

PAPER

Serum lipids, serum insulin, plasma fibrinogen and aerobic capacity in obese and non-obese Singaporean boys

DJ Stensel^{1*}, F-P Lin², TF Ho³ and TC Aw⁴

¹Department of Physical Education, Sports Science and Recreation Management, Loughborough University, Loughborough, Leicestershire, UK; ²School of Physical Education, National Institute of Education, Nanyang Technological University, Singapore; ³Department of Physiology, National University of Singapore, Singapore; and ⁴Department of Laboratory Medicine, National University Hospital, Singapore

OBJECTIVES: To compare blood lipids, lipoproteins, apoproteins, fibrinogen, insulin and aerobic capacity in obese and non-obese Chinese Singaporean boys. To examine relationships between blood metabolites, body composition and aerobic capacity in these groups.

DESIGN: Cross-sectional

SUBJECTS: Forty Chinese Singaporean boys aged 13–15 y. Classified as obese ($n = 20$) or non-obese ($n = 20$) based on adiposity (fat mass/fat free mass): $> 0.60 =$ obese, $< 0.40 =$ non-obese.

MEASUREMENTS: Body composition (dual energy X-ray absorptiometry), waist circumference, peak oxygen consumption (VO_2 peak), serum concentrations of total cholesterol, triacylglycerol, high density lipoprotein cholesterol (HDL-C), total cholesterol/HDL-C, apoproteins A1 and B, lipoprotein(a), insulin and glucose. Plasma concentration of fibrinogen.

RESULTS: Obese boys had significantly ($P < 0.01$) higher (mean \pm s.d.) concentrations of serum triacylglycerol (1.51 ± 0.65 vs 1.04 ± 0.34 mmol/l), serum insulin (24.1 ± 11.5 vs 12.3 ± 4.45 mU/l) and plasma fibrinogen (4.01 ± 0.54 vs 3.35 ± 0.76 g/l) than non-obese boys. Within the non-obese group plasma fibrinogen concentration was significantly related to percentage body fat ($r = 0.546$, $P < 0.05$). VO_2 peak relative to body mass (ml/kg/min or ml/kg^{-0.67}/min) was significantly ($P < 0.001$) lower in obese compared to non-obese boys but absolute VO_2 peak (l/min), adjusted for fat-free mass via analysis of covariance, was higher in obese than non-obese boys ($P < 0.01$). Partial correlations revealed that none of the blood metabolites were significantly related to VO_2 peak independent of body fatness.

CONCLUSIONS: Obesity was related to elevated concentrations of serum triacylglycerol, serum insulin and plasma fibrinogen in Chinese Singaporean boys. These elevated concentrations did not appear to be associated with a lower aerobic capacity (independent of body fatness) in the obese.

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Introduction

Obesity prevalence is increasing in both children and adults in many countries around the world.^{1,2} This trend has not escaped the small city state of Singapore where the percentage of schoolchildren (aged 6–18 y) classified as obese

($> 120\%$ of age and sex specific 50th percentile weight-for-height) increased from 5.4 to 15.1% between 1980 and 1991.³ This is a cause for concern because obesity increases all-cause mortality risk⁴ and there is evidence that obesity in youth is a more powerful predictor of this risk than obesity in adulthood.^{5,6}

One morbidity associated with obesity is cardiovascular disease (CVD),⁷ which is invariably related to dyslipidaemia.⁸ This suggests that obesity promotes dyslipidaemia and, although this interpretation is generally accepted, evidence from studies with adults has been equivocal at times with associations varying from weak to very significant.⁹ In chil-

*Correspondence: DJ Stensel, Department of Physical Education, Sports Science and Recreation Management, Loughborough University, Loughborough, Leicestershire LE11 3TU, UK.
E-mail: D.J.Stensel@lboro.ac.uk.

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dren, several studies support the contention that obesity causes dyslipidaemia.^{10–15} This requires verification, however, since these studies used surrogate measures of body fatness such as weight-for-height,^{14,15} body mass index (BMI)^{10,13} or skinfold thickness.^{11,12} Recently attention has focused on abdominal/visceral fat, which appears to be more closely associated with dyslipidaemia than body fatness *per se*.^{16–18}

Additionally, at least one study has now shown a positive relationship between subcutaneous abdominal adipose tissue and plasma fibrinogen concentration in obese children.¹⁹

A possible link between obesity and dyslipidaemia is physical activity. In adults both physical activity and aerobic fitness are associated with healthy lipid profiles and these relationships may be mediated, in part, through body fatness.²⁰ Whether these findings are applicable to children is uncertain. There is evidence that obese children are less active than non-obese children and that they have a lower aerobic capacity.^{21,22} There are also data linking both physical activity and aerobic capacity (VO₂ peak) with favourable blood lipid profiles in children, although results from longitudinal studies are 'unimpressive'²³ and the traditional practice of using body mass as a denominator for VO₂ peak may have led to erroneous conclusions in cross-sectional studies.²⁴

Therefore, the purpose of the present study was to re-examine the links between obesity, aerobic capacity and blood lipids in children using dual-energy X-ray absorptiometry (DXA) to quantify body fat and more appropriate data normalisation procedures for expressing VO₂ peak.^{25,26} In addition to blood lipids, several other blood metabolites indicative of CVD risk were examined in this study including apoproteins AI and B, lipoprotein(a) (Lp(a)) and fibrinogen.

Methods

Subjects

The subjects in this study were 20 obese and 20 non-obese Chinese Singaporean boys aged 12.8–15.1 y (Table 1). Written informed consent was obtained from these boys and from their parents, prior to the commencement of the study. The Ethical Advisory Committee of the School of Physical Education (Nanyang Technological University) also gave its

permission for the study. Boys were classified as obese or non-obese based on their adiposity (ratio of fat mass to fat free mass). Boys with an adiposity of > 0.60 were classified as obese whereas those with an adiposity of < 0.40 were classified as non-obese. These values were chosen arbitrarily to obtain two distinctly different groups with respect to body fatness. The pubertal status of the boys was not determined but the groups did not differ significantly with respect to age, height and fat-free mass.

Body composition

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain, Dyfed, UK). Body mass was measured to the nearest 0.01 kg on an electronic weighing scale (Mettler Toledo IDL Plus, Eichfahig, Germany), with the subjects wearing socks and shorts. BMI was calculated as weight (kg)/height (m²). The waist/hip ratio (WHR) was determined from waist (minimal circumference of the abdomen) and hip (greatest circumference over the greater trochanters) circumferences measured to the nearest 1.0 cm using a plastic tape. DXA (Lunar DPX-L, software version 1.31, USA) was used to measure each subject's fat mass, fat-free mass and percentage body fat. The DXA scans were performed in the Orthopaedic Diagnostic Centre at the National University Hospital, Singapore.

Blood metabolites

Boys reported to the Blood Donation Centre at the National University Hospital, Singapore, in the morning following a 12 h, overnight fast. They were asked to sit on a couch while nurses collected a 20 ml venous blood sample. These samples were analysed on the day of collection for concentrations of serum total cholesterol, triacylglycerol, HDL-C, insulin and glucose. Plasma fibrinogen was also determined on the day of collection. Further samples of serum were frozen at –70°C and analysed within 1 week to determine serum concentrations of apoprotein AI, apoprotein B and Lp(a).

Blood biochemistry was conducted in the Department of Laboratory Medicine (National University Hospital, Singapore), which is accredited by the College of American Pathologists. Total cholesterol, triacylglycerol and HDL-C were measured by standard colorimetry using an auto-analyser (Integra 700, Roche Diagnostics). Apoprotein AI, apoprotein B and Lp(a) were measured by an automated immuno-nephelometric method (Array 360, Beckman-Coulter Diagnostics). Glucose was measured by an enzymatic method on an auto-analyser (Vitros 950, Ortho Clinical Diagnostics). Insulin was measured by micro-particle enzyme immunoassay (MEIA) on an automated immunoassay analyser (AxSYM, Abbott Diagnostics). Fibrinogen was measured by a photo-optical clot detection method on an automated coagulation analyser (ACL Futura Plus, Instrumentation Laboratory).

Table 1 Age, height and weight of obese and non-obese boys

	Obese (n = 20)	Non-obese (n = 20)	Significance (two-tailed)
Age (y)	13.6 ± 0.5 (12.8–14.5)	13.7 ± 0.6 (12.8–15.1)	0.580
Height (m)	1.64 ± 0.09 (1.46–1.83)	1.64 ± 0.06 (1.48–1.73)	0.822
Weight (kg)	74.8 ± 12.3 (51.1–100.8)	61.8 ± 10.9 (43.0–85.0)	0.001

Values are mean ± s.d. (minimum value and maximum value).

VO₂ peak

Each boy performed a treadmill familiarisation on a separate day prior to VO₂ peak testing. VO₂ peak was determined via a maximal treadmill (18-60, Quinton Instrument, USA) walking test using a modified Balke protocol. A metabolic cart (Sensormedics 2900Z, USA) was used to collect and analyse expired gas samples. This metabolic cart was calibrated prior to each testing session using reference gases. Following calibration a half-face mask with a two-way breathing valve (2700, Hans Rudolph Inc., Kansas, USA) was fitted to each subject and connected by respiratory tubes to the metabolic cart. The subjects then walked for 1 min at 5.0 km/h on a level gradient. The test started immediately following this warm-up. The walking speed for the test was pre-selected and varied between 5.0 and 5.5 km/h based on data from familiarisation tests. The initial gradient was 6% and there was a 3% increase in gradient every 3 min. Subjects were verbally encouraged to continue walking until they could no longer maintain the required speed. The test was terminated when the subjects indicated that they were unable to continue. Expired air was collected throughout the test and heart rate was monitored continuously using short range telemetry (Polar PE 4000, Finland). Finger-prick blood samples were collected 2 min after the test for determination of whole blood lactate concentration via a portable lactate analyser (Accusport: Boehringer Mannheim, Germany). Criteria for maximal effort included two or more of the following: a respiratory exchange ratio ≥ 1.0 ; a heart rate ≥ 195 beat/min; or a blood lactate concentration ≥ 5.8 mmol/l.

Statistics

The Statistical Package for Social Sciences (SPSS Version 9.0) was used to analyse the data. Differences between obese and non-obese boys were assessed using two tailed *t*-tests for independent samples. When comparing VO₂ peak values (l/min), analysis of covariance was employed to adjust for the confounding influence of fat free mass as suggested by Toth and colleagues.²⁵ VO₂ peak values were also compared between obese and non-obese boys using a mass exponent of 0.67 (ml/kg^{-0.67}/min) as suggested by Welsman and co-workers.²⁶ Relationships between variables were analysed using Pearson's product moment correlation with obese and non-obese boys analysed separately. Where necessary, partial correlation was used to control for possible confounding variables. Significance was set at $P < 0.05$. Data are displayed as mean \pm standard deviation (s.d.).

Results

Age and height did not differ significantly between groups but the obese boys were an average of 13 kg heavier than the non-obese boys (Table 1). This excess weight was wholly due to excess fat (Table 2). The average obese boy carried 16 kg excess fat compared to the average non-obese boy. Fat-free mass was lower in obese compared to non-obese boys but

this difference was not significant. The percentage body fat data shows that these two groups were very different and there was no overlap between groups. Body mass index, waist circumference and WHR were also significantly different between groups.

Table 3 displays the blood metabolites examined in this study for obese and non-obese boys. Serum triacylglycerol, serum insulin and plasma fibrinogen concentration were all significantly higher in obese compared to non-obese boys. None of the other blood metabolites differed significantly between the obese and non-obese groups.

VO₂ peak values (Table 4) did not differ between groups when expressed in absolute terms (l/min) but were significantly lower in the obese boys when divided by body mass (ml/kg/min) or scaled using a mass exponent of 0.67 (ml/kg^{-0.67}/min). Since fat-free mass was highly correlated with absolute VO₂ peak ($r = 0.93$ for obese boys and $r = 0.91$ for non-obese boys, both $P < 0.001$) these values (l/min) were compared using analysis of covariance to control for differences in fat-free mass. When this was done, VO₂ peak was

Table 2 Body composition variables for the obese and non-obese boys

	Obese (n = 20)	Non-obese (n = 20)	Significance (two-tailed)
Percentage body fat (%)	42.5 \pm 3.5 (39.0–51.3)	24.1 \pm 4.3 (12.4–28.3)	0.001
BMI (kg/m ²)	27.7 \pm 2.9 (23.1–35.4)	22.8 \pm 3.0 (19.3–30.6)	0.001
Adiposity (FM/FFM)	0.75 \pm 0.12 (0.64–1.06)	0.32 \pm 0.07 (0.14–0.39)	0.001
Fat mass (kg)	30.1 \pm 6.2 (19.4–41.7)	14.1 \pm 4.1 (7.5–23.0)	0.001
Fat-free mass (kg)	40.5 \pm 6.9 (26.7–56.0)	44.1 \pm 7.6 (31.7–58.4)	0.188
Waist circumference (cm)	90.8 \pm 8.1 (76.2–110.2)	76.1 \pm 6.0 (66.7–89.8)	0.001
Waist-hip ratio	0.90 \pm 0.04 (0.80–0.96)	0.84 \pm 0.04 (0.77–0.91)	0.001

Values are mean \pm s.d. (minimum value and maximum value). FM = fat mass; FFM = fat-free mass.

Table 3 Blood metabolites in obese and non-obese boys

	Obese (n = 20)	Non-obese (n = 20)	Significance (two-tailed)
TC (mmol/l)	4.41 \pm 0.60	4.04 \pm 0.89	0.132
Triacylglycerol (mmol/l)	1.51 \pm 0.65	1.04 \pm 0.34	0.007
HDL-C (mmol/l)	1.05 \pm 0.16	1.11 \pm 0.24	0.421
TC/HDL-C	4.30 \pm 0.97	3.77 \pm 1.00	0.096
Apoprotein AI (mg/dl)	118.5 \pm 11.2	113.9 \pm 16.4	0.315
Apoprotein B (mg/dl)	88.0 \pm 22.8	76.5 \pm 18.8	0.090
Lp(a) (mg/dl)	15.7 \pm 14.2	24.4 \pm 25.3	0.188
Fibrinogen (g/l)	4.01 \pm 0.54	3.35 \pm 0.76	0.003
Insulin (mU/l)	24.1 \pm 11.5	12.3 \pm 4.45	0.001
Glucose (mmol/l)	4.48 \pm 0.36	4.34 \pm 0.37	0.247

Values are mean \pm s.d.; TC = total cholesterol (all values are for serum excepting fibrinogen, which was measured in plasma).

significantly higher in the obese compared to the non-obese boys ($P < 0.009$).

Within the non-obese group percentage body fat correlated significantly ($r = 0.546$, $P < 0.05$) with plasma fibrinogen concentration (Figure 1). There were no significant correlations between any of the other blood metabolites and percentage body fat within either the obese or the non-obese group. Moreover, no significant relationships emerged between any of the blood metabolites and relative VO_2 peak (ml/kg/min) when partial correlation was used to control for the confounding influence of body fatness.

Discussion

The main findings of this study were that the obese boys had significantly higher concentrations of serum triacylglycerol, serum insulin and plasma fibrinogen than the non-obese boys. Within the non-obese group percentage body fat correlated significantly with plasma fibrinogen concentration. Using non-traditional but more appropriate data normalisation procedures, obese boys were found to have higher absolute VO_2 peak values (l/min , adjusted for fat free mass via analysis of covariance) but lower relative VO_2 peak values

($\text{ml/kg}^{-0.67}/\text{min}$) than non-obese boys. VO_2 peak was not related to any of the blood metabolites examined in this study independent of body fatness.

A variety of methods and classification systems are available for determining obesity.² Each method has its merits but there are also inherent limitations with any method. In this study, a ratio of fat mass to fat-free mass was used. This resulted in two distinctly different groups with respect to fat mass and percentage body fat. The obese boys were carrying 16 kg excess fat, although their age and height did not differ significantly from that of the non-obese boys. An unusual finding from this study was that the fat-free mass values did not differ significantly between groups and the mean fat-free mass value was lower in the obese than the non-obese group. Usually obesity is associated with excess fat-free mass as well as excess fat mass. There is evidence that tissue thickness affects the accuracy of DXA measurement²⁷ and therefore it is possible that fat-free mass was underestimated in the obese boys or overestimated in the non-obese boys. However, excellent agreement has been found between DXA (Lunar DPX-L) measures of fat-free mass and values determined by chemical analysis in seven pigs weighing 35–95 kg and ranging in body fat from 10 to 50%.²⁸ Therefore, we do not believe that the DXA values were inaccurate. An alternative explanation is that the lower fat-free mass values in our obese boys arose as a peculiarity of the selection criteria employed ie a ratio of fat mass to fat-free mass > 0.60 . This may have encouraged recruitment of boys with a particularly low fat-free mass to the obese group.

Our finding that triacylglycerol concentration is elevated in obese boys is consistent with the findings of previous studies that have used larger samples but less precise methods (BMI, skinfold thickness) to determine body fatness.^{10–12,17} It is also consistent with a previous report of elevated triacylglycerol in a group of 59 obese (weight $> 120\%$ of age and sex specific 50th percentile weight-for-height) Chinese Singaporean boys.¹⁵ The fact that none of the other lipids/lipoproteins examined in the present study differed significantly between obese and non-obese boys suggests that triacylglycerol is more closely associated with body fatness than other lipids/lipoproteins. This suggestion is supported by the Bogalusa Heart Study,¹² which found a 'relatively strong' association between obesity (assessed by subscapular skinfold thickness) and serum triacylglycerol concentration, whereas associations between obesity and other lipids/lipoproteins were of a 'lesser magnitude'.

A high fasting insulin concentration has been identified as an independent CVD risk factor²⁹ and there is now considerable evidence demonstrating a link between obesity (and in particular central obesity) and hyperinsulinaemia in children.¹⁸ Previous research in Singaporean adults has revealed a relationship between obesity (BMI, WHR and abdominal diameter) and insulin resistance.³⁰ To our knowledge the present study is the first to document insulin resistance in obese Singaporean children. Serum insulin was on average twice as high in the obese boys in our

Table 4 VO_2 peak data for obese and non-obese boys

	Obese ($n = 20$)	Non-obese ($n = 20$)	Significance (two-tailed)
VO_2 peak (l/min)	2.52 ± 0.38	2.57 ± 0.50	0.767
VO_2 peak (l/min) adjusted ^a	2.63 ± 0.18	2.46 ± 0.18	0.009
VO_2 peak (ml/kg/min)	33.8 ± 3.0	41.6 ± 3.1	0.001
VO_2 peak ($\text{ml/kg}^{-0.67}/\text{min}$)	140.3 ± 11.7	161.2 ± 15.7	0.001

Values are mean \pm s.d.

^aAdjusted using analysis of covariance to remove the influence of fat-free mass.

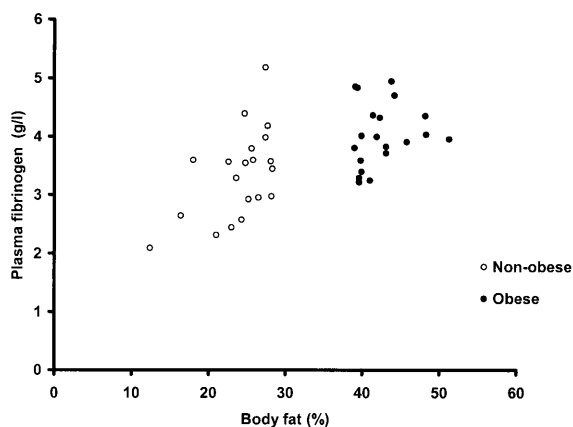


Figure 1 Relationship between percentage body fat and plasma fibrinogen concentration in 20 obese ($r = 0.180$, NS) and 20 non-obese ($r = 0.546$, $P < 0.05$) Chinese Singaporean boys aged 13–15 y.

study compared to the non-obese boys. This confirms reports from the Bogalusa Heart Study^{12,17} of a positive association between obesity (skinfold thickness) and plasma insulin concentration in a large group of North American children.

Fibrinogen is also related to CVD risk³¹ and a recent study¹⁹ has shown that plasma fibrinogen concentration is positively associated with percentage body fat, subcutaneous abdominal adipose tissue, fat mass and BMI in obese boys and girls aged 7–11 y. The findings of our study are consistent with this. Moreover, our findings are also consistent with the observation¹⁹ that percentage body fat is the best single predictor of plasma fibrinogen concentration since percentage body fat was the only body composition variable that correlated significantly with plasma fibrinogen.

The lack of a significant difference in total cholesterol, HDL-C, total cholesterol/HDL-C and apoproteins AI and B between our obese and non-obese boys conflicts with the findings of some previous studies linking body fatness to the concentration of these variables.^{10–15} A possible explanation is the relatively small sample size used in the present study. The probability values for total cholesterol/HDL-C and apoprotein B approached significance in the present study ($P < 0.10$) which would support this suggestion. Regardless of this interpretation the data demonstrate that there is a great metabolic heterogeneity amongst obese children. This observation has been noted previously with reference to obese adults.⁹

It is possible that the interaction between obesity and physical inactivity may promote dyslipidaemia in children. Although VO_2 peak is not always a good surrogate marker for physical activity it does provide an objective measure of aerobic capacity in children.²² In the present study VO_2 peak values were expressed using a mass exponent of 0.67 ($\text{ml/kg}^{-0.67}/\text{min}$). This was done because VO_2 peak does not increase in direct proportion to body size and use of the simple per body mass ratio ($\text{ml/kg}/\text{min}$) may overcompensate for the effects of body mass resulting in unrealistically low values for obese individuals.^{24,26} Although use of the 0.67 exponent attenuated the difference in VO_2 peak between groups, values were still significantly lower in the obese boys. However, absolute VO_2 peak values (l/min), adjusted for fat-free mass via analysis of covariance, were significantly higher in obese compared to non-obese boys. Moreover, VO_2 peak was not related to any of the blood metabolites examined in this study independent of body fatness. This remained true whether correlations were performed within groups or with obese and non-obese groups combined. Thus, a low aerobic capacity does not appear to be related to the elevated triacylglycerol, insulin and fibrinogen values seen in the obese boys.

In conclusion, this study examined the interrelationships between obesity, aerobic capacity and various blood metabolites related to CVD risk in Chinese Singaporean boys. Relatively, novel aspects of the study were the use of DXA to assess body composition, the use of allometric scaling/analysis of covariance for comparing VO_2 peak values between

groups and the measurement of fibrinogen, apoproteins AI and B and Lp(a) in a paediatric sample. The findings suggest that serum triacylglycerol, serum insulin and plasma fibrinogen are most closely associated with obesity in children. Moreover, these differences do not appear to be related to differences in aerobic capacity.

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