Early origins of diabetes: an update from the EarlyBird study

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Summary

The EarlyBird diabetes study has followed over 300 healthy children and their parents for 12 years. The aim has been to understand which children would be most at risk of adult onset diabetes. Repeated measures in the same individuals reveal trends which may be associated with diabetes risk, and will help determine the direction of causality. This report draws on 12 years of EarlyBird data, giving an insight into diabetes risk in three areas:

- Trends in both insulin supply (secretion) and demand (tissue resistance) over the period of pubertal development affect diabetes risk. This paper compares insulin resistance and beta cell function in children who develop impaired fasting glucose with those whose glucose levels remain within the normal range.
- Epigenetics is a new area of research. While genetic make-up cannot change, the way genes are expressed (turned on or off) can vary in relation to environmental stressors. This paper reviews preliminary analyses of epigenetic variation in a gene associated with diabetes risk, and how this variation affects adiposity during childhood.
- The association between low mood (tendency towards depression) and diabetes risk in teenagers is explored.

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Key words

Type 2 diabetes; insulin resistance; children; adolescents; diabetes risk; mood; epigenetics; beta cell function

Introduction

The incidence of type 2 diabetes in young people continues to rise and it is worrying that adolescents, and even children, are now increasingly diagnosed with type 2.[1] The causes for this burgeoning demand on health services are the subject of much research, and it is likely that both lifestyle and genetic factors are implicated.

The concept underpinning the EarlyBird Study is that the process, which eventually leads to type 2 diabetes, begins early and may be identifiable during childhood, years before diabetes is diagnosed. This paper reviews the evidence for three factors which may influence that process – firstly the inborn ability of pancreatic beta cells to respond to insulin demand, secondly the evidence that modifiable environmental factors may alter the genetic risk of a given individual to diabetes and obesity, and thirdly the effect of an individual's mood on his or her diabetes risk. Together, these factors form pieces of the complex jigsaw puzzle that together may explain why some people, but not others, develop diabetes.

Methods

EarlyBird is a non-intervention study following a cohort of healthy children from the age of five through to 16. The study commenced recruitment in 2000 from randomly selected schools in Plymouth, UK and formed a cohort of 347 healthy children, closely grouped in age (standard deviation ± three months). The majority were white Caucasian, with a wide socio-economic mix. Local research ethics committee approval was granted in 1999 and full details of recruitment and methodology were described by Voss *et al.*[2] Annual measures include detailed anthropometry, including body mass index (BMI SDS, standardised for age and gender), body fat percentage (dual-energy x-ray absorptiometry), seven-day physical activity monitoring (accelerometry), blood pressure, and fasting blood tests: glucose, insulin, insulin resistance (HOMA-IR), beta-cell function (HOMA-B), total cholesterol, HDL cholesterol, triglycerides. The same fasting blood tests were made on the parents when the child was aged five years.

Pubertal development was assessed as the age at which the child reached their peak height velocity, determined as the tangential velocity at the middle time-point of three consecutive height measurements taken six months apart.

Participant retention was good: a full data set from 78% of the cohort was obtained at the conclusion of the twelfth study year in 2012, with the children aged 16 years.

Insulin supply and demand

Hyperglycaemia ensues when demand for insulin increases beyond the capability of the beta cell to respond.[3] Cross-sectional studies suggest that the demand for insulin is greater in children with a higher fat percentage, although diabetes will only develop if the supply is unable to meet it.[4,5]

Impaired fasting glucose (IFG) occurs when the control loop is

unable to maintain glucose levels within the normal range in the fasting state. The American Diabetes Association's definition is a fasting glucose more than 5.6–6.9 mmol/l.[6] Studies in adults and children suggest that future diabetes risk increases continually with increasing fasting plasma glucose.[7,8,9]

Hosking *et al.* identified 55 EarlyBird children (39 boys) with IFG at least once between five and 15 years of age (17% of the cohort), and over 70% of them at 11 years or older.[10] Interestingly, there was no difference in BMI between those who showed IFG and those who did not (BMI SDS at five years: 0.27 versus 0.33 respectively, p=0.70). Indeed, only 9.6% of the 55 children who developed IFG were overweight or obese compared with 16.9% of those who did not. Neither were there any differences between the two groups for physical activity, pubertal development or insulin resistance (all p>0.49). However, those with IFG had significantly lower beta cell function than those who did not (HOMA-B at five years: 82.5 versus 95.0 units respectively, p=0.001).

Longitudinal modelling of the trends in beta cell function between the ages of 5 and 16 showed it to be consistently lower in those with IFG than those without (Figure 1). This difference remained statistically significant after adjustment for gender, BMI SDS, age at peak height velocity and physical activity (p=0.03).

It was also interesting to note that the mean fasting glucose of the mothers whose children showed IFG was significantly higher than that of the mothers whose children did not (4.9 versus 4.6, p<0.001), and their HOMA-B was also lower (p=0.02). Mothers' BMI and HOMA-IR was the same in both groups (p>0.6). Again, mean fasting glucose of the fathers of IFG children was higher than that of normal glucose children (5.0 versus 4.8, p=0.04).

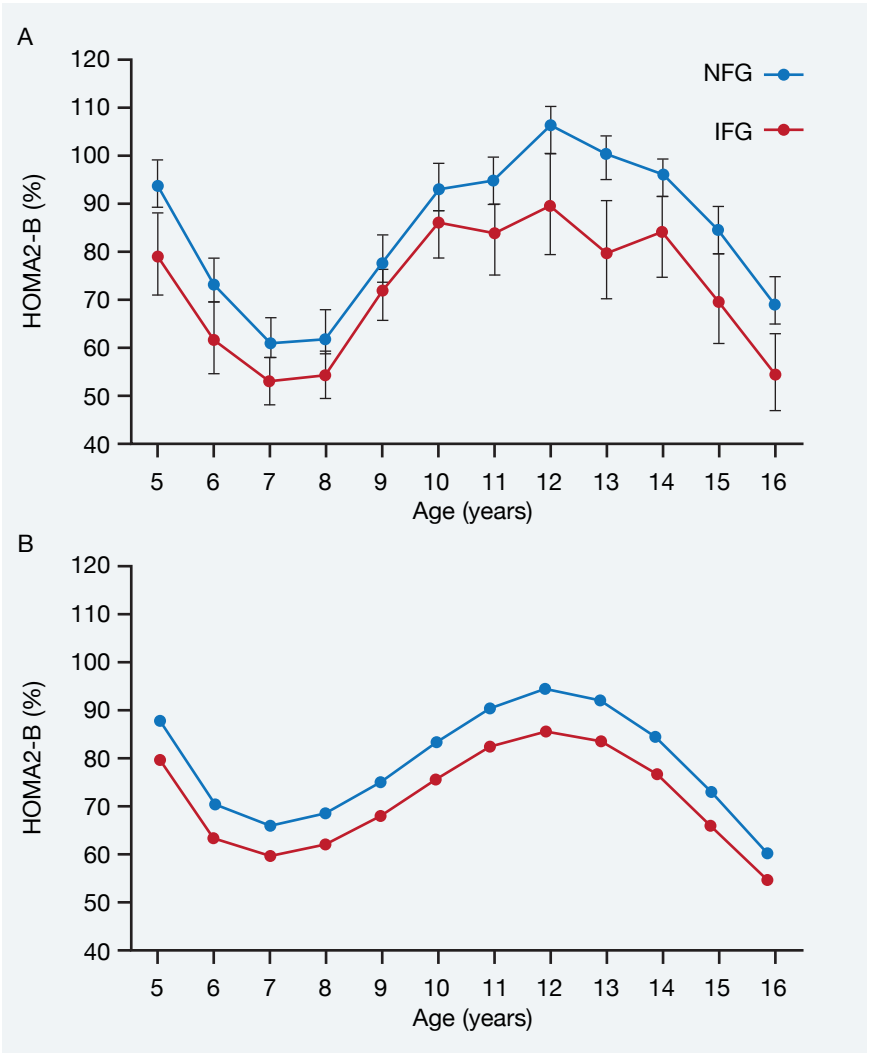

Figure 1. Trajectories of beta cell function (HOMA-B) between 5 years and 15 years in children according to IFG status (a. unadjusted, b. adjusted for age, gender, BMI standards, age at peak height velocity, height standards, and physical activity)[10]

Hosking *et al.*[10] speculated that further weight gain and demand for insulin would be needed for the IFG children to progress to frank diabetes. What distinguished those who developed IFG was a defect in beta cell function, already present at five years of age, and also present in their parents.

Changes in gene expression and diabetes risk

Epigenetics is the study of heritable changes in gene function that occur without a change in DNA sequence. *Epi*, Greek for 'outside', implies that the changes are outside, or in addition to, genetics. Epigenetic changes affect how well, or in what way, individual genes work, and may be thought of as 'turning on' or 'turning off' genes, and all the stages in between . These epigenetic changes may last for that cell's lifetime or through multiple cell divisions and generations, even though there is no change to the gene sequence itself. An example of this process is evident in the families of those who

experienced the Dutch 'Hunger Winter' between 1944–45. Adults who were in utero during this period of famine had increased risk of hypertension, obesity and insulin resistance.[11] Crucially, the effects of the famine continued through the generations, such that the grandchildren of women who were exposed to famine during their pregnancy also showed this increased risk.[12]

Triggers of epigenetic change remain speculative, but probably include periods of rapid growth (gestation, infancy, puberty), diet, exposure to chemicals and drugs, and the ageing process. Epigenetic changes have been implicated in the development of some cancers[13] and autoimmune diseases.[14]

Most epigenetic studies relating to diabetes have been in animals, where it is possible to manipulate the diet of mothers and their offspring. When pregnant rats and mice were fed diets of reduced protein, calories, or a high fat content, the offspring had impaired blood pressure, insulin resistance and obesity, and these effects continued in future generations.[15]

There have so far been very few studies of epigenetic changes in relation to diabetes in humans, and the number of genes studied so far is limited. EarlyBird conducted the first longitudinal epigenetic study[16] in 40 children, stratified according to their insulin resistance at 14 years of age. The first aim was to examine the stability of the PGC1 gene over a 10-year period during childhood. The PGC1 gene is central to energy homeostasis through regulation of pancreatic beta-cell function and adipogenesis (differentiation of fat cells). Clarke-Harris *et al.*[16] studied seven different sites (CpG loci) located on the PGC1 gene, and measured how well the gene was expressed at each of those sites (the percentage methylation). Increased methylation corresponds, in the case of the PGC1 gene, to switching off or lower activity of the gene[16]. The authors found that the amount of methylation at each CpG locus was remarkably stable during childhood: mean (±SD) methylation of individual CpG loci varied from 20.4 ± 3.5% to 64.9 ± 2.8% across all subjects and years and did not differ between boys and girls, and longitudinal tracking co-efficients were high for all loci. This was important to establish as it implies that the function of that gene is not being affected by any environmental stimulus during this period of time, and that an early measure of methylation can accurately predict methylation 10 years later.

The second aim was to establish whether methylation was associated with body fat (measured by dual energy x-ray absorptiometry). A clear differentiation was seen, with greater methylation (suppression of gene activity) associated with greater body fat at each age. This was statistically significant ($p \leq 0.03$) in four CpG sites for both boys and girls, and the pattern was replicated, but not statistically significant, at the other three sites. The magnitude of the association suggested that a 10% difference in methylation predicted up to 12% difference in fat mass. This is important as it is the first evidence that epigenetic changes early in childhood may predict adiposity later, and that epigenetic marks in childhood could be used as biomarkers on which to base interventions to reduce risk of obesity.

The next step is to examine the association between methylation of the PGC1 gene and insulin resistance. This study is ongoing, and results will be available from EarlyBird in due course. The same group is also studying methylation in other genes thought to affect diabetes risk (SIRT 1, HNF4 , glucokinase). If the expected associations are found, this could mean that an individual's diabetes risk could be predicted from a single blood test early in childhood.

Mood and diabetes risk

The prevalence of depression is doubled in individuals with type 2 diabetes compared with those without diabetes.[17] It is not known whether depression develops as a result of living with the burden of diabetes,[18] or whether depression is an independent risk factor for the development of type 2 diabetes.[19,20] In a meta-analysis of longitudinal studies in adults, Knol *et al.*[21] concluded that those with depressive symptoms had a 37% increased risk of developing type 2 diabetes in the future, after accounting for confounders such as BMI. There are a number of pathways by which this could happen: depressive symptoms may activate stress pathways, including upregulating the hypothalamic-pituitary-adrenal axis, altering insulin signalling in the brain or increasing pro-inflammatory factors.[22]

Mood is associated with mental health problems in both adults[23] and children[24,25] and comprises two components: negative and positive affect. Negative affect is a general factor of emotional distress. Positive affect is represented by pleasant feelings (eg interested, strong and active), and low positive affect is specific to depression. Postive affect is represented by pleasant feelings and low positive effect is specific to depression. Both negative and positive affect are relatively stable as people move from childhood into adulthood,[26] and are associated with diabetes in adults.[27,28]

The link between obesity and depression in children is estab-

lished,[29] and depressive symptoms in adolescents have been shown to predict obesity a year later.[30] Cross-sectional studies of adolescents have found depressive symptoms to be associated with insulin sensitivity, both dependent[31] and independent of adiposity.[32,33]

In a recent publication,[34] EarlyBird hypothesised that:

1. Lower mood and increased diabetes risk in teenagers would be correlated, and that adiposity would explain some of this association
2. Increases in diabetes risk during childhood would predict lower mood at 16 years of age.

Mood was measured by the positive affect and negative affect schedule – child form (PANAS-C) is a widely used scale which has been validated in a UK adult population[35,36] and in children aged 11–18 years.[37] The scale consists of 30 adjectives, 15 positive and 15 negative. The child was asked 'How do you normally feel?' in the way described by the adjective on a five-point scale ranging from 1 (very slightly or not at all) to 5 (extremely). The scores for each affect were added and an overall mood score calculated (positive minus negative). A higher mood score denoted a happier child.

A mean diabetes risk z-score was calculated comprising sex and age-specific standardised measures of insulin resistance (IR), cholesterol: HDL cholesterol ratio, triglycerides, systolic and diastolic blood pressure.[38] A higher score represented poorer metabolic health and increased diabetes risk.

Jeffery *et al.*[34] found that teenagers with a higher fat percentage had significantly lower mood than those who did not, and that this relationship was partly explained by the fact that girls have a higher fat percentage than boys, as well as lower mood at 16.

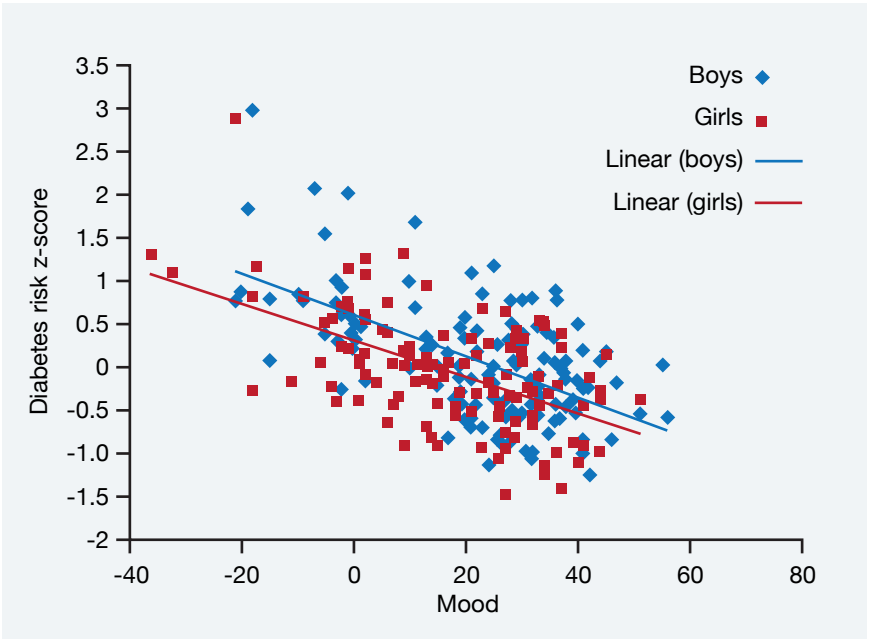

Figure 2. Scatterplot of mood and diabetes risk score in boys and girls[10]

Low mood in otherwise healthy adolescents was associated with increased diabetes risk at 16 years (= -0.26, p=0.008), confirming our first hypothesis. Figure 2 shows the scatterplot between mood and diabetes risk (r= -0.19, p=0.003).

We also found evidence to support our second hypothesis – lower mood was associated with increasing diabetes risk during childhood, independent of adiposity and sex (= -0.40, p=0.004). This is the first study to suggest that those children, whose metabolic health deteriorates over time, appear to be the most at risk for exhibiting depressive symptoms. Earlier developing children had higher negative affect than those developing later[39] Further research is needed to determine whether early intervention to relieve depressive symptoms in teenagers could ameliorate pubertal insulin resistance and/or reduce the risk of developing type 2 diabetes in the future.

Summary and conclusion

This article has highlighted three ways in which factors during childhood can lead to increased diabetes risk for teenagers. By following a cohort of normal youngsters during their childhood certain risk factors have become evident. The study identified an early deficit in beta cell function among otherwise healthy children who went on to develop impaired fasting glucose during puberty. Further work to better understand the origins of the deficit (genetic, epigenetic or environmental) may be important in reducing diabetes risk.

It was previously thought impossible to alter one's genes, but the science of epigenetics illustrated here shows that gene expression could potentially be modifiable (*eg* dietary, weight management). Furthermore, these new biomarkers for obesity, and potentially for diabetes, are identifiable early in life.

Finally, we report that teenagers who have a more buoyant mood tend to be leaner and at lower diabetes risk than their peers. This has implications for the future physical and mental health of teenagers with diabetes risk factors.

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KEY POINTS

- Some teenagers who develop impaired fasting glucose have an inherited beta cell defect which can be detected as early as five years old
- Epigenetic markers (how well diabetes genes are expressed) in early childhood may predict adiposity later in childhood
- Happier children have lower diabetes risk, even after accounting for their level of adiposity

References

1. Ehtisham S, Barrett TG. The emergence of type 2 diabetes in childhood. *Ann Clin Biochem* 2004;41:10–16.
2. Voss LD, Kirkby J, Metcalf BS, *et al.* Preventable factors in childhood that lead to insulin resistance, diabetes mellitus and the metabolic syndrome: the EarlyBird diabetes study 1. *J Pediatr Endocrinol Metab* 2003; 16:1211–1224.
3. Cnop M, Vidal J, Hull RL, *et al.* Progressive loss of beta-cell function leads to worsening glucose tolerance in first-degree relatives of subjects with type 2 diabetes. *Diabetes Care* 2007;30:677–682.
4. Bergsten P. Pathophysiology of impaired pulsatile insulin release. D*iabetes Metab Res Rev* 2000;16:179–191.
5. Polonsky KS, Given BD, Hirsch LJ, *et al.* Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 1988;318:1231–1239.
6. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2011;34(Suppl 1):S62–69.
7. Nichols GA, Hillier TA, Brown JB. Normal fasting plasma glucose and risk of type 2 diabetes diagnosis. *Am J Med* 2008; 121:519–524.
8. Tirosh A, Shai I, Tekes-Manova D, *et al.* Normal fasting plasma glucose levels and type 2 diabetes in young men. *N Engl J Med* 2005;353:1454–1462.
9. Tfayli H, Lee S, Arslanian S. Declining beta-cell function relative to insulin sensitivity with increasing fasting glucose levels in the nondiabetic range in children. *Diabetes Care* 2010;33:2024–2030.
10. Hosking J, Metcalf BS, Jeffery AN, *et al.* Evidence of early beta-cell deficiency among children who show impaired fasting glucose: 10-yr cohort study (EarlyBird 56). *Pediatr Diabetes* 2013;14:481–489.
11. Kaati G, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. Eur *J Hum Genet* 2002;10:682–688.
12. Stein AD, Lumey LH. The relationship between maternal and offspring birth weights after maternal prenatal famine exposure: the Dutch famine birth cohort study. *Hum Biol* 2000;72:641–654.
13. Vucic EA, Brown CJ, Lam WL. Epigenetics of cancer progression. *Pharmacogenomics* 2008; 9:215–234.
14. Richardson BC, Patel DR. Epigenetics in 2013. DNA methylation and miRNA—key roles in systemic autoimmunity. *Nat Rev Rheumatol* 2014;10:72–74.
15. Burdge GC, Lillycrop KA, Jackson AA. Nutrition in early life, and risk of cancer and metabolic disease: alternative endings in an epigenetic tale? *Br J Nutr* 2009; 101:619–630.
16. Clarke-Harris R, Wilkin TJ, Hosking J, *et al.* Peroxisomal proliferator activated receptor-gamma-co-activator-1alpha promoter methylation in blood at 5-7 years predicts adiposity from 9 to 14 years (EarlyBird 50). *Diabetes* 2014 [epub ahead of print].
17. Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care* 2001;24:1069–1078.
18. Collins MM, Corcoran P, Perry IJ. Anxiety and depression symptoms in patients with diabetes. *Diabet Med* 2009;26:153–161.
19. Everson-Rose SA, Meyer PM, Powell LH, *et al.* Depressive symptoms, insulin resistance, and risk of diabetes in women at midlife. *Diabetes Care* 2004; 27:2856–2862.
20. Golden SH, Williams JE, Ford DE, *et al.* Depressive symptoms and the risk of type 2 diabetes: the Atherosclerosis Risk in Communities study. *Diabetes Care* 2004;27:429–435.
21. Knol MJ, Twisk JW, Beekman AT, *et al.* Depression as a risk factor for the onset of type 2 diabetes mellitus. A meta-analysis. *Diabetologia* 2006;49:837–845.
22. Golden SH. A review of the evidence for a neuroendocrine link between stress, depression and diabetes mellitus. *Curr Diabetes Rev* 2007;3:252–259.
23. Clark LA, Watson D. Tripartite model of anxiety and depression: psychometric evidence and taxonomic implications. *J Abnorm Psychol* 1991;100:316–336.
24. Garber J, Weersing VR. Comorbidity of Anxiety and Depression in Youth: Implications for Treatment and Prevention. *Clin Psychol* 2010;17:293–306.
25. Phillips BM, Lonigan CJ, Driscoll K, *et al.* Positive and negative affectivity in children: a multitrait-multimethod investigation. *J Clin Child Adolesc Psychol* 2002;31:465–479.
26. Caspi A, Roberts BW, Shiner RL. Personality development: stability and change. *Annu Rev Psychol* 2005;56:453–84.
27. Golden SH, Lazo M, Carnethon M, *et al.* Examining a bidirectional association between depressive symptoms and diabetes. *JAMA 2008*;29:2751–2759.
28. Holt RI, Phillips DI, Jameson KA, *et al.* The relationship between depression and diabetes mellitus: findings from the Hertfordshire Cohort Study. *Diabet Med* 2009;26:641–648.
29. Erickson SJ, Robinson TN, Haydel KF, *et al.* Are overweight children unhappy? Body mass index, depressive symptoms, and overweight concerns in elementary school children. *Arch Pediatr Adolesc Med* 2000;154:931–935.
30. Goodman E, Whitaker RC. A prospective study of the role of depression in the development and persistence of adolescent obesity. *Pediatrics* 2002;110:497–504.
31. Jaser SS, Holl MG, Jefferson V, *et al.* Correlates of depressive symptoms in urban youth at risk for type 2 diabetes mellitus. *J Sch Health* 2009;79:286–292.
32. Louise S, Warrington NM, McCaskie PA, *et al.* Associations between anxious-depressed symptoms and cardiovascular risk factors in a longitudinal childhood study. *Prev Med* 2012;54:345–350.
33. Shomaker LB, Tanofsky-Kraff M, Young-Hyman D, *et al.* Psychological symptoms and insulin sensitivity in adolescents. *Pediatr Diabetes* 2010; 11:417–423.
34. Jeffery AN, Hyland ME, Hosking J, *et al.* Mood and its association with metabolic health in adolescents: a longitudinal study, EarlyBird 65. *Pediatr Diabetes* 2014 [epub ahead of print].
35. Crawford JR, Henry JD. The positive and negative affect schedule (PANAS): construct validity, measurement properties and normative data in a large non-clinical sample. *Br J Clin Psychol* 2004;43:245–265.
36. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol* 1988; 54:1063–1070.
37. Laurent J, Catanzaro SJ, Rudoph KD, *et al.* A Measure of Positive and Negative Affect for Children: Scale Development and Preliminary Validation. *Psychol Assess* 1999; 11:326–338.
38. Metcalf BS, Voss LD, Hosking J, *et al.* Physical activity at the government-recommended level and obesity-related health outcomes: a longitudinal study (Early Bird 37). *Arch Dis Child* 2008;93:772–777.
39. Fernandez-Guasti A, Fiedler JL, Herrera L, *et al.* Sex, stress, and mood disorders: at the intersection of adrenal and gonadal hormones. *Horm Metab Res* 2012; 44:607–618.