Symptoms of stress and depression effect percentage of body fat and insulin resistance in healthy youth: LOOK longitudinal study

Introduction

Type 2 diabetes is a common condition in today's society and an emerging health concern among children. Increased insulin resistance is a physiological precursor of type 2 diabetes (Martin et al., 1992; Taylor, 2012), and psychological factors, including depression and psychosocial stress have been associated with both insulin resistance (Kan et al., 2013; Räikkönen, Keltikangas-Järvinen, Adlercreutz, & Hautanen, 1996) and increased risk of developing type 2 diabetes in adults (Anderson, Freedland, Clouse, & Lustman, 2001; Heraclides, Chandola, Witte, & Brunner, 2009; Knol et al., 2006; Mezuk, Eaton, Albrecht, & Golden, 2008). Meta-analytic examinations undertaken with adults have shown that depression in non-diabetic populations increases the risk of developing diabetes by 37-60% (Kan et al., 2013; Knol et al., 2006; Mezuk et al., 2008), with further evidence indicating that diabetics who go on to experience depression also have a poorer prognosis and increased risk of mortality (van Dooren et al., 2013). Also among adults, stressful life events, daily hassles and work stress have been associated with both insulin resistance and type 2 diabetes (Fang, Boden, Siu, & Tseng, 2015; Heraclides et al., 2009). While the adult evidence for a causal link between psychological factors and metabolic factors is compelling, it is not fully reflected in a much smaller volume of evidence coming from studies of children and adolescents. However, the persuasive nature of the evidence from adult populations likely points to similar pathways between earlier psychological disturbance and metabolic risk in children and adolescents.

Based on a framework that draws from the adult empirical data there is a clear need for investigations among younger populations, which use strong methodology and longitudinal designs. From the existing pediatric literature, a positive association between low mood and depression with insulin resistance has been reported (e.g. Jeffery, Hyland,

Hosking, & Wilkin, 2014; Shomaker et al., 2011; Shomaker et al., 2010). However, the majority of these studies have been cross sectional, with only one longitudinal study published to date. Shoemaker et al. (2011), in their prospective study, provided evidence that earlier depression was associated with higher insulin resistance among children aged 5-13 years, although, this study's design precluded investigations of any effect of change in depression on change in insulin resistance. No longitudinal studies investigating the effect of psychosocial stress on insulin resistance in children or adolescents have been published. However, there is cross sectional evidence of an association between the homeostatic model of insulin resistance (HOMA-IR) and a range of potentially stressful experiences in adolescents, including high levels of internalized racism (Chambers et al., 2004) and low socio-economic status (SES; Goodman, Daniels, & Dolan, 2007).

In addition to these reported associations, a number of plausible pathways that may link depression and psychosocial stress to insulin resistance exist. One potential pathways is via behavioral changes often associated with poor mental health, including low physical activity and poor diet, both factors that influence body fat accumulation and impaired blood glucose control (Telford et al., 2009a; Telford, Cunningham, Telford, Riley, & Abhayaratna, 2012). Moreover, psychosocial stress and depression may have a more direct effect on increased fatness via physiological pathways (Rosmond & Bjorntorp, 2000); this in itself providing a link between psychological characteristics and insulin resistance. It is well known that increased body fat and potentially specific distribution of fat, such as intra-abdominal (visceral) fat, increase the risk for insulin resistance and type 2 diabetes in adults (Goran, Ball, & Cruz, 2003; Venables & Jeukendrup, 2009). In children and adolescents, fatness has been associated with greater insulin resistance (Freedman et al., 1987; Gutin et al., 1994) and greater insulin resistance has been documented among obese youth compared to their non-obese counterparts (Legido et al., 1989). This was the case for children of the Australian

LOOK longitudinal study, at least during late childhood between the ages of 8 and 12 years, where it was observed that a one-unit increase in percent body fat resulted in a 2.2% and 1.6% increase in HOMA-IR for boys and girls respectively (Telford et al., 2012).

Although the mechanisms explaining the link between psychosocial stress, depression and increased adiposity and insulin resistance are complex and likely to be multifactorial, pertinent to this discussion is that obesity related cytokine inflammatory responses have been associated with disruption of insulin sensitivity and pancreatic β-cell function, and contribute to the development of type 2 diabetes (Stuart & Baune, 2012; Wang, Guan, & Yang, 2010). In addition to the potential pathway mediated by adiposity, depression and stress-related disorders are thought to directly affect cell-mediated cytokine production via hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA-axis; Bao, Meynen, & Swaab, 2008) and sympathetic nervous system (SNS; Barton et al., 2007). Moreover, psychosocial stress related stimulation of the HPA-axis and SNS has been shown to result in the secretion of hormones including glucocorticoids (cortisol in humans), adrenaline, and neuropeptide Y, placing stress on blood glucose control (see Stuart & Baune, 2012). Therefore, it appears that plausible biological mechanisms exist that can potentially explain the link between psychological factors and insulin resistance, and that this is likely mediated via the stress response.

In light of this evidence, the aim of the present study was to investigate the links between psychosocial stress and depressive symptoms with insulin resistance in healthy children as they move into adolescence. This may help to inform us of the potential benefits of early psychologically based interventions targeting the prevention of metabolic dysfunction. It is hypothesized that children reporting more psychosocial stress and higher levels of depressive symptoms will also display greater insulin resistance. With obesity a known risk factor for type 2 diabetes, we also investigate the effect of stress and depression on body composition. It is hypothesized that children reporting more depressive symptoms

and perceived stress will have greater percent body fat, raising the possibility, at least in part, of an adiposity mediated effect of stress or depression on insulin resistance in youth.

Method

Participants. A summary of participant characteristics can be seen in Table 1. Children were all participants of the LOOK longitudinal study (Telford et al., 2009b). Participant numbers, based on blood collections taken at each year of measurement were 747, 490 and 247 in grade 2, grade 6 and grade 10 respectively. A similar number of participants also competed psychological, percent body fat, physical activity and puberty assessments. All data were used to quantify cross sectional (between-child) relationships for insulin resistance and psychological factors. For longitudinal relationships (within-child), our general linear mixed model adjusts for any missed measurements and so maximizes use of existing data. Children were recruited into the study via the participating school principal and parents. Initially, thirty outer suburban schools, selected for their relative homogeneity to each other and in relation to the Australian average in terms of SES were approached to participate, of which 29 schools agreed. From the 890 grade two children enrolled in these schools, 853 children with parental consent agreed to participate. At baseline, approximately 86% of the children had one or both parents of Caucasian descent, 8% of Asian descent, 3% Australian Aboriginal or Torres Strait Islander and 1% Polynesian; no data were available on 2% of the families.

Attrition. Fifteen children withdrew from the study. The remaining missed assessments were due to either absence from school on the day of assessment, relocation to a school outside the jurisdiction, technical difficulties with blood collections or inadequate compliance with test procedures, including failure to fast and an inability to make another appointment. Children who missed an assessment in a particular year remained in the study and were included in the analysis, with the statistical model adjusting for missing values.

Despite the fact that attrition was unlikely to affect relationships (in contrast with an intervention study) we compared the characteristics at baseline of those children who later left the study. These analyses revealed that children remaining in the study had significantly healthier psychological profiles (e.g. were less stressed, $p = .002$; and less depressed, $p = .013$) and were thinner ($p = .044$) than children leaving the study (or not participating in a given year). No further differences were observed.

Measures.

Psychosocial stress and depressive symptoms. Psychosocial stress was assessed using the Children's Stress Questionnaire (Byrne, Thomas, Burchell, Olive, & Mirabito, 2011). The CSQ is a 50-item self-report questionnaire that assesses the self-reported impact of a range of stressor experiences relevant to children occurring over the past 12-months. Children are asked to report their stressor experience on a 5-point Likert scale, ranging from 1 = "This didn't happen to me", 2 = "It happened to me but it didn't matter", 3 = "It made me a bit upset", 4 = "It made me quite upset"; 5 = "It made me very upset". Response items are then summed to form the CSQ Full scale, resulting in a full scale spanning 50 to 250, with higher scores indicating greater psychosocial stress. The CSQ has been shown to have good internal and test-retest reliability (Cronbach's alpha > 0.9) and both construct and predictive validity (Byrne et al., 2011).

Depression was assessed using a modified version of the Children's Depression Inventory (CDI; Kovacs, 1990), the validity and reliability of which has been tested and reported elsewhere (Byrne, Thomas, Burchell, Olive, & Mirabito, 2011; Olive, Telford, Byrne, Abhayaratna, & Telford, 2016). Modification to this scale was required to gain approval from the Jurisdiction's Department of Education. The full scale of this modified version comprised 19 items, with response choices limited to two (symptom present or

absent). This resulted in full scale score of depression that ranged from 19 to 38, with higher scores indicating greater severity of depressive symptoms.

Blood collection and the homeostasis model of insulin resistance (HOMA-IR). Morning fasting blood samples were collected at participating schools by trained phlebotomists experienced with children. Breakfast was provided to children subsequent to blood collection. Serum samples were mixed and allowed to clot for up to 30 minutes before centrifugation. Blood was not taken from children where there was doubt as to whether they had fasted or refrained from vigorous exercise; instead a new appointment was made. Samples were centrifuged on site for 10 min at 2850 rpm (Spintron GT-25P; Spintron Pty Ltd., Australia) and then either immediately frozen in ice and stored at -80°C for subsequent analysis or taken to the hospital pathology laboratories for immediate analysis. Care was taken to maximize consistency of laboratory handling of samples. All samples were subject to the same procedures, which were carried out according to instrument manufacturers' standards, and biochemical analysis was performed within acceptable limits of internal quality control. Insulin concentration was measured using microparticle enzyme immunoassay on the AXSYM (Abbott laboratories). The HOMA-IR was our surrogate of insulin resistance, where;

$$HOMA\text{-}IR = [fasting\ insulin\ (mU/L) \times fasting\ glucose\ (mmol/L)]/22.5$$

This measure has been validated for use with children (Gungor, Saad, Janosky, & Arslanian, 2004; Huang, Johnson, & Goran, 2002) and has shown to be of acceptable reliability (Keskin, Kurtoglu, Kendirci, Atabek, & Yazici, 2005). Whilst insulin sensitivity may be a more appropriate term in asymptomatic children, HOMA-IR can be compared with previous publications in children (Jeffery et al., 2007; Metcalf, Voss, Hosking, Jeffery, & Wilkin, 2008; Srinivasan, Myers, & Berenson, 2006).

Percent body fat. Percent body fat was assessed using dual energy x-ray absorptiometry (DEXA; Hologic Discovery QDR Series; Hologic Inc., Bedford, MA). All scans were performed with children wearing light clothing and total body scans were analyzed using QDR Hologic Software Version 12.4.7 to generate total lean tissue mass and fat mass from which percent body fat was calculated.

Physical activity. Physical activity was measured using New Lifestyle pedometers (Lee's Summit, MO, USA), which record the number of steps taken per day. The validity of New Lifestyle pedometers detection mechanism has been previously demonstrated in children (Beets, Patton, & Edwards, 2005). Children wore pedometers on their hip (in line with their left patella) for seven consecutive days. Measurements taken on the first day were excluded to account for the potential of artificially increased physical activity, and because they did not form a full day (24-hours). Pedometer data were collected on both weekdays and weekends and at least three days data, comprising at least two weekdays and one weekend day were required for inclusion in the analyses. A physical activity index was calculated using best linear unbiased predictor (BLUPS; Robinson, 1994) or "shrunken mean". The BLUPS index is calculated for a given individual by providing a weighted combination of the population mean and the mean for that individual. The BLUPS method was chosen for its desirable statistical properties (see Telford, Cunningham, & Telford, 2009c).

Height and weight. Height was measured by a portable stadiometer to the nearest 0.01 m and body weight by portable electronic scales to the nearest 0.05 kg. Assessments were conducted in private with individual children in either a hospital or school setting.

Pubertal Status. Pubertal maturation was assessed in the current study using the self-report Tanner stages of pubic hair, breast development, and date of menarche (Tanner, 1962) using diagrams based on those previously described (Duke, Litt, & Gross, 1980). In grade 2, the self-assessment took place at home with guidance from parents, and in grade 6 and grade

10 the self-assessment was completed in a hospital setting under the supervision of an experienced teacher.

Socio-economic status. The Australian Bureau of Statistics Socio-economic Indexes for Areas (SEIFA) was used as a measure of SES (Australian Bureau of Statistics, 2006). This index represents different aspects of relative socio-economic advantage and/or disadvantage in a geographic area, including education and occupation, and economic and social resources, as measured by the government Census. The average SES index of the suburbs in our study (1085 ± SD 40 and range 982-1160) was higher and less variable than the average and spread of all towns and cities throughout Australia (980 ± 84, 598-1251).

Procedure. Assessments for the present study were collected at three time points. Baseline assessments occurred in 2005 when children were in grade 2 (7 to 9 years), with follow-up assessments occurring four- and eight-years later in grade 6 (11 to 13 years) and grade 10 (15 to 17 years) respectively. Psychological data was collected at participating schools in class groups. Self-report measures of stress and depression were administered by a psychologist and were presented to children via a PowerPoint presentation using TurningPoint software. Children responded using hand-held keypads utilizing KEEpad interactive software, which was relayed back via wireless connection and saved on a laptop. Each individual item was read out to children and the psychologist was able to answer any queries the children may have had regarding the meaning of questionnaire items. During the administration, time anchors, such as birthdays and major school holidays were utilized to assist children with recall and to reduce the likelihood of recall bias relating to under reporting of symptoms due to failure to recall, or from over-reporting symptoms occurring outside the measurement period. Morning fasting blood samples were collected at school by nursing staff as outlined previously. Physical activity and fitness assessments were conducted in whole class groups by the same exercise scientist across each year of measurement. For the

most part, measures of psychological health, physical activity and fitness, and blood samples were collected on different days within a two month window, all occurring within the same season (summer).

Ethics approval for the study was obtained from the ACT Department of Education, the Human Research Ethics Committee (HREC) of the Australian National University, and the Ethics Committee of the Australian Sports Commission (for the entire LOOK Study). All parents gave informed and written consent for their children to participate in the LOOK study, as well as the psychological component specifically. The children also gave written consent, a condition required by the HREC of the Australian National University.

Statistical analysis. The statistical model reported here fits within the general framework of general linear mixed models (Galway, 2006) and was developed to account for the dependence structure that is a result of the current studies sampling design. This model has been previously presented (see Olive et al., 2016). The mixed model used in the present study allows for analyses of regression relationships between the response variable (e.g. insulin resistance) and candidate explanatory variables (e.g. stress and depression) at both the within-child (longitudinal) and between-children (cross sectional) level.

Our statistical model estimates effect sizes for the expected change in the response variable (e.g. insulin resistance) over time per unit change in the explanatory variable (e.g. depression), using repeated measures obtained from the same child. Restricted maximum likelihood is used to estimate variance components and weighted least squares for estimating fixed effects. Statistical significance of effects was assessed by calculating adjusted Wald statistics (Kenward & Roger, 1997). HOMA-IR was scaled by natural logarithm to better meet linearity assumptions and a physical activity index (PAI) was calculated as previously described (Telford et al., 2009c). Final models were adjusted for percent body fat (where percent body fat was not the response variable), physical activity, puberty and SES. General

model checking procedures were routinely used to identify aberrant data and to check the model assumptions. Analyses were conducted separately for boys and girls and this approach has been supported in the literature (Kurtoglu et al., 2010).

Results

A summary of participant characteristics, based on raw scores can be seen in Table 1. Participant levels of stress and depression based on mean values can be seen in Table 1. Mental health trajectories in the current cohort were characterized by a decline in stress and depression for both boys and girls between grade 2 and grade 6, followed by a significant increase in depression for both boys and girls and a significant increase in stress for girls only between grade 6 and grade 10. With the exception of stress at baseline, where girls where significantly more stressed than boys (p = .009), no further sex differences in stress or depression were found at age 8- or 12-years. However, by age 16 years, girls had significantly greater stress (p = .030) and depression (p = .028), which is consistent with previous literature (Nolen-Hoeksema, 2001).

To provide an idea of the physical health of the current cohort, by age 16 years, 13.6% of girls and 15.7% of boys had evidence of an elevated HOMA-IR of greater than 3, the suggested cut-point for risk of metabolic syndrome (Tresaco et al., 2005). Whilst suggested cut-points for HOMA-IR among pediatric populations have varied across samples (ranging from 2.5 to 4), a cut-point of 3 was chosen as characteristics of the sample used in Tresaco et al. (2005) more closely reflected the current sample compared to other studies (Aradillas-García et al., 2012; Madeira et al., 2008; Singh, Garg, Tandon, & Marwaha, 2013). This reflects a decrease from previous reports of the same cohort based on data from children at age 12 years (23% of boys and 31% of girls; Telford et al., 2012). The median HOMA-IR vales were greater in girls than boys at ages 8, 12, and 16 years respectively. In addition, girls had significantly greater percent body fat than boys at each measurement period (all p <

.001). While boys showed a small but significant increase in percent body fat between 8 and 12 years ($p < .001$), followed by a significant decrease between 12 and 16 years ($p < .001$), girls were measured with a non-significant increase in percent body fat between 8 and 12 years, and a significant increase during the transition into adolescence between 12 and 16 years ($p < .001$). However, it should be noted that as previously reported in Telford, Cunningham and Abhayaratna (2014) a plateau period was evident among this cohort between ages 10 and 12 years, with no significant change in percent body fat being observed at this time. These findings were divergent to BMI measures taken from the same children at this time, where a linear increase in BMI was observed (see Telford et al., 2014).

Notwithstanding the limitations associated with BMI in longitudinal studies with children (Telford et al., 2014), the body composition of children in the current cohort were classified according to their body mass index (BMI) to assist a comparison with other studies. Among LOOK participants included in the current investigation, by the end of grade 2 (8-years) approximately 20% of girls and 24% of boys were classified as overweight or obese; in grade 6 (12 years) this increased to 24% of girls and 25% of boy; and by grade 10 (age 16 years) 10% of girls and 7% of boys were classified as being in this category. In terms of pubertal maturations, by age 16 years, a greater proportion of girls were assessed as being further along in pubertal maturation with 27.2% of girls and 25.4% of boys self-reporting they were in stage 5 of the Tanner stages of pubertal maturation.

Effect of depression and stress on HOMA-IR

Longitudinal analyses, based on repeated measures of the same child provided evidence of a trend that increases in depressive symptoms were associated with increases in HOMA-IR for boys, although this did not reach statistical significance ($\beta = 0.011$, $p = .073$). We found no evidence for a similar effect among girls ($\beta = -0.008$, $p = .429$), however, we would expect that the decrease in IR observed among girls between age 12 and 16 years

would tend to offset any relationship between depression and IR. Similarly, we found no evidence for a longitudinal effect of stress on HOMA-IR for either boys (β = -0.001, *p* = .394) or girls (β = <0.001, *p* = .791). While this study focuses on longitudinal effects, it is still of interest that cross sectional (between-child) relationships between depression and HOMA-IR were evident among boys only (β = 0.012, *p* = .016). Associations between perceived stress and HOMA-IR were not significant at the cross sectional level for either boys (β = 0.002, *p* = .081) or girls (β = 0.001, *p* = .149). A summary of these effects can be seen in Table 2.

Effects of depression and stress on percent body fat

A significant longitudinal effect of depression on %BF was found among boys, whereby boys with greater depressive symptoms also had higher %BF (β = 0.127, *p* = .046). No evidence for a similar effect was found among girls (β = 0.048, *p* = .412), nor did we find any longitudinal effect of stress on %BF for either boys (β = -0.014, *p* = .116) or girls (β = 0.006, *p* = .504). Between-child analyses revealed a significant effect of depression on %BF for boys (β = 0.193, *p* = .011) and a significant effect of stress (β = 0.033, *p* = .020) on %BF for girls, such that children with greater stress and depression also had higher %BF.

Discussion

The current study shows that apparently healthy boys who report more symptoms of depression have higher insulin resistance. In other words, there is a statistically significant 'dose-response' relationship, whereby higher depressive symptoms are associated with higher insulin resistance, independent of adiposity. When we consider this finding along with the longitudinal trend whereby boys displaying increased depressive symptoms develop higher insulin resistance, together with the above outlined physiological mechanistic links between stress, depression and insulin resistance, the case that early depressive symptoms in boys may be the cause of this metabolic dysfunction is strengthened. Furthermore, that depressive

symptoms emerge as a significant explanator of insulin resistance independently of percent body fat (a well-established contributor to insulin resistance), our data provide support for the premise that early attention to depression per se in childhood and adolescence may reduce insulin resistance and ongoing metabolic dysfunction.

Two other avenues of support emerge for the premise that depression might have a direct effect on metabolic dysfunction. Firstly, we found that boys with higher depressive symptoms had higher percent body fat, and boys whose depressive symptoms increased became fatter. It is well established that overweight or obese children are prone to higher insulin resistance (Telford et al., 2012) and obesity is a well-established risk factor for type 2 diabetes (Bell, Kivimaki, & Hamer, 2014). Our finding that depression is linked to overweight and obesity in a dose-response manner provides further reason to suggest that early treatment of depression, even in its very earliest stage, may contribute to the prevention of chronic disease in later life. Secondly, in an earlier study of our cohort, is was reported that the lower the fitness and physical activity of a child, the higher their depression was, independent of adiposity (Olive et al., 2016), and physical inactivity, like obesity is associated with insulin resistance (Telford et al., 2009a; Telford et al., 2012). Therefore, given that a boy with depressive symptoms is more likely to be insulin resistant irrespective of adiposity; that a depressed child is more likely to be fatter and less physically active; and considering the positive relationship between depression and stress (another potential risk for insulin resistance; Kendler, Karkowski, & Prescott, 1999), there is a broad bank of evidence that symptoms of depression during childhood constitute an early and real risk for increased insulin resistance.

Consequently, our data suggest that identification and treatment of early non-clinical or clinically diagnosed depression may reduce early onset insulin resistance. In turn, given that metabolic diseases may have their roots in childhood (Ehtisham, Barrett, & Shaw, 2000),

early attention to children with depression, even in the early stages, may reduce the risk for chronic disease in later life. To date, no such intervention has been investigated among younger populations but the current findings lend support for psychological interventions that target depressive symptoms among children and adolescents as a preventative measure, or indeed in treating metabolic disorders during youth

Our findings are supported by previous work among youth, where depression was associated with greater insulin resistance in both longitudinal (Shomaker et al., 2011) and cross-sectional investigations (Jeffery et al., 2014; Shomaker et al., 2010); and in adults, where depressive symptoms were related to increased risk for developing type 2 diabetes (Mezuk et al., 2008; Mezuk, Heh, Prom-Wormley, Kendler, & Pedersen, 2015). However, in contrast to prior studies, where participants were selected on the basis of being overweight or obese, participants in the current study were healthy children drawn from the general community, allowing the current findings to be generalizable to the broader population.

Furthermore, our findings linking depressive symptoms in a boy with his adiposity are supported by prior longitudinal studies among adolescents. For example, Stice, Presnell, Shaw, and Rohde (2005) reported that among adolescents of healthy weight, baseline depression was associated with a four-fold increased risk for obesity four years later. Similar effects did not extend to girls in our cohort, which is in contrast to a previous report (Elizabeth. Goodman & Whitaker, 2002). There is no obvious reason as to why these relationships did not extend to girls, given adjustments were made for puberty and physical activity. In any case, complementing reports focusing on the link between increased fatness and aspects of poor mental health in girls (Lawler & Nixon, 2011; Olive, Byrne, Cunningham, & Telford, 2012), our data indicate that boys may face similar pressure to meet an ideal body type, and that this may relate to negative mood states.

Despite commonly held beliefs that depression and childhood obesity are related, longitudinal evidence has been sparse. In contrast to the adolescent literature, the majority of longitudinal studies among children report that depressive symptoms do not predict later BMI change, body fat or obesity status (for a review see Incledon, Wake, & Hay, 2011). The weaker findings reported among children compared to adolescents may indicate that the relationship may not emerge until a certain age. Alternatively, this may reflect methodological limitations, with much of the childhood literature being confounded by low methodological quality, including the use of non-validated assessments and in some instances, relying on self-reported height and weight when calculating BMI (Incledon, Wake, & Hay, 2011). Further to these limitations, while BMI may provide a reasonable means of classifying fatness, it is problematic and misleading when used in longitudinal analyses with growing children (Telford et al., 2014).

The current study extends prior research among children and adolescents by utilizing a longitudinal design spanning eight years, with multiple follow-up assessments spanning both child and adolescent development. A strength of our work was the use an objective assessment of body fat and therefore not having to rely on BMI, which, as indicated above, is flawed in pediatric longitudinal work. Another strong aspect was the statistical model with its adjustment for a number of potentially confounding covariates, each of which became evident during our multidisciplinary LOOK study including pubertal stage, the absence of which has been identified as a weakness in prior studies (Incledon et al., 2011).

On the other hand, a number of limitations exist, including the rate of attrition from baseline. Although our analyses of those who left the study and those who remained did not reveal any significant differences in percent body fat or insulin resistance, children who remained in the study were less stressed and depressed at baseline. While the effect is likely to be minimal, the accompanying reduction in the range of any variable would tend to reduce

the likelihood of detecting relationships. The reliance on self-report measures to assess both psychosocial stress and depressive symptoms may also be seen as a limitation. However, self-reported assessments of psychosocial stress and depression are a common practice in field work and the current study was not focused on assessing clinical depression per se but rather on early symptoms, within a preventive medicine framework in a sample of apparently healthy youth. A final limitation was that, although we investigated a community-dwelling sample of apparently healthy and predominantly white children, the findings may not be generalizable to youth of different nationality with differing SES and ethnicity.

Conclusion

In summary, the current study provides evidence that healthy boys who report more symptoms of depression have higher insulin resistance; a relationship well supported by the trend for a direct (longitudinal) effect of depressive symptoms on insulin resistance. In addition, boys whose depressive symptoms increased were more likely to become fatter. Taken together, alongside the plausible biological mechanisms linking psychological risk factors with insulin resistance, the case for early depressive symptoms to constitute a cause of this metabolic dysfunction is strengthened. Our data obtained from healthy children points to the potential efficacy of a novel approach to the prevention of metabolic and chronic diseases; one which focuses on the treatment of early onset depression and depressive symptoms in children and adolescents.

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References

Anderson, R. J., Freedland, K. E., Clouse, R. E., & Lustman, P. J. (2001). The prevalence of comorbid depression in adults with diabetes: A meta-analysis. *Diabetes Care, 24*, 1069-1078. doi: 10.2337/diacare.24.6.1069

Aradillas-García, C., Rodríguez-Morán, M., Garay-Sevilla, M. E., Malacara, J. M., Rascon-Pacheco, R. A., & Guerrero-Romero, F. (2012). Distribution of the homeostasis model assessment of insulin resistance in Mexican children and adolescents. *European Journal of Endocrinology, 166*, 301-306. doi: 10.1530/eje-11-0844

Bao, A. M., Meynen, G., & Swaab, D. F. (2008). The stress system in depression and neurodegeneration: focus on the human hypothalamus. *Brain Research Reviews, 57*, 531-553. doi: 10.1016/j.brainresrev.2007.04.005

Barton, D. A., Dawood, T., Lambert, E. A., Esler, M. D., Haikerwal, D., Brenchley, C., . . . Lambert, G. W. (2007). Sympathetic activity in major depressive disorder: identifying those at increased cardiac risk? *Journal of Hypertension, 25*, 2117-2124. doi: 10.1097/HJH.0b013e32829baae7

Beets, M. W., Patton, M. M., & Edwards, S. (2005). The accuracy of pedometer steps and time during walking in children. *Medicine and Science in Sports and Exercise, 37*, 513-520.

Bell, J. A., Kivimaki, M., & Hamer, M. (2014). Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. *Obesity Reviews, 15*, 504-515. doi: 10.1111/obr.12157

Byrne, D. G., Thomas, K. A., Burchell, J. L., Olive, L. S., & Mirabito, N. S. (2011). Stressor experience in primary school-aged children: Development of a scale to assess profiles of exposure and effects on psychological well-being. *International Journal of Stress Management, 18*, 88-111. doi: 10.1037/a0021577

Chambers, E. C., Tull, E. S., Fraser, H. S., Mutunhu, N. R., Sobers, N., & Niles, E. (2004). The relationship of internalized racism to body fat distribution and insulin resistance among African adolescent youth. *Journal of the National Medical Association, 96*, 1594-1598.

Duke, P. M., Litt, I. F., & Gross, R. T. (1980). Adolescents' self-assessment of sexual maturation. *Pediatrics, 66*, 918–920.

Ehtisham, S., Barrett, T. G., & Shaw, N. J. (2000). Type 2 diabetes mellitus in UK children – an emerging problem. *Diabetic Medicine, 17*, 867-871. doi: 10.1046/j.1464-5491.2000.00409.x

Fang, C. Y., Boden, G., Siu, P. T., & Tseng, M. (2015). Stressful life events are associated with insulin resistance among Chinese immigrant women in the United States. *Preventive Medicine Reports, 2*, 563-567. doi: http://dx.doi.org/10.1016/j.pmedr.2015.06.013

Freedman, D. S., Srinivasan, S. R., Burke, G. L., Shear, C. L., Smoak, C. G., Harsha, D. W., . . . Berenson, G. S. (1987). Relation of body fat distribution to hyperinsulinemia in children and adolescents: the Bogalusa Heart Study. *American Journal of Clinical Nutrition, 46*, 403-410.

Garnett, S. P., Hogler, W., Blades, B., Baur, L. A., Peat, J., Lee, J., & Cowell, C. T. (2004). Relation between hormones and body composition, including bone, in prepubertal children. *American Journal of Clinical Nutrition, 80*, 966-972

Goodman, E., Daniels, S. R., & Dolan, L. M. (2007). Socioeconomic disparities in insulin resistance: results from the Princeton school district study. *Psychosomatic Medicine, 69*, 61-67. doi: 10.1097/01.psy.0000249732.96753.8f

Goodman, E., & Whitaker, R. C. (2002). A Prospective Study of the Role of Depression in the Development and Persistence of Adolescent Obesity. *Pediatrics, 110*, 497-504. doi: 10.1542/peds.110.3.497

Goran, M. I., Ball, G. D., & Cruz, M. L. (2003). Obesity and risk of type 2 diabetes and cardiovascular disease in children and adolescents. *Journal of Clinical; Endocrinology Metabolism, 88*, 1417-1427. doi: 10.1210/jc.2002-021442

Group, D. C. a. C. T. R. (1994). Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. Diabetes Control and Complications Trial Research Group. *Journal of Pediatrics, 125*, 177-188.

Gungor, N., Saad, R., Janosky, J., & Arslanian, S. (2004). Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. *Journal of Pediatrics, 144*, 47-55. doi: 10.1016/j.jpeds.2003.09.045

Gutin, B., Islam, S., Manos, T., Cucuzzo, N., Smith, C., & Stachura, M. E. (1994). Relation of percentage of body fat and maximal aerobic capacity to risk factors for atherosclerosis and diabetes in black and white seven- to eleven-year-old children. *Journal of Pediatrics, 125*, 847-852.

Heraclides, A., Chandola, T., Witte, D. R., & Brunner, E. J. (2009). Psychosocial stress at work doubles the risk of type 2 diabetes in middle-aged women: Evidence from the Whitehall II Study. *Diabetes Care, 32*, 2230-2235. doi: 10.2337/dc09-0132

Howard, G., O'Leary, D. H., Zaccaro, D., Haffner, S., Rewers, M., Hamman, R., . . . Bergman, R. (1996). Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Circulation, 93*, 1809-1817.

Huang, T. T., Johnson, M. S., & Goran, M. I. (2002). Development of a prediction equation for insulin sensitivity from anthropometry and fasting insulin in prepubertal and early pubertal children. *Diabetes Care, 25*, 1203-1210.

Incledon, E., Wake, M., & Hay, M. (2011). Psychological predictors of adiposity: systematic review of longitudinal studies. *International Journal of Pediatric Obesity, 6*, e1-11. doi: 10.3109/17477166.2010.549491

Jeffery, A. N., Alba, S., Murphy, M. J., Voss, L. D., Metcalf, B. S., Hosking, J., . . . Wilkin, T. J. (2007). Behavior of insulin resistance and its metabolic correlates in prepubertal children: a longitudinal study (EarlyBird 32). *Diabetes Care, 30*, 2962-2964. doi: 10.2337/dc07-0178

Jeffery, A. N., Hyland, M. E., Hosking, J., & Wilkin, T. J. (2014). Mood and its association with metabolic health in adolescents: a longitudinal study, EarlyBird 65. *Pediatric Diabetes, 15*, 599-605. doi: 10.1111/pedi.12125

Kan, C., Silva, N., Golden, S. H., Rajala, U., Timonen, M., Stahl, D., & Ismail, K. (2013). A systematic review and meta-analysis of the association between depression and insulin resistance. *Diabetes Care, 36*, 480-489. doi: 10.2337/dc12-1442

Kendler, K. S., Karkowski, L. M., & Prescott, C. A. (1999). Causal relationship between stressful life events and the onset of major depression. *American Journal of Psychiatry, 156*, 837-841. doi.org/10.1176/ajp.156.6.837

Keskin, M., Kurtoglu, S., Kendirci, M., Atabek, M. E., & Yazici, C. (2005). Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. *Pediatrics, 115*, e500-503. doi: 10.1542/peds.2004-1921

Knol, M. J., Twisk, J. W., Beekman, A. T., Heine, R. J., Snoek, F. J., & Pouwer, F. (2006). Depression as a risk factor for the onset of type 2 diabetes mellitus. A meta-analysis. *Diabetologia, 49*, 837-845. doi: 10.1007/s00125-006-0159-x

Kurtoglu, S., Hatipoglu, N., Mazicioglu, M., Kendirici, M., Keskin, M., & Kondolot, M. (2010). Insulin resistance in obese children and adolescents: HOMA-IR cut-off levels in the prepubertal and pubertal periods. *Journal of Clinical Research in Pediatric Endocrinology, 2*, 100-106. doi: 10.4274/jcrpe.v2i3.100

Laakso, M., & Kuusisto, J. (2014). Insulin resistance and hyperglycaemia in cardiovascular disease development. *Nature Reviews Endocrinology, 10*, 293-302. doi: 10.1038/nrendo.2014.29

Lawler, M., & Nixon, E. (2011). Body dissatisfaction among adolescent boys and girls: the effects of body mass, peer appearance culture and internalization of appearance ideals. *Journal of Youth and Adolescence, 40*, 59-71. doi: 10.1007/s10964-009-9500-2

Legido, A., Sarria, A., Bueno, M., Garagorri, J., Fleta, J., Ramos, F., . . . Perez-Gonzalez, J. (1989). Relationship of body fat distribution to metabolic complications in obese prepubertal boys: gender related differences. *Acta Paediatrica Scandinavica, 78*, 440-446.

Madeira, I. R., Carvalho, C. N., Gazolla, F. M., de Matos, H. J., Borges, M. A., & Bordallo, M. A. (2008). [Cut-off point for Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index established from Receiver Operating Characteristic (ROC) curve in the detection of metabolic syndrome in overweight pre-pubertal children]. *Archives of Endocrinology and Metabolism, 52*, 1466-1473.

Martin, B. C., Warram, J. H., Krolewski, A. S., Soeldner, J. S., Kahn, C. R., Martin, B. C., & Bergman, R. N. (1992). Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *The Lancet, 340*, 925-929. doi: http://dx.doi.org/10.1016/0140-6736(92)92814-V

Metcalf, B. S., Voss, L. D., Hosking, J., Jeffery, A. N., & Wilkin, T. J. (2008). Physical activity at the government-recommended level and obesity-related health outcomes: a longitudinal study (Early Bird 37). *Archives of Disease in Childhood, 93*, 772-777. doi: 10.1136/adc.2007.135012

Mezuk, B., Eaton, W. W., Albrecht, S., & Golden, S. H. (2008). Depression and type 2 diabetes over the lifespan: a meta-analysis. *Diabetes Care, 31*, 2383-2390. doi: 10.2337/dc08-0985

Mezuk, B., Heh, V., Prom-Wormley, E., Kendler, K. S., & Pedersen, N. L. (2015). Association between major depression and type 2 diabetes in midlife: findings from the screening across the lifespan twin study. *Psychosomatic Medicine, 77*, 559-566. doi: 10.1097/psy.0000000000000182

Nolen-Hoeksema, S. (2001). Gender Differences in Depression. *Current Directions in Psychological Science, 10*, 173-176. doi: 10.1111/1467-8721.00142

Olive, L. S., Byrne, D. G., Cunningham, R. B., & Telford, R. D. (2012). Effects of physical activity, fitness and fatness on children's body image: The Australian LOOK longitudinal study. *Mental Health and Physical Activity, 5*, 116-124. doi: http://dx.doi.org/10.1016/j.mhpa.2012.08.004

Olive, L. S., Telford, R. M., Byrne, D. G., Abhayaratna, W. P., & Telford, R. D. (2016). Psychological distress leads to reduced physical activity and fitness in children: the Australian longitudinal LOOK study. *International Journal of Behavioral Medicine*, *39*, 587. doi:10.1007/s10865-016-9723-0

Räikkönen, K., Keltikangas-Järvinen, L., Adlercreutz, H., & Hautanen, A. (1996). Psychosocial stress and the insulin resistance syndrome. *Metabolism, 45*, 1533-1538. doi: http://dx.doi.org/10.1016/S0026-0495(96)90184-5

Robinson, G. K. (1994). That BLUP is a good thing: the estimation of random effects. *Statistical Science, 6*, 15-32.

Rosmond, R., & Bjorntorp, P. (2000). Occupational status, cortisol secretory pattern, and visceral obesity in middle-aged men. *Obesity Research, 8*, 445-450. doi: 10.1038/oby.2000.55

Shomaker, L. B., Tanofsky-Kraff, M., Stern, E. A., Miller, R., Zocca, J. M., Field, S. E., . . . Yanovski, J. A. (2011). Longitudinal study of depressive symptoms and progression of insulin resistance in youth at risk for adult obesity. *Diabetes Care, 34*, 2458-2463.

Shomaker, L. B., Tanofsky-Kraff, M., Young-Hyman, D., Han, J. C., Yanoff, L. B., Brady, S. M., . . . Yanovski, J. A. (2010). Psychological symptoms and insulin sensitivity in adolescents. *Pediatric Diabetes, 11*, 417-423. doi: 10.1111/j.1399-5448.2009.00606.x

Singh, Y., Garg, M. K., Tandon, N., & Marwaha, R. K. (2013). A study of insulin resistance by HOMA-IR and its cut-off value to identify metabolic syndrome in urban Indian adolescents. *Journal of Clinical Research in Pediatric Endocrinology, 5*, 245-251. doi: 10.4274/Jcrpe.1127

Srinivasan, S. R., Myers, L., & Berenson, G. S. (2006). Changes in metabolic syndrome variables since childhood in prehypertensive and hypertensive subjects: the Bogalusa Heart Study. *Hypertension, 48*, 33-39. doi: 10.1161/01.HYP.0000226410.11198.f4

Stice, E., Presnell, K., Shaw, H., & Rohde, P. (2005). Psychological and behavioral risk factors for obesity onset in adolescent girls: a prospective study. *Journal of Consulting and Clinical Psychology, 73*, 195-202. doi: 10.1037/0022-006x.73.2.195

Stuart, M. J., & Baune, B. T. (2012). Depression and type 2 diabetes: Inflammatory mechanisms of a psychoneuroendocrine co-morbidity. *Neuroscience & Biobehavioral Reviews, 36*, 658-676. doi: http://dx.doi.org/10.1016/j.neubiorev.2011.10.001

Taylor, R. (2012). Insulin resistance and type 2 diabetes. *Diabetes, 61*, 778-779. doi: 10.2337/db12-0073

Telford, R. D., Bass, S. L., Budge, M. M., Byrne, D. G., Carlson, J. S., Coles, D., … Waring, P. (2009b). The lifestyle of our kids (LOOK) project: outline of methods. *Journal of Science & Medicine in Sport, 12,* 156-63.

Telford, R. D., & Cunningham, R. B. (2008). Reformulation of BMI and percent body fat to remove the height bias in 8-year-olds. *Obesity (Silver Spring), 16*, 2175-2181. doi: 10.1038/oby.2008.332

Telford, R. D., Cunningham, R. B., & Abhayaratna, W. P. (2014). Temporal divergence of percent body fat and body mass index in pre-teenage children: the LOOK longitudinal study. *Pediatric Obesity, 9*, 448-454. doi: 10.1111/j.2047-6310.2013.00194.x

Telford, R. D., Cunningham, R. B., Shaw, J. E., Dunstan, D. W., Lafferty, A. R., Reynolds, G. J., . . . Telford, R. M. (2009a). Contrasting longitudinal and cross-sectional relationships between insulin resistance and percentage of body fat, fitness, and physical activity in children-the LOOK study. *Pediatric Diabetes, 10*, 500-507. doi: 10.1111/j.1399-5448.2009.00513.x

Telford, R. D., Cunningham, R. B., & Telford, R. M. (2009). Day-dependent step-count patterns and their persistence over 3 years in 8-10-year-old children: the LOOK project. *Annals of Human Biology, 36*, 669-679.

Telford, R. D., Cunningham, R. B., Telford, R. M., Kerrigan, J., Hickman, P. E., Potter, J. M., & Abhayaratna, W. P. (2012). Effects of changes in adiposity and physical activity on preadolescent insulin resistance: the Australian LOOK longitudinal study. *PLoS ONE, 7*, e47438. doi: 10.1371/journal.pone.0047438

Telford, R. D., Cunningham, R. B., Telford, R. M., Riley, M., & Abhayaratna, W. P. (2012). Determinants of Childhood Adiposity: Evidence from the Australian LOOK Study. *PLoS ONE, 7*, e50014. doi: 10.1371/journal.pone.0050014

Tresaco, B., Bueno, G., Pineda, I., Moreno, L. A., Garagorri, J. M., & Bueno, M. (2005). Homeostatic model assessment (HOMA) index cut-off values to identify the metabolic syndrome in children. *Journal of Physiology and Biochemistry, 61*, 381-388.

van Dooren, F. E. P., Nefs, G., Schram, M. T., Verhey, F. R. J., Denollet, J., & Pouwer, F. (2013). Depression and Risk of Mortality in People with Diabetes Mellitus: A Systematic Review and Meta-Analysis. *PLoS ONE, 8*, e57058. doi: 10.1371/journal.pone.0057058

Wang, C., Guan, Y., & Yang, J. (2010). Cytokines in the Progression of Pancreatic β-Cell Dysfunction. *International Journal of Endocrinology, 2010*. doi: http://dx.doi.org/10.1155/2010/515136

Whittle, S., Yücel, M., Lorenzetti, V., Byrne, M. L., Simmons, J. G., Wood, S. J., . . . Allen, N. B. (2012). Pituitary volume mediates the relationship between pubertal timing and depressive symptoms during adolescence. *Psychoneuroendocrinology, 37*, 881-891. doi: 10.1016/j.psyneuen.2011.10.004

Zhang, M., Hu, T., Zhang, S., & Zhou, L. (2015). Associations of Different Adipose Tissue Depots with Insulin Resistance: A Systematic Review and Meta-analysis of Observational Studies. *Scientific Reports, 5*, 18495. doi: 10.1038/srep18495