell function in adults with a higher physical fitness⁽⁴¹⁾, which lends support to the result of improved insulin sensitivity in participants with a higher physical fitness in the current dataset. There is also a

strong body of evidence that chronic exercise interventions improve insulin sensitivity in obese youth⁽⁴²⁾. Whilst there has been suggestion that these improvements might be due to increased capillarisation of skeletal muscle⁽⁴³⁾ and increased GLUT4 translocation⁽⁴³⁾, others have suggested that the chronic improvements are largely mediated through weight loss⁽⁴⁴⁾. Identifying a mechanism, through which physical fitness improves postprandial insulinaemia was not in the scope of the present study. However, it is interesting that physical fitness remained in the final model, even in the presence of adiposity. Nonetheless, it is important that future research investigates the mechanisms through which physical fitness leads to better postprandial insulinaemia, and whether this differs from those as a result of acute and chronic exercise. The present study is the first to show a beneficial relationship between physical fitness and postprandial insulinaemia in adolescents, suggesting that physical fitness may be a key predictor for this outcome even when considering the role of other predictors. This has important practical implications that highlight the need to promote physical fitness in youth, given the strong role it has in metabolic health.

The present study also demonstrates that HOMA-IR provides a significant explanation of the variance in postprandial insulinaemia. These data support and extend previous findings following a standardised breakfast⁽¹¹⁾ and an OGTT⁽⁴⁵⁾. Previous work has shown that HOMA-IR is positively correlated ($r = .63$) with insulin tAUC following an OGTT⁽⁴⁵⁾. This is of similar magnitude to the present study ($r = .53$), however the previous association was only applicable to adolescent boys in response to an OGTT⁽⁴⁵⁾. The present study extends this relationship to a sample of adolescent boys and girls, in response to an ecologically valid mixed-meal. Although the meals provided between the present study and previous work⁽¹¹⁾ were different, they offered the same relative energy provision ($1.5 \text{ g} \cdot \text{kg}^{-1}$ body mass of carbohydrate). Collectively, these results suggest that basal metabolic function is important for determining the physiological response to test meals. The results from the present study also suggest that an increase in HOMA-IR (higher basal insulin resistance) will lead to greater postprandial insulinaemic responses, even when other strong predictors such as waist circumference and physical fitness are controlled for.

The present study suggests that when considering fasting metabolic status (using HOMA-IR), BMI and maturity offset were the most informative explanatory variables. Independently, BMI was the stronger explanatory variable which is consistent with previous work in this population stating that adiposity has a strong predictive role in fasting measures of insulin resistance^(24,46,47), despite using different surrogate measures of adiposity. The current study

advances previous work in obese adolescents⁽⁴⁶⁾ to demonstrate that BMI is strongly related to HOMA-IR in healthy, asymptomatic (from cardiometabolic health conditions) adolescents. Maturity offset was also positively related with HOMA-IR, which is consistent with previous literature stating that there is a degree of pubertal insulin resistance during adolescence^(10–13), which is sometimes more profound in girls^(11,12). The role of maturity and sex, in the present study, seemed to only be reflected in the fasting proxy of insulin resistance, whereas previously it has been shown that girls are hyperinsulinaemic compared to boys, following the same standard meal⁽¹¹⁾. This is an interesting observation which may be indicative of potentially differential insulin resistance development during puberty, where fasting hepatic insulin resistance occurs at the earlier stages, with postprandial peripheral insulin resistance developing in the latter stages. However, there are currently no data to support this suggestion which would require the measurement of postprandial insulinaemia in adolescents at different stages of puberty, or a longitudinal follow-up throughout the course of adolescence.

The results of the present study demonstrate that the use of low-cost, non-invasive measures of adiposity and physical fitness provides a much greater explanation of variance in postprandial insulinaemia than the traditional fasting marker of metabolic health, HOMA-IR. This has important practical implications, given the invasive and costly nature of HOMA-IR, and the potential use of these measurements (especially waist circumference) in predicting postprandial insulinaemia. However, there are still other characteristics that might provide additional information about the variance in postprandial insulinaemia. Habitual physical activity is known to attenuate the puberty-related insulin resistance seen in adolescence⁽⁴⁸⁾. Furthermore, in adults matched for $\dot{V}O_{2\max}$, those with greater levels of habitual physical activity were more insulin sensitive in response to an OGTT⁽⁴⁹⁾. Given this evidence, it would be worthwhile including habitual physical activity as an explanatory variable in future work. In addition, this work could be extended by incorporating participants across the age of adolescence, which would help to identify if the relationships highlighted in the present study exist across different age groups and stages of pubertal development.

The present study has a number of limitations that need consideration. Firstly, a mixed-meal was consumed rather than a traditional OGTT. The OGTT is a valid test meal when examining postprandial responses and the consumption of a solid mixed-meal will have different gastric emptying rates compared to a drink solution, thus comparisons may be limited⁽³²⁾. However, examining the postprandial responses to a mixed-meal has been favoured in recent paediatric research given that young people spend most of awake time in

the postprandial state. The present study also used maturity offset as a marker of maturation status⁽²⁹⁾; which is based on predictive modelling using anthropometric measurements. Despite being a prediction of maturation, maturity offset is often favoured in a non-clinical setting over traditional measures (such as the Tanner scale, which examines secondary sex characteristics), which are deemed invasive⁽⁵⁰⁾. Whilst the present study included examined several relevant predictors of metabolic health, there were also a number of predictors not included (such as the habitual dietary intake and physical activity levels of participants, mode of transport to school and socioeconomic status), which should be examined in future research. Furthermore, as the present study is cross-sectional, causality between the chosen predictors and postprandial responses cannot be inferred. Finally, it is important to consider that the participants in the current study are considered healthy and asymptomatic from cardiometabolic health conditions. Indeed, it might be more appropriate to study the relationships examined in the present study in populations with increased prevalence of risk factors for cardiometabolic diseases, given they would be the target of future interventions. Nonetheless, identifying these relationships in healthy adolescents provides important information, given the role of postprandial hyperinsulinaemia in the pathophysiology of insulin resistance and related cardiometabolic health issues⁽⁵⁾ and the suggested early manifestation of such conditions⁽⁴⁾.

In conclusion, the findings of the present study demonstrate that over half of the variance in postprandial insulinaemia in response to a standard mixed-meal, in adolescents, can be explained by measurements that are frequently employed to characterise participants in paediatric exercise literature; waist circumference, multi-stage fitness test performance and HOMA-IR. Overall, measures of body composition (particularly waist circumference) were key when explaining the variance in metabolic health in this sample. These data extend previous work using different surrogates of body composition and fasting indices of insulin resistance, thus demonstrating that body composition (particularly waist circumference) is important for postprandial metabolic responses and cardiometabolic health. These findings have important practical implications, as the predictors identified are easily measurable in young people and considered modifiable. Future work should investigate additional variables that might help explain the variance in postprandial insulinaemia and glycaemia, such as habitual physical activity, and how the impact of these participant characteristics may change throughout the course of adolescence.

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Conflict of Interest

None.

Authorship

The author contributions are as follows: R.W contributed to the design of the study, the collection and analysis of the data and the writing of the manuscript. K.J.D contributed to the design of the study and the collection and analysis of the data. S.B.C contributed to the design of the study and the collection and analysis of the data. J.G.M contributed to the design of the study and collection of the data. C.S contributed to the design of the study and collection of the data. M.E.N contributed to the design of the study and the collection of the data. R.W, K.J.D, S.B.C and M.E.N drafted the manuscript. All authors read and approved the final version of the manuscript.

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6. Tables

Table 1. Participant characteristics and metabolic markers split into boys and girls. Data are presented as mean, standard deviation and range.

| Variable | Group | | | | | |
|--|---------------|-------|------------|----------------|-------|------------|
| | Boys (n = 52) | | | Girls (n = 56) | | |
| | M | SD | Range | M | SD | Range |
| <i>Characteristics</i> | | | | | | |
| Age (y) | 12.4 | 0.5 | 11.4-13.4 | 12.4 | 0.6 | 11.1-13.5 |
| Height (m) | 1.59 | 0.09 | 1.43-1.81 | 1.59 | 0.07 | 1.45-1.77 |
| Body Mass (kg) | 48.4 | 10.7 | 31.9-78.1 | 48.2 | 9.0 | 32.6-74.3 |
| BMI (kg·m ⁻²) | 19.0 | 2.7 | 14.0-24.9 | 19.1 | 2.7 | 14.1-28.3 |
| BMI Percentile | 61.2 | 29.8 | 1.2-98.6 | 52.4 | 27.3 | 0.6-99.5 |
| Maturity Offset (y) | -1.0 | 0.6 | -2.0-0.6 | 0.6 | 0.6 | -0.8-2.1 |
| Sum of Skinfolks (mm) | 48.0 | 21.8 | 14.1-102.5 | 52.4 | 21.9 | 24.0-127.0 |
| Waist Circumference (cm) | 67.4 | 6.7 | 54.5-86.4 | 66.2 | 6.8 | 53.4-92.3 |
| Multi-Stage Fitness Test Distance (m) | 1240 | 420 | 420-2160 | 1080 | 340 | 360-1740 |
| <i>Metabolic Markers</i> | | | | | | |
| Fasting Blood Glucose (mmol·L ⁻¹) | 4.5 | 0.6 | 2.6-5.7 | 4.3 | 0.7 | 2.4-6.1 |
| Fasting Plasma Insulin (pmol·L ⁻¹) | 54.1 | 28.6 | 11.3-120.0 | 59.1 | 27.1 | 13.8-138.6 |
| HOMA-IR (AU) | 1.73 | 0.92 | 0.33-3.81 | 1.93 | 0.94 | 0.43-4.01 |
| Insulin tAUC (pmol·L ⁻¹ x 120 min) | 27590 | 16419 | 9288-97148 | 28679 | 13400 | 8240-73224 |
| Glucose tAUC (mmol·L ⁻¹ x 120 min) | 587 | 73 | 453-791 | 582 | 78 | 443-791 |

Abbreviations: M = Mean. SD = Standard Deviation. BMI = Body Mass Index. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. tAUC = Total Area Under the Curve.

Table 2. Example of the standard and vegetarian options for the test meal, with energy and macronutrient breakdown, based on a hypothetical 50 kg individual.

| Ingredient | Meal Option | | | | | | | | | |
|------------------------------------|-------------|--------------------|------------------|---------|-------------|------------|--------------------|------------------|---------|-------------|
| | Standard | | Vegetarian | | | | | | | |
| | Amount (g) | Energy (kJ) [kcal] | Carbohydrate (g) | Fat (g) | Protein (g) | Amount (g) | Energy (kJ) [kcal] | Carbohydrate (g) | Fat (g) | Protein (g) |
| <i>White Bread</i> ^a | 70 | 690 [165] | 32 | 1 | 6 | 70 | 690 [165] | 32 | 1 | 6 |
| <i>Flora Original</i> ^b | 8 | 134 [32] | 0 | 4 | 0 | 8 | 134 [32] | 0 | 4 | 0 |
| <i>Chicken</i> ^c | 115 | 544 [130] | 0 | 2 | 27 | | | | | |
| <i>Cheese</i> ^d | | | | | | 34 | 556 [133] | 0 | 11 | 9 |
| <i>Baked Crisps</i> ^e | 35 | 598 [143] | 26 | 3 | 2 | 35 | 598 [143] | 26 | 3 | 2 |
| <i>Apple</i> ^f | 120 | 230 [55] | 13 | 0 | 0 | 120 | 230 [55] | 13 | 0 | 0 |
| <i>Total</i> | | 2197 [526] | 71 | 10 | 36 | | 2209 [529] | 71 | 19 | 17 |

^a White bread (Kingsmill soft white thick slice, UK)

^b Margarine (Flora Original, UK)

^c Sainsbury's roast chicken slices (Sainsbury's Ltd., UK)

^d Sainsbury's medium cheddar (Sainsbury's Ltd., UK)

^e Walkers ready salted baked crisps (Walkers, UK)

^f Braeburn apple

Table 3. Correlation matrix for all independent variables.

| | Sex | BM | BMI | MO | SumSF | WC | MSFT | HOMA-IR |
|----------------|------------|-----------|------------|-----------|--------------|-----------|-------------|----------------|
| Sex | . | | | | | | | |
| BM | .01 | . | | | | | | |
| BMI | .00 | .88** | . | | | | | |
| MO | -.80** | .38** | .21 | . | | | | |
| SumSF | -.09 | .64** | .80** | .18 | . | | | |
| WC | .09 | .85** | .88** | .20 | .76** | . | | |
| MSFT | .23 | -.31* | -.45** | -.14 | -.59** | -.38** | . | |
| HOMA-IR | -.05 | .41** | .38** | .21 | .26 | .35** | -.17 | . |

Abbreviations: BM = Body Mass. BMI = Body Mass Index. MO = Maturity Offset. SumSF = Sum of Skinfolts. WC = Waist Circumference. MSFT = Distance run on Multi-Stage Fitness Test. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance.

Holm correction for multiple testing used. * = $p < .01$. ** = $p < .001$.

Table 4. A summary of simple linear regression outputs for each variable predicting plasma insulin tAUC.

| Predictor | β_0 | β_1 | SE | <i>t</i> | <i>p</i> | R ² | Adj. R ² |
|----------------|-----------|-----------|------|----------|----------|----------------|---------------------|
| <i>Sex</i> | 28679 | -1088 | 2886 | -0.38 | .707 | .001 | -.008 |
| <i>WC</i> | 28105 | 1364 | 169 | 8.07 | <.001*** | .383 | .377 |
| <i>SumSF</i> | 28138 | 366 | 55 | 6.62 | <.001*** | .294 | .287 |
| <i>BMI</i> | 28143 | 3226 | 442 | 7.29 | <.001*** | .336 | .330 |
| <i>MSFT</i> | 44969 | -14 | 3 | -4.21 | <.001*** | .148 | .139 |
| <i>MO</i> | 28524 | 2538 | 1431 | 1.77 | .079 | .029 | .020 |
| <i>HOMA-IR</i> | 12268 | 8780 | 1324 | 6.63 | <.001*** | .299 | .292 |

Abbreviations: β_0 = Intercept. β_1 = Parameter Estimate. SE = Standard Error. WC = Waist Circumference. SumSF = Sum of Skinfolds. BMI = Body Mass Index. MSFT = Distance run on Multi-Stage Fitness Test. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. MO = Maturity Offset. * = $p < .05$. ** = $p < .01$. *** $p < .001$.

Table 5. Summary of the hierarchical regression (backwards elimination) for variables predicting plasma insulin tAUC. 95% CI are for unstandardized coefficients (**B**).

| Variable | B | SE | β | <i>t</i> | <i>p</i> | 95% CI | | Adj. R ² |
|---|-------|------|---------|----------|----------|--------|-------|---------------------|
| | | | | | | Lower | Upper | |
| <i>Step 1</i> ($F_{(7, 94)} = 15.59, p < .001$) | | | | | | | | .503 |
| Intercept | 22269 | 4826 | | | | | | |
| Sex | -1855 | 4276 | -0.06 | -0.43 | .665 | -10346 | 6635 | |
| WC | 950 | 371 | 0.43 | 2.56 | .012* | 214 | 1687 | |
| SumSF | 92 | 99 | 0.14 | 0.93 | .356 | -105 | 290 | |
| BMI | -582 | 948 | -0.10 | -0.61 | .541 | -2466 | 1301 | |
| MSFT | -4 | 3 | -0.12 | 1.30 | .197 | -11 | 2 | |
| MO | -1329 | 2104 | -0.09 | -0.63 | .529 | -5507 | 2849 | |
| HOMA-IR | 6428 | 1307 | 0.40 | 4.92 | <.001*** | 3831 | 9025 | |
| <i>Step 2</i> ($F_{(6, 95)} = 18.32, p < .001$) | | | | | | | | .507 |
| Intercept | 21975 | 4757 | | | | | | |
| WC | 883 | 335 | 0.40 | 2.63 | .009** | 217 | 1550 | |
| SumSF | 101 | 92 | 0.15 | 1.04 | .299 | -91 | 293 | |
| BMI | -569 | 944 | -0.10 | -0.60 | .548 | -2443 | 1305 | |
| MSFT | -4 | 3 | -0.13 | -1.46 | .147 | -11 | 1 | |
| MO | -550 | 1095 | -0.04 | -0.50 | .616 | -2725 | 1623 | |
| HOMA-IR | 6402 | 1300 | 0.39 | 4.92 | <.001*** | 3819 | 8985 | |
| <i>Step 3</i> ($F_{(5, 96)} = 22.10, p < .001$) | | | | | | | | .511 |
| Intercept | 22259 | 4706 | | | | | | |
| WC | 882 | 334 | 0.40 | 2.64 | .009** | 218 | 1546 | |
| SumSF | 101 | 96 | 0.15 | 1.05 | .299 | -90 | 292 | |
| BMI | -585 | 940 | -0.10 | -0.62 | .535 | -2451 | 1280 | |
| MSFT | -4 | 3 | -0.13 | -1.45 | .149 | -11 | 1 | |
| HOMA-IR | 6261 | 1265 | 0.38 | 4.95 | <.001*** | 3749 | 8773 | |
| <i>Step 4</i> ($F_{(4, 97)} = 27.70, p < .001$) | | | | | | | | .514 |
| Intercept | 22479 | 4677 | | | | | | |
| WC | 754 | 263 | 0.34 | 2.87 | .005** | 231 | 1277 | |
| SumSF | 75 | 87 | 0.11 | 0.87 | .388 | -98 | 249 | |
| MSFT | -4 | 3 | -0.13 | -1.44 | .152 | -11 | 1 | |
| HOMA-IR | 6113 | 1239 | 0.38 | 4.94 | <.001*** | 3654 | 8572 | |
| <i>Step 5</i> ($F_{(3, 98)} = 36.78, p < .001$) | | | | | | | | .515 |
| Intercept | 24326 | 4158 | | | | | | |
| WC | 921 | 180 | 0.41 | 5.12 | <.001*** | 564 | 1278 | |
| MSFT | -6 | 3 | -0.16 | -2.19 | .031* | -12 | 0 | |
| HOMA-IR | 6046 | 1234 | 0.37 | 4.90 | <.001*** | 3595 | 8496 | |

Abbreviations: B = Regression Coefficient. SE = Standard Error. β = Standardised Coefficient. WC = Waist Circumference. SumSF = Sum of Skinfolts. BMI = Body Mass Index. MSFT = Distance run on Multi Stage Fitness Test. MO = Maturity Offset. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance.

* = $p < .05$. ** = $p < .01$. *** = $p < .001$.

ΔR^2 : Step 2 = .004. Step 3 = .004. Step 4 = .003. Step 5 = .001.

Table 6. A summary of simple linear regression outputs for each variable predicting blood glucose tAUC.

| Predictor | β_0 | β_1 | SE | <i>t</i> | <i>p</i> | R ² | Adj. R ² |
|----------------|-----------|-----------|-------|----------|----------|----------------|---------------------|
| <i>Sex</i> | 578 | 8.2 | 15.12 | 0.54 | .590 | .003 | -.007 |
| <i>WC</i> | 582 | -0.1 | 1.13 | -0.09 | .930 | .000 | -.009 |
| <i>SumSF</i> | 582 | -0.2 | 0.34 | -0.62 | .537 | .004 | -.006 |
| <i>BMI</i> | 582 | 0.5 | 2.86 | 0.17 | .862 | .000 | -.009 |
| <i>MSFT</i> | 605 | -0.0 | 0.02 | -1.04 | .299 | .011 | .010 |
| <i>MO</i> | 581 | -6.6 | 7.58 | -0.87 | .386 | .007 | -.002 |
| <i>HOMA-IR</i> | 558 | 13.9 | 8.26 | 1.68 | .097 | .027 | .018 |

Abbreviations: β_0 = Intercept. β_1 = Parameter Estimate. SE = Standard Error. WC = Waist Circumference. SumSF = Sum of Skinfolds. BMI = Body Mass Index. MSFT = Distance run on Multi-Stage Fitness Test. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. MO = Maturity Offset.

Table 7. A summary of simple linear regression outputs for each variable predicting HOMA-IR.

| Predictor | β_0 | β_1 | SE | <i>t</i> | <i>p</i> | R ² | Adj. R ² |
|--------------|-----------|-----------|------|----------|----------|----------------|---------------------|
| <i>Sex</i> | 2.02 | -0.21 | 0.19 | -1.08 | .284 | .011 | .002 |
| <i>WC</i> | 1.83 | 0.05 | 0.01 | 4.44 | <.001*** | .161 | .153 |
| <i>SumSF</i> | 1.31 | 0.01 | 0.00 | 2.71 | .008** | .066 | .057 |
| <i>BMI</i> | 1.82 | 0.15 | 0.03 | 4.80 | <.001*** | .183 | .175 |
| <i>MSFT</i> | 2.48 | -0.00 | 0.00 | -2.16 | .033* | .044 | .035 |
| <i>MO</i> | 1.95 | 0.28 | 0.09 | 2.93 | .004** | .076 | .068 |

Abbreviations: β_0 = Intercept. β_1 = Parameter Estimate. SE = Standard Error. WC = Waist Circumference. SumSF = Sum of Skinfolds. BMI = Body Mass Index. MSFT = Distance run on Multi-Stage Fitness Test. MO = Maturity Offset.

* = $p < .05$. ** = $p < .01$. *** = $p < .001$.

Table 8. Summary of the hierarchical regression (backwards elimination) for variables predicting HOMA-IR. 95% CI are for unstandardized coefficients (**B**).

| Variable | B | SE | β | <i>t</i> | <i>p</i> | 95% CI | | Adj. R ² |
|--|-------|------|---------|----------|----------|--------|-------|---------------------|
| | | | | | | Lower | Upper | |
| <i>Step 1</i> ($F_{(6, 95)} = 4.88, p < .001$) | | | | | | | | .188 |
| Intercept | 1.94 | 0.32 | | | | 1.45 | 2.10 | |
| Sex | 0.15 | 0.33 | 0.08 | 0.45 | .657 | -0.52 | 0.82 | |
| WC | 0.02 | 0.03 | 0.17 | 0.82 | .413 | -0.03 | 0.08 | |
| SumSF | -0.01 | 0.01 | -0.22 | -1.18 | .242 | -0.03 | 0.01 | |
| BMI | 0.13 | 0.07 | 0.37 | 1.75 | .083 | -0.02 | 0.27 | |
| MSFT | -0.00 | 0.00 | -0.06 | -0.50 | .616 | 0.00 | 0.00 | |
| MO | 0.25 | 0.16 | 0.27 | 1.49 | .139 | -0.08 | 0.57 | |
| <i>Step 2</i> ($F_{(5, 96)} = 5.87, p < .001$) | | | | | | | | .194 |
| Intercept | 1.97 | 0.32 | | | | 1.68 | 2.01 | |
| WC | 0.03 | 0.03 | 0.21 | 1.12 | .266 | -0.02 | 0.08 | |
| SumSF | -0.01 | 0.01 | -0.24 | -1.31 | .194 | -0.03 | 0.01 | |
| BMI | 0.13 | 0.07 | 0.37 | 1.75 | .083 | -0.02 | 0.27 | |
| MSFT | -0.00 | 0.00 | -0.05 | -0.41 | .686 | -0.00 | 0.00 | |
| MO | 0.18 | 0.08 | 0.20 | 2.16 | .033* | 0.02 | 0.35 | |
| <i>Step 3</i> ($F_{(4, 100)} = 7.59, p < .001$) | | | | | | | | .202 |
| Intercept | 1.85 | 0.08 | | | | 1.69 | 2.01 | |
| WC | 0.02 | 0.03 | 0.15 | 0.83 | .409 | -0.03 | 0.07 | |
| SumSF | -0.01 | 0.01 | -0.21 | -1.37 | .175 | -0.02 | 0.00 | |
| BMI | 0.15 | 0.07 | 0.42 | 2.07 | .041* | 0.01 | 0.29 | |
| MO | 0.17 | 0.08 | 0.19 | 2.06 | .042* | 0.01 | 0.34 | |
| <i>Step 4</i> ($F_{(3, 101)} = 9.92, p < .001$) | | | | | | | | .204 |
| Intercept | 1.85 | 0.08 | | | | 1.69 | 2.01 | |
| SumSF | -0.08 | 0.01 | -0.18 | -1.22 | .224 | -0.02 | 0.01 | |
| BMI | 0.19 | 0.05 | 0.53 | 3.61 | <.001*** | 0.08 | 0.29 | |
| MO | 0.17 | 0.08 | 0.19 | 2.09 | .039* | 0.01 | 0.34 | |
| <i>Step 5</i> ($F_{(2, 102)} = 14.06, p < .001$) | | | | | | | | .201 |
| Intercept | 1.85 | 0.08 | | | | 1.69 | 2.01 | |
| BMI | 0.14 | 0.03 | 0.39 | 4.34 | <.001*** | 0.07 | 0.20 | |
| MO | 0.17 | 0.08 | 0.19 | 2.09 | .039* | 0.01 | 0.34 | |

Abbreviations: B = Regression Coefficient. SE = Standard Error. β = Standardised Coefficient. WC = Waist Circumference. SumSF = Sum of Skinfolts. BMI = Body Mass Index. MSFT = Distance run on Multi Stage Fitness Test. MO = Maturity Offset.

* = $p < .05$. ** = $p < .01$. *** = $p < .001$.

ΔR^2 : Step 2 = .006. Step 3 = .008. Step 4 = .003. Step 5 = -.004.