The metabolic inter-relationships between changes in waist circumference, triglycerides, insulin sensitivity and small dense low density lipoprotein particles with acute weight loss in clinically obese children and adolescents.

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Word count: 3156

Number of tables: 3

Number of figures: 2

References: 31

Key words: Obesity, dyslipidemia, small dense LDL, weight loss, insulin sensitivity.

Running title: Changes in small dense LDL in obese children

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James.Hobkirk@hyms.ac.uk**Abstract:**

Objective: Small, dense LDL particles are highly atherogenic and strongly associated with obesity-related dyslipidemia. The metabolic inter-relationships between weight loss induced changes in waist circumference, triglycerides, insulin sensitivity and small, dense LDL particles in clinically obese children and adolescents has not been studied.

Methods: Seventy five clinically obese boys and girls (standardized body mass index 3.07 ± 0.59, aged 8-18 years) were recruited and anthropometric, body composition and cardio-metabolic risk factors were measured pre and post weight loss.

Results: There were highly significant reductions in anthropometric, body composition and cardio-metabolic risk factors. Triglyceride change was positively correlated to LDL peak particle density and percentage LDL pattern B changes (relative abundance of small, dense LDL particles). Multiple regression analyses showed that changes in triglyceride concentration accounted for between 24% and 18% of the variance in LDL peak particle density and percentage LDL pattern B change, respectively. Changes in waist circumference, and insulin sensitivity did not predict these changes in LDL characteristics.

Conclusion: Acute and highly significant weight loss significantly decreased LDL peak particle density and percentage LDL pattern B. The change in triglycerides were a strong predictor of LDL peak particle density and percentage LDL pattern B change.

Introduction:

The increase in childhood and adolescent obesity is a worldwide problem showing limited signs of abatement or reversal (1). Obesity is associated with a constellation of cardio-metabolic risk factors and increased risk of future of Type 2 Diabetes (T2D)(2). Amongst the obesity-related metabolic consequences, increases in triglycerides (TG) and decreases in high density lipoprotein cholesterol (HDL-c) are commonly observed (3). This clustering is termed the atherogenic lipoprotein phenotype (ALP), and has been described in obese children with T2D (4). Adolescent dyslipidemia combined with overweight or obesity substantially increases risk of early atherosclerosis in adulthood, measured by carotid-intimal medial thickness (5). In a seminal weight loss study, Epstein and colleagues showed that the magnitude of lipoprotein-lipid changes were significantly related to relative weight changes in obese children (6). There is very limited information regarding the modification of small, dense LDL in high-risk clinically obese children/adolescents with lifestyle modification (7-12).

In a small pilot study, we previously examined the effects of weight loss on small, dense LDL in a sample of clinically obese children and adolescents. We showed significant decreases in LDL cholesterol, LDL peak particle density and a significant reduction in cholesterol contained within the small dense LDL subclass (13). Only two small studies have measured LDL peak particle density/size in children and adolescents within an interventional setting(14, 15). However, the metabolic inter-relationships were not reported. Therefore, the aim of the present investigation was to evaluate the acute metabolic inter-relationships amongst anthropometric and body composition variables,clinically routine metabolic variables (lipoprotein-lipids, fasting glucose and insulin) with changes in LDL peak particle density and LDL subclasses in response to acute weight loss in clinically obese children and adolescents.

Materials and Methods:

The obese children and adolescents were attendees of the Carnegie International Camp (CIC), a multi-factorial weight loss programme (16) sampled between the years of 2004 and 2006. Children and adolescents (8-18 years) attended from all over the United Kingdom and a few from Europe, through self/parental-referral, medical, social service referral or educational organizations. Acceptance onto the programme was reliant on having a body mass index (BMI) above the age related International Obesity Task Force (IOTF) cut-offs for overweight proposed by Cole et al (17). There were no other specific inclusion criteria which resulted in a group of heterogeneous children and adolescents with respect to age, sexual maturation and degree of obesity. The subjects were primarily Caucasians (n=72 Asian n=1 and Black n=2). Determination of Tanner stages or a family history of obesity or T2D were not available.

The programme incorporated energy restriction, physical activity and lifestyle education. Every individual undertook a daily schedule of physical activity which combined a range of structured fun-type, skill-based activities consisting of six 1-hour sessions each day. Energy intake ($kcal*day^{-1}$) was provided as three meals and a snack and was based on an approximation of basal metabolic rate (18). The energy restriction provided sufficient daily energy to meet the individuals basal requirements based on their age and body mass. Each child was assigned to one of four diet groups ranging from 1050-3050 $kcal*day^{-1}$ based upon their age and body mass. The daily energy intake contained 15% protein, 30-35% fat, 50-55% carbohydrate. In addition, each individual engaged in four 1-hour educational sessions per week, covering issues such as changing food choices, balance of good health, portion control and stimulus

control. Sessions also included developing skills such as maintaining behavior substitution, cognitive reinforcement, goal setting, problem solving, acquiring social support and addressing issues regarding bullying.

Ethical approval:

Ethical approval was granted by Leeds West National Health Service Research Ethics Committee, Leeds, UK. All children and parents provided informed written consent and verbal assent.

Anthropometric and body composition measures:

Body mass was measured to the nearest 0.01 kg using calibrated electronic scales (Tanita TBF-310 Tanita Corp, Tokyo, Japan). Height was measured to the nearest 0.1 cm using a floor-standing Seca stadiometer (model 220). BMI was calculated (weight [kg]/ height $[m]^{-2}$). Waist circumference was assessed both at the mid-point (narrowest point between the lowest rib and the iliac crest) as recommended by the International Diabetes Federation (IDF). Bioelectrical impedance analysis (BIA) measures were made using a Tanita TBF-310 (Tanita Corp, Tokyo, Japan). BIA estimated total body water and fat mass were obtained using the inbuilt standard manufacturer equations (19). Blood pressure was measured using a mercury sphygmomanometer (Mercurial BK1001) on the left arm. All individuals sat quietly for 5 minutes prior to measurement. A single measurement was taken in the semi-supine position. In a sub-sample of participants (2006 cohort), a digital blood pressure monitoring device as opposed to manual method was used (Omron HEM-773AC,UK). Measurement protocols defined the appropriate cuff size to be used in all measurements.

Biochemical measurements:

Fasting blood samples (12-13.5 hours) were drawn into appropriate BD Vacutainer™ tubes by venepuncture. Analyses for lipids, glucose and insulin were undertaken at the Department of Clinical Biochemistry, Leeds General Infirmary, UK, using routine clinical assays. Insulin was analyzed by an ADVIA chemiluminescent sandwich immunoassay using a monoclonal mouse anti-insulin antibody. The intra-assay coefficient of variation for insulin was 3.5%. Fasting blood glucose and insulin values were used to calculate homeostatic model assessment for insulin resistance score (HOMA-IR), defined as fasting blood glucose ($mmol \cdot L^{-1}$) × insulin ($mU \cdot L^{-1}$)/22.5, and used as a surrogate measure of insulin resistance (20).

Apolipoprotein B:

Apolipoprotein B (Apo B) was analyzed at the Department of Clinical Sciences, Manchester Royal Infirmary, UK, by a sandwich ELISA technique, employing a goat anti-human apoB antibody (Abcam ab7616, Cambridge) and HRP-labelled goat anti-human apo B (Abcam ab20047). Following incubation with horse radish peroxidase substrate (Sigma-Aldrich P-9187) colour development was measured at 490nm on a Dynatech MR 7000 Plate Reader. The within-batch coefficient of variation was 8.7% and the between-batch coefficient of variation was 9.9%.

LDL subclass measurement:

LDL subclasses were measured in the Carnegie Research Institute, Leeds Metropolitan University, by iodixanol gradient ultracentrifugation and digital photography (21). Briefly, 1.52 ml of plasma was added to 0.4 ml of 60% w/v iodixanol (Axis Shield, UK) and stained with coomassie blue (50mg/ml in phosphate buffered saline. This was layered under 3.4 ml of iodixanol 9% (w/v) in Beckman Optiseal tubes. Tubes were housed in a Beckman NVT 65.2 rotor and centrifuged at 65,000 rpm for 2.5 hrs. A Nikon digital camera D-100 was used to photograph the optiseal™ tubes and these images were downloaded into gel scan software (Total Lab, Non Linear Dynamics, UK). The method was calibrated on-site to determine a migration distance (R_f) cut-off for a density of 1.028 g/ml. Following ultracentrifugation, each tube was analysed using gel scan software to determine the percentage area under the curve. The proportional area under the curve (AUC) ≥1.028g/ml indicated the relative distribution and proportion of small, dense LDL particles, expressed as a percentage. This is referred to as percentage LDL pattern B. In contrast, AUC <1.028g/ml indicates the relative distribution and proportion of large, buoyant LDL particles. This is referred to as percentage LDL pattern A. The proportion of LDL band B allows the classification to LDL pattern phenotypes. A percentage abundance <40%, 40-50% and >50% indicated patterns A, I and B respectively (21). This method has been validated against both salt density gradient ultracentrifugation and gradient gel electrophoresis (21).

Statistics:

All data were assessed for normality of distribution using skewness and kurtosis values. TG, insulin and leptin were logarithmically transformed prior to analysis. Pre-intervention sex differences in anthropometric or cardio-metabolic risk factors were established by

independent T test. Paired T tests and the Wilcoxon T test were used to determine the significance of pre to post mean differences for parametric and non-parametric data, respectively. Analysis of covariance (ANCOVA) was used to examine mean differences in metabolic variables between boys and girls in the response to intervention after adjustment for pre-intervention differences in these variables (SBP, TG and LDL peak particle density and percentage LDL pattern B).

One-way analysis of variance was used to establish the differences in metabolic responses according to age, waist circumference and HOMA-IR median values. Partial correlation co-efficients (adjusted for age) were used to assess the relationships between continuous variables. Spearman correlation coefficients assessed the relationships between sex and continuous data.

Stepwise multiple regression analyses were conducted to predict changes in LDL peak particle density and percentage LDL pattern B. The number of independent predictors relative to cases were 1:25 or 1:19 for LDL peak particle density and percentage LDL pattern B, respectively. Independent variables entered into the regression model for LDL peak particle density included sex, changes in waist circumference and Ln TG. Independent variables entered into the regression model for percentage LDL pattern B, included sex and changes in waist circumference, Ln TG and HOMA-IR which all significantly correlated (partial coefficients) with the dependent variable. Stepwise regression methods for predictors of both LDL peak particle density and percentage LDL pattern B were undertaken using change (delta) values. All analyses were performed on SPSS (version 14.0) and statistical significance was assumed at $p<0.05$.

Results:

Anthropometric and cardio-metabolic risk factors in sex specific groups are shown in Table 1. There were significant ($p<0.05$) pre- intervention sex differences in SBP 125 ± 16 vs. 118 ± 10 mmH;, TG 1.25 ± 0.61 vs. 1.02 ± 0.39 $mmol \cdot l^{-1}$; LDL peak particle density 1.026 ± 0.003 vs. 1.024 ± 0.002 $g \cdot ml^{-1}$; and percentage LDL pattern B, 38.9 ± 15.3 vs. 30.3 ± 7.7% in boys and girls, respectively. There were no significant differences between boys and girls in the metabolic responses to lifestyle intervention (after adjustment for pre-intervention differences). There were highly significant reductions ($p<0.001$) in body mass, BMI, fat mass and waist circumference (Table 1). There were also highly significant ($p<0.01$) reductions in SBP, TC, LDL-c, TG, HDL-c, LDL peak particle density, percentage LDL pattern B, apo B, glucose, insulin and HOMA-IR following weight loss (Table 1).

Changes in metabolic responses to acute weight loss were also evaluated in individuals dichotomised above or below the median HOMA-IR value of 3.2. Whilst more insulin resistant participants had significantly larger decreases in waist circumference (-6.4 ± 3.7 vs. -4.4 ± 6.4 cm, $p<0.05$) and fasting insulin concentrations (-9.3 ± 8.3 vs. -0.1 ± 4.3 $mU \cdot L^{-1}$, $p<0.001$), there were no significant differences evident in lipoprotein-lipids, specifically TG. Furthermore, there were no significant differences in metabolic responses in individuals dichotomised above or below the median of waist circumference or age (data not shown).

After adjustment for age, there were significant correlations between changes in waist circumference, Ln TG and Ln insulin; waist circumference and LnTG ($r=0.25$, $p=0.05$), waist circumference and Ln insulin ($r=0.27$, $p<0.05$), and Ln insulin and Ln TG ($r=0.41$.; $p<0.001$). Notably, LDL peak particle density change correlated significantly with changes in Ln TG ($r=-0.47$, $p<0.001$) and sex ($r=0.28$, $p<0.05$), but not with waist circumference ($r=0.20$, $p<0.09$),

Similar correlations existed for percentage LDL pattern B, except for an additional significant correlation with HOMA-IR ($r=0.27$, $p<0.05$). Stepwise multiple regression analysis demonstrated that Ln TG change and sex together accounted for 29.6% of the total variance in changes in LDL peak particle density (Table 2, figure 1). Ln TG change accounted for 24.2%, ($p<0.001$) and sex accounted for 5.4% of the variability ($p=0.013$). Waist circumference was ommited from the regression model ($p=0.451$).

Ln TG change and sex together accounted for 21.8% of the total variance in changes in percentage LDL pattern B (Table 3, figure 2). Ln TG accounted for 18.3% ($p<0.001$) of the variance in percentage LDL pattern B and sex accounted for an additional 3.5% ($p=0.046$) of the variance (Table 3, figure 2). Waist circumference and HOMA-IR were both ommited from the above regression model ($p=0.310$ and $p=0.305$, respectively). Further post-hoc stepwise multiple regression analysis showed that change in fasting insulin concentration was the only predictor of TG change and accounted for 15.6% of the variance, which provides useful mechanistic information.

Discussion

The present study has shown that LDL peak particle density and the distribution of LDL particles (assessed by the relative proportion of LDL pattern *B*) moved towards a greater proportion of larger and buoyant LDL particles. Decreasing the number of small dense LDL particles has also shown to translate into atherosclerotic regression, measured by angiography (22). However, this is the first report to show the importance of TG on modifying the the relative abundance of small, dense LDL particles simulataneously with changes in peak density/size in clinically obese children and adolescents. This is particularly important as both LDL characteristics provide independent and cumulative (23). These data are of relevance when considering the prevalence of hypertriglyceridemia in comparison to hypercholesterolemia. In >4000 6^{th} and 8^{th} graders elevated TG are highly prevalent at ~21%. This contrasts LDL-c (>the AAP cut-point of 3.4mmol/L) which was prevalent in only 3.4% of individuals (24). The regression analyses (tables 2-3 & figure, 1-2) illustrate the important relationship between changes in TG and changes in LDL peak particle density and the percentage of LDL pattern *B.* Notably, TG change accounted for 24.2% of the variance in LDL peak particle density and accounted for 18.3% of the variance in percentage LDL pattern B. Kang et al (25). showed a negative correlation (r=-0.54) between TG and LDL peak particle size in n=80 obese children. Multiple regressions showed that only TG and HDL-c were independent predictors of LDL size, accounting for 31% of the variance. Miyashita et al (26) showed that TG was negatively correlated (r=-0.616) to LDL peak particle size and accounted for 39.6% of the variance. Notably, only a small number of studies have shown that diet and exercise can modify LDL size. For example, Ferguson et al (14) measured LDL size in response to exercise training (four months duration) and showed that percentage body fat (-1.6%), TG (-0.25 $mmol \cdot L^{-1}$) and insulin (-25 $pmol \cdot L^{-1}$) significantly decreased in obese boys

Table 1: Anthropometric, body composition and cardio-metabolic risk factor changes after acute weight loss in boys and girls.

	Pre Mean ±SD		Post Mean ± SD		p-values (within sex)		p-values (between sex)
	Boys	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	
Body mass (kg)	99.3 ± 26.5	91.4 ± 19.0	93.4 ± 23.8	86.3± 18.4	0.001	0.001	0.32
BMI ($kg \cdot m^{-2}$)	33.6 ± 6.5	34.5 ± 6.4	31.6 ± 5.6	32.5 ± 6.2	0.001	0.001	0.94
Sds BMI	3.09 ± 0.60	3.05 ±0.59	2.88 ± 0.61	2.79 ± 0.65	0.001	0.001	0.07
Waist Mid-point (cm)	101.4 ± 13.0	96.7 ± 11.2	95.3 ± 10.8	91.5 ± 10.5	0.001	0.001	0.30
Fat mass (kg)	35.9 ± 14.9	40.7 ± 12.4	29.0 ± 11.5	36.1 ± 11.9	0.001	0.001	**0.01**
SBP (mmHg)	125 ± 16	117 ± 10	119 ± 11	115 ± 11	**0.03**	**0.001**	0.20
DBP (mmHg)	74 ± 14	70 ± 8	66 ± 9	69 ± 11	**0.01**	**0.05**	**0.01**
TC ($mmol \cdot L^{1}$)	4.17 ± 0.62	4.13 ± 0.73	3.15 ± 0.42	3.33 ±0.64	**0.001**	**0.001**	0.05
LDL-c ($mmol \cdot L^{1}$)	2.47 ± 0.55	2.47 ± 0.59	1.72 ± 0.37	1.84 ± 0.49	**0.001**	**0.001**	0.15
HDL-c ($mmol \cdot L^{1}$)	1.15 ± 0.22	1.23 ± 0.25	1.07 ± 0.22	1.13 ± 0.24	**0.01**	**0.001**	0.6
TG ($mmol \cdot L^{1}$)	1.25 ± 0.61	1.03 ± 0.39	0.86 ± 0.31	0.83 ± 0.31	**0.001**	**0.001**	**0.05**
LDL density ($g \cdot mL^{-1}$)	1.026 ± 0.003	1.024 ± 0.002	1.023 ± 0.001	1.023 ± 0.002	**0.001**	**0.001**	**0.01**
LDL pattern B (%)	38.9 ± 15.3	30.2 ± 7.7	32.9 ± 10.7	30.2 ± 7.7	**0.001**	**0.95**	**0.01**
Apo B ($mg \cdot dL^{-1}$)	86.9 ± 21.3	90.6 ± 22.2	66.8 ± 13.5	70.7 ± 18.4	**0.001**	**0.001**	0.11
Leptin ($ng.mL^{-1}$)	87.4 ± 39.6	148.1 ±72.9	31.1 ± 19.5	70.9 ± 55.9	**0.001**	**0.001**	0.96
Glucose ($mmol \cdot L^{1}$)	4.85 ± 0.40	4.76 ± 0.40	4.64 ± 0.22	4.51 ± 0.35	**0.01**	**0.001**	0.18
Insulin ($mU \cdot L^{-1}$)	20.7 ± 13.7	15.7 ± 9.0	14.4 ± 10.4	11.6 ±5.5	**0.01**	**0.001**	0.29
HOMA IR	4.4 ± 3.2	3.4 ± 1.9	3.0 ± 2.3	2.3 ± 1.1	**0.01**	**0.001**	0.43

Table 2: Summary table of multiple regression models for the prediction of LDL peak particle density change.

	Adjusted r^2	**Beta**	**p**
Model 1			
Ln TG	0.296	0.458	p<0.001
Sex		0.255	p=0.013

Predictors included in the model; sex, waist, Ln TG

Table 3: Summary of multiple regression models for the prediction of percentage LDL pattern B change.

	Adjusted r^2	**Beta**	**p**
Model 1			
Log TG	0.218	0.408	p<0.001
Sex		0.217	p=0.046

and girls of varying ethnicities. Despite these cardio-metabolic changes, LDL size showed no mean change (p=0.83), despite the change in untransformed TG being inversely correlated to changes in LDL size r= -0.38, p=0.02 (14). Kang et al. (15) randomly assigned obese children to either lifestyle education or exercise and lifestyle education. IAAT, TG and insulin decreased simultaneously (-42cm^{-3} -0.22 $mmol \cdot L^{-1}$ and -18 $pmol \cdot L^{-1}$) respectively) in the combined group and LDL size increased by 4.18Å. The metabolic inter-relationships of anthropometrical or body composition variables, TG, insulin and LDL size were not clarified in these studies using multiple regression analyses. In contrast to our findings, both these studies, did not evaluate the distribution or relative abundance of small dense LDL particles.

Figure 1: A scatter plot representing the relationship between between changes in Ln TG and LDL peak particle density.

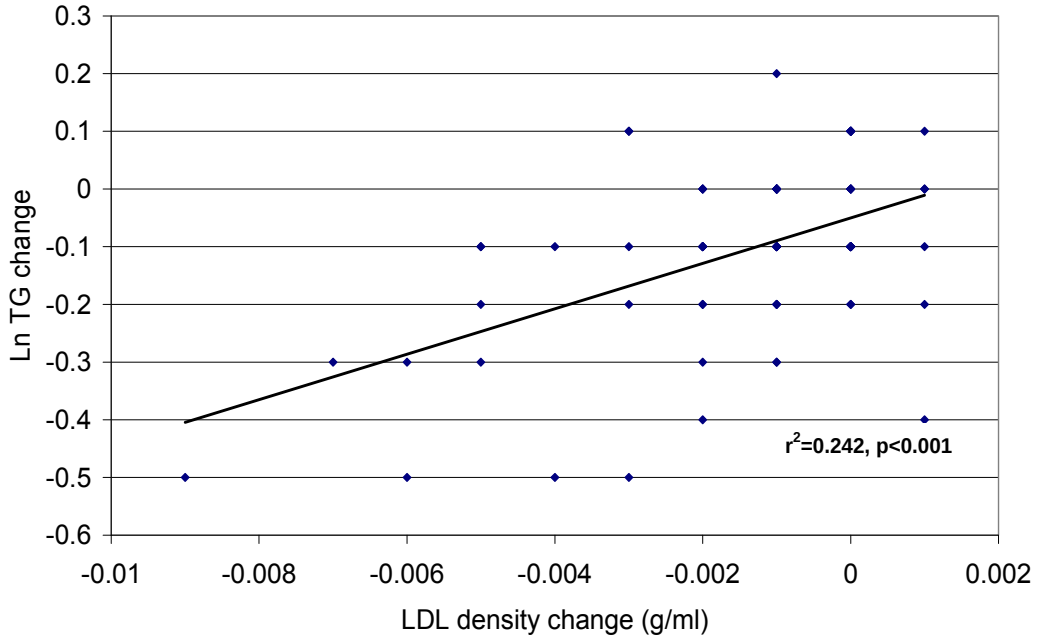
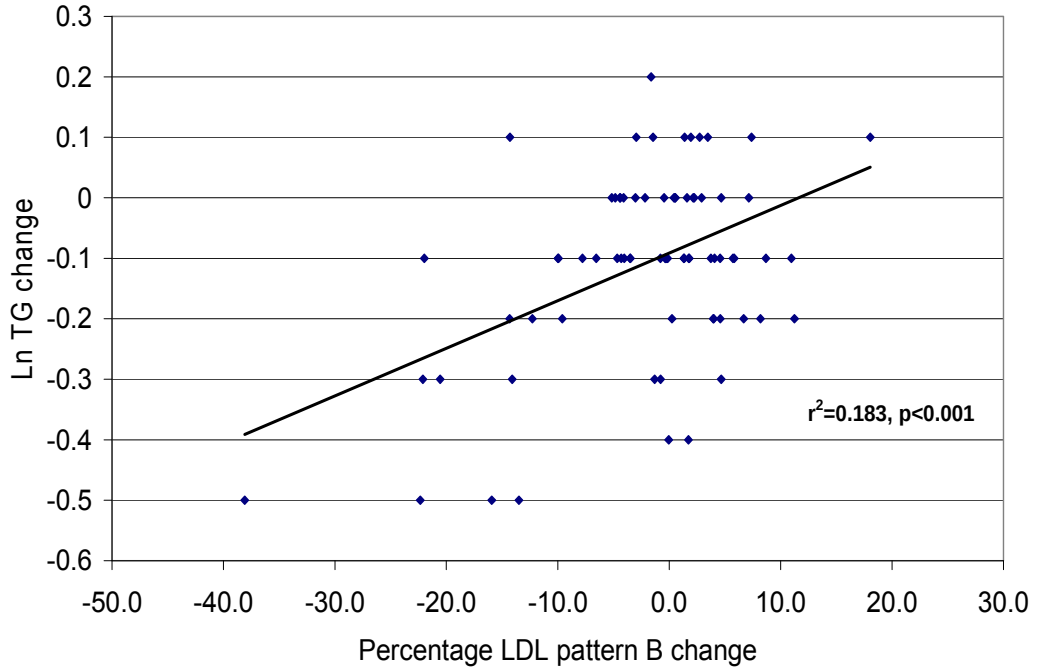


Figure2: A scatter plot representing the relationship between between changes in Ln TG and percentage LDL pattern *B*.



Despite the highly significant role of TG in modifying LDL peak particle density and LDL pattern B in our study,, changes in waist circumference and HOMA-IR did not provide an independent contribution to changes in LDL peak particle density or percentage LDL pattern B. The relationship between insulin sensitivity, LDL peak particle density and percentage LDL pattern B is exerted via improved TG metabolism, and therefore HOMA-IR was not significant in regression analyses. In an elegant study of VLDL-LDL kinetics, Bioletto et al (27) conducted multiple regression analyses and showed that insulin sensitivity accounted for 37% of the variance in TG rich VLDL1 secretion, but only TG (not insulin sensitivity) was a predictor of small dense LDL, suggesting that the mass and activity of TG metabolising enzymes (lipoprotein lipase, hepatic lipase and cholesteryl ester transfer protein) are critical in the generation of small, dense LDL. However, our data indicate that the decrease in TG was partially mediated by a significant increase in insulin sensitivity.

There are some inherent limitations in our study. The study group was heterogeneous in age, obesity status and sexual maturation. We assessed the changes in metabolic risk according to median age (14.5 years) and found no significant differences in the metabolic response to intervention. Furthermore, there was no significant differences in metabolic responses to weight loss according to those above or below the median waist circumference. Furthermore, we were not able to consider the effect of differences in sexual maturity within this cohort. However, published data are somewhat inconsistent on the effect of sexual maturation on blood lipids in obese adolescents. In one study, TG levels did not change during puberty in both sexes (28), whereas dyslipidemia worsened at the onset of puberty and then remained stable during all other pubertal stages(29).

In the present study, our measure of insulin sensitivity was limited to fasting insulin or HOMA-IR. However, both variables have been shown to be highly correlated with more invasive and precise measures of insulin sensitivity and appear valid in obese children and adolescents(30). It is also important that IAAT was not directly quantified in the present study. The number of participants is relatively small for examining independent associations using multivariate analyses. However, we limited the number of independent variables entered within multivariate models to three or four, which is appropriate. The present study was not randomised and did not recruit a comparison or control group of participants, although the efficacy of this programme has been reported. However, in contrast to previous studies (8-12) we have used a standardized acute or "immersion" lifestyle intervention incorporating both substantive calorie restriction and physical activity. This approach may be most appropriate to assess the acute metabolic inter-relationships associated with weight loss. A recent study has shown the efficacy of weight maintenance on cardio-metabolic risk factors (31). Further studies need to assess the durability of changes in LDL characteristics with weight stabilisation and further weight loss

Conclusion:

This is the first study providing insight into the potential mechanisms by which acute lifestyle modifications improves the atherogenic lipoprotein phenotype in clinically obese children and adolescents.

Author Contributions: JPH and PG collected all data. JPH and SC conducted all statistical analyses and wrote the manuscript. RFK, PP, AS, JHB, NH, ID, PG reviewed/edited manuscript.

Conflicts of interest: None

Acknowledgements: The authors would like to thank the children who participated in the study.

References:

1. Wang, Y., and Lobstein, T. 2006. Worldwide trends in childhood overweight and obesity. *Int J Pediatr Obes* 1:11-25.

2. Morrison, J.A., Friedman, L.A., Wang, P., Glueck, C.J. 2008. Metabolic syndrome in childhood predicts adult metabolic syndrome and type 2 diabetes mellitus 25 to 30 years later. *J Pediatr* 152:201-206.

3. Steinberger, J., Moorehead, C., Katch, V., Rocchini, A.P. 1995. Relationship between insulin resistance and abnormal lipid profile in obese adolescents. *J Pediatr* 126:690-695.

4. Albers, J.J., Marcovina, S.M., Imperatore, G., et al. 2008. Prevalence and determinants of elevated apolipoprotein B and dense low-density lipoprotein in youths with type 1 and type 2 diabetes. *J Clin Endocrinol Metab* 93:735-742.

5. Magnussen, C.G., Raitakari, O.T., Thomson, R. et al. 2008. Utility of currently recommended pediatric dyslipidemia classifications in predicting dyslipidemia in adulthood: evidence from the Childhood Determinants of Adult Health (CDAH) study, Cardiovascular Risk in Young Finns Study, and Bogalusa Heart Study. *Circulation* 117:32-42.

6. Epstein, L.H., Kuller, L.H., Wing, R.R., Valoski, A., and McCurley, J. 1989. The effect of weight control on lipid changes in obese children. *Am J Dis Child* 143:454-457.

7. Huelsing, J., Kanafani, N., Mao, J., and White, N.H. 2010. Camp jump start: effects of a residential summer weight-loss camp for older children and adolescents. *Pediatrics* 125:e884-890.

8. Kolsgaard, M.L., Joner, G., Brunborg, C., Anderssen, S.A., Tonstad, S., and Andersen, L.F. 2011. Reduction in BMI z-score and improvement in cardiometabolic risk factors in obese children and adolescents. The Oslo Adiposity Intervention Study - a hospital/public health nurse combined treatment. *BMC Pediatr* 11:47.

9. Monzavi, R., Dreimane, D., Geffner, M.E.et al. 2006. Improvement in risk factors for metabolic syndrome and insulin resistance in overweight youth who are treated with lifestyle intervention. *Pediatrics* 117:e1111-1118.

10. Reinehr, T., Kleber, M., and Toschke, A.M. 2009. Lifestyle intervention in obese children is associated with a decrease of the metabolic syndrome prevalence. *Atherosclerosis* 207:174-180.

11. Savoye, M., Shaw, M., Dziura, J. et al. 2007. Effects of a weight management program on body composition and metabolic parameters in overweight children: a randomized controlled trial. *JAMA* 297:2697-2704.

12. Shalitin, S., Ashkenazi-Hoffnung, L., Yackobovitch-Gavan, M.et al. 2009. Effects of a twelve-week randomized intervention of exercise and/or diet on weight loss and weight maintenance, and other metabolic parameters in obese preadolescent children. *Horm Res* 72:287-301.

13. King, R.F., Hobkirk, J.P., Cooke, C.B., Radley, D., and Gately, P.J. 2008. Low-density lipoprotein sub-fraction profiles in obese children before and after attending a residential weight loss intervention. *J Atheroscler Thromb* 15:100-107.

14. Ferguson, M.A., Gutin, B., Le, N.A.et al. 1999. Effects of exercise training and its cessation on components of the insulin resistance syndrome in obese children. *Int J Obes Relat Metab Disord* 23:889-895.

15. Kang, H.S., Gutin, B., Barbeau, P.et al. 2002. Physical training improves insulin resistance syndrome markers in obese adolescents. *Med Sci Sports Exerc* 34:1920-1927.

16. Gately, P.J., Cooke, C.B., Barth, J.H., Bewick, B.M., Radley, D., and Hill, A.J. 2005. Children's residential weight-loss programs can work: a prospective cohort study of short-term outcomes for overweight and obese children. *Pediatrics* 116:73-77.

17. Cole, T.J., Bellizzi, M.C., Flegal, K.M., and Dietz, W.H. 2000. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 320:1240-1243.

18. Schofield, W.N. 1985. Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr* 39 Suppl 1:5-41.

19. Jebb, S.A., Cole, T.J., Doman, D., Murgatroyd, P.R., and Prentice, A.M. 2000. Evaluation of the novel Tanita body-fat analyser to measure body composition by comparison with a four-compartment model. *Br J Nutr* 83:115-122.

20. Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., and Turner, R.C. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419.

21. Davies, I.G., Graham, J.M., and Griffin, B.A. 2003. Rapid separation of LDL subclasses by iodixanol gradient ultracentrifugation. *Clin Chem* 49:1865-1872.

22. Zambon, A., Hokanson, J.E., Brown, B.G., and Brunzell, J.D. 1999. Evidence for a new pathophysiological mechanism for coronary artery disease regression: hepatic lipase-mediated changes in LDL density. *Circulation* 99:1959-1964.

23. Lamarche, B., Tchernof, A., Moorjani, S., et al. 1997. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Circulation* 95:69-75.

24. Willi, S.M., Hirst, K., Jago, R. et al. 2012. Cardiovascular risk factors in multi-ethnic middle school students: the HEALTHY primary prevention trial. *Pediatr Obes* 7:230-239.

25. Kang, H.S., Gutin, B., Barbeau, P., Litaker, M.S., Allison, J., and Le, N.A. 2002. Low-density lipoprotein particle size, central obesity, cardiovascular fitness, and insulin resistance syndrome markers in obese youths. *Int J Obes Relat Metab Disord* 26:1030-1035.

26. Miyashita, M., Okada, T., Kuromori, Y., and Harada, K. 2006. LDL particle size, fat distribution and insulin resistance in obese children. *Eur J Clin Nutr* 60:416-420.

27. Bioletto, S., Golay, A., Munger, R., Kalix, B., and James, R.W. 2000. Acute hyperinsulinemia and very-low-density and low-density lipoprotein subfractions in obese subjects. *Am J Clin Nutr* 71:443-449.

28. Pinhas-Hamiel, O., Lerner-Geva, L., Copperman, N.M., and Jacobson, M.S. 2007. Lipid and insulin levels in obese children: changes with age and puberty. *Obesity (Silver Spring)* 15:2825-2831.

29. Reinehr, T., and Toschke, A.M. 2009. Onset of puberty and cardiovascular risk factors in untreated obese children and adolescents: a 1-year follow-up study. *Arch Pediatr Adolesc Med* 163:709-715.

30. Conwell, L.S., Trost, S.G., Brown, W.J., and Batch, J.A. 2004. Indexes of insulin resistance and secretion in obese children and adolescents: a validation study. *Diabetes Care* 27:314-319.

31. Davis, J.N., Ventura, E.E., Tung, A., et al. 2012. Effects of a randomized maintenance intervention on adiposity and metabolic risk factors in overweight minority adolescents. *Pediatr Obes* 7:16-27.