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ORIGINAL ARTICLE

Metabolic, cardiovascular and anthropometric differences between prepubertal girls and boys

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Summary

Objective We aimed to assess possible differences in insulin sensitivity and other metabolic, anthropometric and cardiovascular parameters between boys and girls prior to puberty.

Methods We studied 85 healthy prepubertal children (33 girls and 52 boys) aged 8·7 ± 1·9 years (range 4·0–11·9 years), born 38–40 weeks gestation, and of birth weight appropriate-for-gestational-age. Insulin sensitivity was measured using frequently sampled intravenous glucose tests and Bergman's minimal model. Other clinical assessments included anthropometric measures, fasting lipid and hormonal profiles, body composition from whole-body dual-energy X-ray absorptiometry and 24-h ambulatory blood pressure monitoring.

Results Prepubertal girls and boys were of similar parent-adjusted height SDS ($P = 0{\cdot}26$), but girls had considerably more body fat ($P < 0{\cdot}0001$), less fat-free mass ($P = 0{\cdot}0002$) and greater abdominal adiposity ($P < 0{\cdot}0001$). These differences in body composition were independent of adrenal androgens. Insulin sensitivity was 18% lower in girls (11·0 vs $13{\cdot}4 \times 10^{-4}$/min (mU/l); $P = 0{\cdot}028$), but this difference disappeared with adjustment for adiposity and DHEAS concentrations. There were, however, some apparent sex differences in cardiovascular parameters, with girls displaying increased heart rate and reduced blood pressure dipping. Girls also had higher triglyceride concentrations (+23%; $P = 0{\cdot}036$).

Conclusion There are a number of anthropometric, metabolic and cardiovascular differences between sexes prior to the appearance of external signs of puberty. Although differences in insulin sensitivity were eliminated when adiposity and DHEAS concentrations were accounted for, there were independent differences in body composition and cardiovascular parameters. Thus, gender, adrenarche and adiposity should be accounted for in studies examining metabolic and cardiovascular outcomes prior to puberty.

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Abbreviations

BMI	body mass index
BMI SDS	body mass index standard deviation score
CV	coefficient of variation
DHEAS	dehydroepiandrosterone sulphate
HDL-C	high-density lipoprotein cholesterol
Height SDS – MPH SDS	height standard deviation score corrected for mid-parental height standard deviation score
IGF-I	insulin-like growth factor I
IGFBP-3	insulin-like growth factor binding protein-3
LDL-C	low-density lipoprotein cholesterol
MPH SDS	mid-parental height standard deviation score
SDS	standard deviation score

Introduction

There are known differences in body composition between males and females that appear to occur early in childhood and accentuate with pubertal development.[1,2] However, there is a paucity of data on sex differences in metabolic and cardiovascular parameters in healthy developmentally normal children. In particular, there is a lack of robust data on insulin sensitivity in boys and girls prior to pubertal onset. Hence, in this study, we aimed to assess possible differences in insulin sensitivity and other metabolic, anthropometric and cardiovascular parameters between prepubertal boys and girls.

Methods

Ethics approval

Ethics approval for this study was provided by the Northern Y Regional Ethics Committee (Ministry of Health, New Zealand).

Written informed consent was obtained from parents or guardians, as well as verbal or written consent from each child as was appropriate to their age.

Participants

This study involved the retrospective analyses of a cohort recently studied to identify the effects of birth order on insulin sensitivity.[3] In summary, all recruited children were healthy, naturally conceived, aged 4–11 years, born at term (38–40 weeks gestation), from singleton pregnancies and of birth weight appropriate-for-gestational-age [birth weight −2 to 2 standard deviation scores (SDS)]. All participants were evaluated by a single paediatric endocrinologist and determined to be Tanner stage 1. Any subjects with signs of puberty (Tanner stage 2 breast development in girls and testicular volume >3 ml in boys) or evidence of pubarche (i.e. pubic or axillary hair) were excluded. Other exclusion criteria were genetic syndromes, receiving medication that could affect insulin sensitivity, as well as having a first-degree relative or grandparent with diabetes, the metabolic syndrome or any of its features other than central adiposity. Children were also excluded if born to mothers with gestational diabetes, pre-eclampsia, gestational or pre-existing hypertension, chronic illnesses or maternal drug use during pregnancy (including tobacco and alcohol).

Clinical assessments

All children were assessed at the Maurice & Agnes Paykel Clinical Research Unit (Liggins Institute, University of Auckland). Data on each child were collected during a single visit to the clinic. A number of neonatal parameters were recorded, including birth weight and gestational age. Birth weight data were transformed into SDS.[4]

Insulin sensitivity was assessed using a 90-min modified frequently sampled intravenous glucose test (FSIGT), modified with insulin, and analysed using Bergman's minimal model software.[5] Three baseline samples were drawn at −20, −10 and 0 min. A 25% dextrose infusion (at 0·3 g/kg) started at 0 min and lasted for 1 min. Blood samples were drawn at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 and 19 min. Insulin (0·015 units/kg) was then intravenously administered as a bolus at 20 min, and further samples were drawn at 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 60, 70, 80 and 90 min. Note that no episodes of hypoglycaemia (blood glucose concentration <4 mmol/l) were recorded in any of the participants throughout the study. Secondary outcomes from the FSIGT included acute insulin release (insulin secretory capacity) and glucose effectiveness (glucose-mediated glucose uptake).

Children's heights were measured using a Harpenden stadiometer. Weight and body composition data were obtained using whole-body dual-energy X-ray absorptiometry (DXA, Lunar Prodigy 2000; General Electric, Madison, WI, USA), namely total body fat, truncal fat and android fat to gynoid fat ratio (a more specific measure of central adiposity). The latter parameters are provided by the manufacturer's software based on automated sectioning of specific areas of the body.[6] Height SDS was derived from Tanner/Whitehouse reference data[7] and body mass index (BMI) SDS according to British 1990 standards.[8] Parental weights and heights were measured by the investigators in 95% of mothers and 80% of fathers, with the remaining measurements reported. Mean parental BMI was calculated as the average of maternal and paternal BMI. Mid-parental height SDS (MPH SDS) was calculated using standard formulae.[9] Each child's height SDS was then individually corrected for their genetic potential (parents' heights), using the formula: 'Height SDS – MPH SDS'.[9] Ethnicity was recorded by self-report using a prioritized system, such that if multiple ethnicities were selected, the patient was assigned to a single category, following a hierarchical system of classification.[10]

Following an overnight fast, baseline blood samples were drawn to measure serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), insulin-like growth factor I (IGF-I), IGF binding protein 1 (IGFBP-1), IGFBP-3, leptin, adiponectin, androstenedione, dehydroepiandrosterone sulphate (DHEAS), glucose and insulin concentrations.

Twenty-four-hour ambulatory blood pressure was assessed following the clinical visit, when participants were fitted with a Spacelabs 90217 monitor (Spacelabs Medical Inc., Redmond, WA, USA) on the nondominant arm. Measurements were performed every 20 min from 07:00 to 22:00, and every 30 min from 22:00 to 07:00. Only profiles with more than 14 daytime and 7 nocturnal readings over a 24-h period were included for analysis (as per British Hypertension Society recommendations). Reported parameters included daytime and nocturnal systolic and diastolic blood pressure, and nocturnal systolic and diastolic blood pressure dipping.

Assays

Glucose concentrations were measured on a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) by enzymatic colorimetric assay (Roche, Mannheim, Germany), with an interassay coefficient of variation (CV) of 2·1%. Insulin concentrations were measured using an Abbott AxSYM system (Abbott Laboratories, Abbott Park, IL, USA) by microparticle enzyme immunoassay, with a CV of 5·7%. HDL-C, LDL-C and total cholesterol concentrations were measured using a Hitachi 902 autoanalyser, with CV of 11·4, 10·1 and 8·9%, respectively. Commercially available ELISA kits E20, E01, E03A, E07 and E09 (Mediagnost, Reutlingen, Germany) were used for quantitative determination of serum IGF-I, IGFBP-1, IGFBP-3, leptin and adiponectin concentrations, respectively; assay sensitivities were 0·09, 0·2, 0·1, 1·0 and 0·6 ng/ml, with CV of 3·1, 9·4, 9·6, 6·7 and 3·0%, respectively. DHEAS and androstenedione concentrations were measured using Finnigan TSQ Quantum Ultra AM triple quadrupole mass spectrometer controlled by Finnigan Xcaliber software (Thermo Electron Corporation, San Jose, CA, USA), with mean CV of 18·4 and 8·2%, respectively.

Statistical analyses

Baseline data were compared using one-way ANOVA and Fisher's exact tests (Minitab v.16, Pennsylvania State University, State College, PA, USA). Random-effect mixed models were used to compare the outcomes of interest between girls and boys. Models included maternal identification number as a random factor to account for the clustering of siblings. Initially, age and birth parameters were adjusted for in the analyses, namely ethnicity, gestational age, birth weight SDS and birth order. Subsequent models were run with the inclusion of DHEAS concentrations and total body fat percentage as confounders. Note when examining cardiovascular parameters, height was included instead of age; for body composition, total body fat percentage was replaced by mean parental BMI. Multivariate analyses were performed using SAS v.9.3 (SAS Institute Inc. Cary NC, USA). Parameters associated with glucose homeostasis were log-transformed to approximate normality. All statistical tests were two-tailed and maintained at a 5% significance level. Age and birth data are presented as means ± standard deviations. Outcome data are presented as means ± standard errors of the mean (SEM), as well as model-adjusted means (estimated marginal means adjusted for the confounding factors in the models), with associated 95% confidence intervals.

Results

In total, 85 children aged 8·7 ± 1·9 years participated in the study, including 33 girls (age range 4·0–11·6 years) and 52 boys (4·0–11·9 years). Children of both sexes were of similar age and ethnic composition and were also born of similar gestational age and birth weight (Table 1). Girls had higher concentrations of both androstenedione and DHEAS (Table 1).

Anthropometry

Girls and boys were of similar parent-adjusted height SDS, but there were major differences in body composition between sexes, independently of adrenal androgens (Table 2). Girls had more body fat ($P = 0{\cdot}0002$) than boys, as well as greater central adiposity, with higher android to gynoid fat ratio ($P = 0{\cdot}006$) and more truncal fat ($P < 0{\cdot}0001$; Table 2).

Table 1. Demographic and adrenal androgen data for prepubertal boys and girls. Demographic and adrenal androgen data are means ± standard deviations

	Girls	Boys	*P*-value
n	33	52	
Demography			
Age (years)	8·4 ± 1·9	8·9 ± 1·9	0·19
Gestational age (weeks)	39·4 ± 0·7	39·4 ± 0·7	0·80
Birth weight (kg)	3·42 ± 0·35	3·52 ± 0·38	0·24
Birth weight SDS	0·14 ± 0·79	0·12 ± 0·86	0·92
Ethnicity (New Zealand European)	79%	77%	0·99
Adrenal androgens			
Androstenedione (nmol/l)	0·59 ± 0·34	0·42 ± 0·29	0·009
DHEAS (nmol/l)	4·66 ± 3·75	3·15 ± 3·23	0·026

Insulin sensitivity and parameters of glucose homeostasis

Insulin sensitivity was 18% lower in girls (11·0 vs $13{\cdot}4 \times 10^{-4}{\cdot}\,min^{-1}{\cdot}$(mU/l); $P = 0{\cdot}028$), but this difference disappeared with adjustment for adiposity and DHEAS concentrations (Table 2). Although the addition of either of these two parameters to the model eliminated the significant gender difference in insulin sensitivity, there was a strong negative association of adiposity and insulin sensitivity ($P = 0{\cdot}0002$), which was much stronger than that of DHEAS ($P = 0{\cdot}15$). Acute insulin response, disposition index and glucose effectiveness were similar in boys and girls (Table 2).

Lipid profile

Total cholesterol, LDL-C and HDL-C concentrations were similar in girls and boys. However, girls displayed triglyceride concentrations that were consistently higher than that of boys ($P = 0{\cdot}036$; Table 2).

Twenty-four-hour ambulatory blood pressure monitoring

Prior to adjustment for body fat and adrenal androgens, there were many cardiovascular differences between girls and boys (Table 2). Girls displayed higher heart rate ($P = 0{\cdot}003$), higher nocturnal systolic ($P = 0{\cdot}050$) and diastolic ($P = 0{\cdot}023$) blood pressure, as well as lower nocturnal systolic ($P = 0{\cdot}003$) and diastolic ($P = 0{\cdot}005$) dipping (Table 2). However, even after adjustment for body fat and DHEAS concentrations, girls still displayed higher heart rate (+5·6 bpm; $P = 0{\cdot}044$) and lower systolic dip ($P = 0{\cdot}013$; Table 2).

Growth factors and hormone concentrations

Leptin concentrations were 67% higher in girls than in boys ($P = 0{\cdot}002$), consistent with body composition data (i.e. greater body fat). Not surprisingly, leptin differences disappeared following adjustment for body fat and DHEAS concentrations (Table 2). There were no differences between genders in concentrations of adiponectin or growth factors, but girls had IGFBP-3 concentrations that were 16% higher than those of boys ($P = 0{\cdot}016$; Table 2).

Ethnicity

Statistical models were re-run including solely the 66 participants of New Zealand European ethnicity. The results were nearly identical (data not shown) except that most *P*-values changed due a 22% reduction in *n*, corroborating the findings obtained for the overall cohort.

Table 2. Study outcomes among prepubertal boys and girls

	Unadjusted means ± SEM		Adjusted means and 95% confidence intervals		
	Girls	Boys	Girls	Boys	*P*-value
n	33	52	33	52	
Anthropometry					
Height SDS – MPH SDS	−0·24 ± 0·18	−0·07 ± 0·12	−0·06 (−0·49–0·36)	0·25 (−0·10–0·61)	0·18
BMI SDS	0·06 ± 0·20	0·32 ± 0·11	−0·01 (−0·37–0·35)	0·41 (0·10–0·72)	0·025
Fat-free mass (%)	78·0 ± 1·3	83·1 ± 0·9****	77·4 (74·4–80·3)	83·3 (80·8–85·9)	0·0003
Total body fat (%)	22·8 ± 1·3	17·6 ± 0·9****	22·9 (19·6–26·8)	16·5 (14·4–18·9)	0·0002
Android fat to gynoid fat ratio	0·66 ± 0·03	0·59 ± 0·02****	0·65 (0·57–0·73)	0·54 (0·48–0·60)	0·006
Truncal fat (%)	20·0 ± 1·5	14·6 ± 1·0****	19·2 (15·8–23·3)	12·5 (10·6–14·8)	<0·0001
Glucose homeostasis					
Insulin sensitivity ($\times 10^{-4}\cdot min^{-1}\cdot$(mU/l))	11·0 ± 0·9	13·4 ± 0·8*	9·3 (7·8–11·1)	9·7 (8·4–11·2)	0·84
Acute insulin response (mU/l)	357 ± 66	281 ± 24	310 (237–406)	277 (223–345)	0·49
Disposition index	3029 ± 369	3485 ± 326	2706 (2006–3649)	2711 (2124–3460)	0·93
Glucose effectiveness (10^{-2}/min)	2·75 ± 0·28	2·92 ± 0·19	2·63 (2·00–3·25)	2·50 (2·00–3·00)	0·73
Growth factors					
IGF-I (ng/ml)	189 ± 13	173 ± 8	193 (161–224)	181 (156–206)	0·55
IGFBP-1 (ng/ml)	14·5 ± 1·3	17·3 ± 1·7	10·8 (7·7–15·1)	10·8 (8·2–14·2)	0·99
IGFBP-3 (ng/ml)	3454 ± 154	3209 ± 90	3428 (3110–3746)	2958 (2700–3216)	0·016
Hormones					
Adiponectin (μg/ml)	10 161 ± 570	10 639 ± 600	9257 (7572–11317)	10 220 (8655–12069)	0·41
Leptin (ng/ml)	5·71 ± 1·11	3·42 ± 0·52**	3·29 (2·63–4·11)	3·14 (2·61–3·77)	0·73
Lipid profile					
Total cholesterol (mmol/l)	3·88 ± 0·12	3·90 ± 0·11	3·86 (3·49–4·23)	3·85 (3·54–4·15)	0·95
HDL-C (mmol/l)	1·23 ± 0·04	1·36 ± 0·04	1·29 (1·17–1·42)	1·33 (1·22–1·44)	0·53
LDL-C (mmol/l)	2·31 ± 0·10	2·24 ± 0·09	2·27 (1·97–2·57)	2·20 (1·96–2·44)	0·68
Triglycerides (mmol/l)	0·79 ± 0·05	0·70 ± 0·04*	0·76 (0·64–0·91)	0·62 (0·54–0·72)	0·036
Total cholesterol to HDL-C ratio	3·19 ± 0·10	3·02 ± 0·10	2·98 (2·69–3·30)	2·95 (2·71–3·20)	0·87
24-h ambulatory blood pressure					
Heart rate (bpm)	88·0 ± 1·6	80·0 ± 1·5**	84·5 (79·9–89·1)	78·9 (74·7–83·1)	0·044
Daytime systolic (mmHg)	110·3 ± 1·4	112·6 ± 1·5	107·8 (103·2–112·4)	112·8 (108·8–116·9)	0·059
Daytime diastolic (mmHg)	67·4 ± 1·1	68·6 ± 1·0	66·9 (63·5–70·3)	68·4 (65·4–71·4)	0·44
Nocturnal systolic (mmHg)	99·9 ± 1·6	97·9 ± 1·2*	99·0 (94·1–103·9)	98·5 (94·3–102·8)	0·86
Nocturnal diastolic (mmHg)	57·2 ± 1·1	54·9 ± 0·8*	56·4 (53·1–59·8)	54·9 (52·0–57·8)	0·40
Systolic dip (%)	9·2 ± 0·9	13·0 ± 0·7**	7·9 (5·1–10·7)	11·9 (9·4–14·4)	0·013
Diastolic dip (%)	14·6 ± 1·3	19·7 ± 1·0**	14·4 (10·4–18·4)	18·3 (14·7–21·9)	0·068

*$P < 0·05$, **$P < 0·01$ and ****$P < 0·0001$ for boys vs girls accounting for birth parameters (ethnicity, gestational age, birth weight SDS and birth order) and age (or height for blood pressure). Adjusted means and 95% confidence intervals have been adjusted for all confounding factors in the multivariate models, including the above-described parameters, as well as total body fat percentage (except for anthropometry) and DHEAS concentrations.

Discussion

Our study shows that there are anthropometric, metabolic and cardiovascular differences between boys and girls prior to the appearance of any external signs of puberty. Girls displayed lower insulin sensitivity, higher triglyceride concentrations, as well as more body fat and greater abdominal adiposity, increased heart rate, higher nocturnal blood pressure and lower nocturnal blood pressure dipping than boys. Although all children were prepubertal, girls had higher adrenal androgen concentrations and greater adiposity than boys throughout childhood, which accounted for the differences in insulin sensitivity.

The limited data on differences in insulin sensitivity between prepubertal boys and girls have been inconsistent. A previous study using FSIGT did not observe a gender effect on insulin sensitivity during the prepubertal years.[11] Conversely, Moran *et al.*[12] suggested that there were differences between boys and girls during all Tanner stages, but they only examined three girls in Tanner stage 1. Our analyses showed differences in insulin sensitivity between sexes, which were primarily associated with girls' greater adiposity, with an added contribution from higher adrenal androgen concentrations.

Our findings corroborate previous studies showing that greater fat mass is associated with reduced insulin sensitivity in children and adolescents.[5,13] Increased adiposity in prepubertal 7-year-old children has been shown to be associated with increased DHEAS concentrations.[14] Thus, it is not easy to distinguish the effects of greater adiposity from higher DHEAS levels on insulin sensitivity in children. There is indirect evidence to suggest that adrenarche is associated with reduced insulin sensitivity.[15] A longitudinal study found that early reductions in insulin sensitivity (from the age of 7 years) occur 3–4 years before the onset of puberty,[15]

which would be consistent with adrenarche. Thus, we propose that the lower insulin sensitivity seen in girls was due to increased adiposity and higher adrenal androgen levels.

Importantly, there were differences in body composition and cardiovascular parameters between the sexes that were not attributable to adrenal androgen levels and/or adiposity. Our observation that girls had more body fat than boys is in agreement with previous data showing similar findings prior to puberty.[16–18] Girls in our cohort also displayed greater abdominal adiposity than boys. While a similar finding was observed in 11-year-old children,[19] most previous studies in prepubertal boys and girls showed no gender differences in visceral and/or abdominal fat.[18,20,21] In this study, apart from contributing to the lower insulin sensitivity, increased fat mass was also likely associated with higher nocturnal blood pressure in girls.

The persisting differences in nocturnal blood pressure dipping and heart rate between prepubertal boys and girls appear to be novel findings. Although it has been shown in late childhood and adolescence that girls have lower blood pressure than boys,[22,23] data from ambulatory blood pressure monitoring comparing sexes in nonobese prepubertal children appear to be lacking. The higher heart rate and lower nocturnal blood pressure dipping in girls are not easily explained, but possible differences in fitness between the two sexes may exist.

Although all children in this study were assessed as prepubertal by a paediatric endocrinologist, the observed sex differences in DHEAS and androstenedione concentrations indicate that there were underlying hormonal differences between boys and girls. Rising plasma levels of adrenal androgens, particularly DHEAS, are the first recognizable hormonal changes associated with pubertal development and generally occur independently of sexual maturation.[24] These hormonal differences could contribute to the increased fat mass deposition in girls. In addition, we also observed higher leptin concentrations in girls than in boys, differences that were eliminated following adjustment for body fat and DHEAS concentrations. Leptin signals the amount of adipose tissue deposition to the brain and exerts major effects on energy homeostasis and neuroendocrine function, and a leptin surge is observed prior to pubertal onset.[24]

There are a number of anthropometric, metabolic and cardiovascular differences between sexes prior to the appearance of external signs of puberty. Although differences in insulin sensitivity are eliminated when adiposity and DHEAS concentrations are accounted for, there are independent differences in body composition and cardiovascular parameters between boys and girls. Thus, gender, adrenarche and adiposity should be accounted for in studies examining metabolic and cardiovascular outcomes prior to puberty.

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Author contributions

AA, WSC, PLH and JGBD conceived and designed the study. AA and JB recruited and performed the tests. AA and JGBD collected and compiled the data, which were analysed by JGBD. JGBD wrote the manuscript with input from other authors. All authors have approved the submission of the final version of this manuscript.

References

1 Kirchengast, S. (2010) Gender differences in body composition from childhood to old age: an evolutionary point of view. *Journal of Life Science*, **2**, 1–10.
2 Kirchengast, S. & Marosi, A. (2008) Gender differences in body composition, physical activity, eating behavior and body image among normal weight adolescents–an evolutionary approach. *Collegium Antropologicum*, **32**, 1079–1086.
3 Ayyavoo, A., Savage, T., Derraik, J.G.B. *et al.* (2013) First-born children have reduced insulin sensitivity and higher daytime blood pressure compared to later-born children. *Journal of Clinical Endocrinology & Metabolism*, **98**, 1248–1253.
4 Niklasson, A., Ericson, A., Fryer, J. *et al.* (1991) An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age. *Acta Paediatrica*, **80**, 756–762.
5 Cutfield, W.S., Bergman, R.N., Menon, R.K. *et al.* (1990) The modified minimal model: a novel tool to measure insulin sensitivity in children. *Journal of Clinical Endocrinology & Metabolism*, **70**, 1644–1650.
6 Novotny, R., Going, S., Teegarden, D. *et al.* (2007) Hispanic and Asian pubertal girls have higher android/gynoid fat ratio than whites. *Obesity*, **15**, 1565–1570.
7 Tanner, J. & Whitehouse, R. (1976) Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Archives of Disease in Childhood*, **51**, 170–179.
8 Cole, T.J., Freeman, J.V. & Preece, M.A. (1995) Body mass index reference curves for the UK, 1990. *Archives of Disease in Childhood*, **73**, 25.
9 Tanner, J., Goldstein, H. & Whitehouse, R. (1970) Standards for children's height at ages 2-9 years allowing for height of parents. *Archives of Disease in Childhood*, **45**, 755–762.
10 Douglas, N.M. & Dockerty, J.D. (2007) Survival by ethnicity for children diagnosed with cancer in New Zealand during 1990–1993. *Journal of Paediatrics & Child Health*, **43**, 173–177.
11 Gower, B.A., Nagy, T.R. & Goran, M.I. (1999) Visceral fat, insulin sensitivity, and lipids in prepubertal children. *Diabetes*, **48**, 1515–1521.

12 Moran, A., Jacobs, D.R., Steinberger, J. *et al.* (1999) Insulin resistance during puberty: results from clamp studies in 357 children. *Diabetes*, **48**, 2039–2044.
13 Cali, A.M. & Caprio, S. (2008) Obesity in children and adolescents. *Journal of Clinical Endocrinology & Metabolism*, **93**, S31–S36.
14 Corvalan, C., Uauy, R. & Mericq, V. (2013) Obesity is positively associated with dehydroepiandrosterone sulfate concentrations at 7 y in Chilean children of normal birth weight. *American Journal of Clinical Nutrition*, **97**, 318–325.
15 Jeffery, A.N., Metcalf, B.S., Hosking, J. *et al.* (2012) Age before stage: insulin resistance rises before the onset of puberty: a 9-year longitudinal study (EarlyBird 26). *Diabetes Care*, **35**, 536–541.
16 Taylor, R.W., Gold, E., Manning, P. *et al.* (1997) Gender differences in body fat content are present well before puberty. *International Journal of Obesity & Related Metabolic Disorders*, **21**, 1082–1084.
17 Cole, Z.A., Harvey, N.C., Kim, M. *et al.* (2012) Increased fat mass is associated with increased bone size but reduced volumetric density in pre pubertal children. *Bone*, **50**, 562–567.
18 Arfai, K., Pitukcheewanont, P.D., Goran, M.I. *et al.* (2002) Bone, muscle, and fat: sex-related differences in prepubertal children. *Radiology*, **224**, 338–344.
19 Fox, K., Peters, D., Armstrong, N. *et al.* (1993) Abdominal fat deposition in 11-year-old children. *International Journal of Obesity & Related Metabolic Disorders*, **17**, 11–16.
20 Liem, E.T., De Lucia Rolfe, E., L'Abee, C. *et al.* (2009) Measuring abdominal adiposity in 6 to 7-year-old children. *European Journal of Clinical Nutrition*, **63**, 835–841.
21 Nagy, T.R., Gower, B.A., Trowbridge, C.A. *et al.* (1997) Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children. *Journal of Clinical Endocrinology & Metabolism*, **82**, 2148–2152.
22 Harshfield, G.A., Alpert, B.S., Pulliam, D.A. *et al.* (1994) Ambulatory blood pressure recordings in children and adolescents. *Pediatrics*, **94**, 180–184.
23 Syme, C., Abrahamowicz, M., Leonard, G.T. *et al.* (2009) Sex differences in blood pressure and its relationship to body composition and metabolism in adolescence. *Archives of Pediatrics & Adolescent Medicine*, **163**, 818–825.
24 Mantzoros, C.S., Flier, J.S. & Rogol, A.D. (1997) A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. *Journal of Clinical Endocrinology & Metabolism*, **82**, 1066–1070.