Original Article

Low-Density Lipoprotein Sub-Fraction Profiles in Obese Children Before and After Attending a Residential Weight Loss Intervention

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Aim: Small dense LDL particles are associated with an increased risk of coronary heart disease and are prevalent in obesity related dyslipidaemia. This study evaluated the effect of weight loss in nine children (BMI 33.4 ± 8.4 kg.m⁻² and age 15.1 ± 2.9 years) on LDL peak particle size, and cholesterol concentrations within particular LDL sub-fractions.

Methods: Each child undertook fun based physical activity, dietary restriction and modification and lifestyle education classes in a residential summer weight loss intervention. Blood was drawn before and after intervention and LDL heterogeneity measured by ultracentrifugation.

Results: The mean change in body weight were −6.8 ± 4.9 kg, BMI units −2.5 ± 1.4 kg.m⁻², and waist circumference −6.3 ± 6.3 cm (all p < 0.01). Absolute LDL-c concentration reduced from 106.2 mg/dL to 88.3 mg/dL (p < 0.01). The cholesterol contained within the small dense LDL sub-fraction (LDL-c III) reduced from 54.1 mg/dL to 40.4 mg/dL (p < 0.01). Peak particle density decreased from 1.041 g/mL to 1.035 g/mL (p < 0.01). At pre intervention 50.9% of absolute cholesterol was within LDL-c III particles, changing to 46.2%.

Conclusion: Mean weight loss of −6.8 ± 4.9 kg lowers absolute LDL-c and the cholesterol specifically within LDL-c III particles. LDL peak particle size increased and a degree of LDL particle remodelling occurred. These favourable adaptations, accrued in a matter of 4 weeks, maybe associated with a reduction in CHD risk.


Key words; Pattern A, Pattern B, LDL-c III

Background

Childhood obesity is regarded as the “pandemic of the new millennium” reflecting the rising worldwide prevalence, the increased risk for adulthood obesity and the impact on health. Obesity is a heterogeneous condition, which is influenced by genetic and environmental factors. A positive energy intake and a hypo-kinetic behaviour is associated with an increased risk of obesity. There is a significant contribution of child obesity to pre clinical conditions such as dyslipidemia, insulin resistance and an array of other metabolic aberrations. With particular relevance to coronary heart disease (CHD), lipids have long been recognised to play an aetiological role. Of particular significance is the relationship between elevated low density lipoprotein cholesterol (LDL-c) and the finding of atheroma in young children and fibrous plaques in adolescents.

It is now recognised that certain lipid classes, such as absolute LDL-c may not distinguish an individual's true CHD risk. The atherogenic potential of LDL-c may not be based purely on cholesterol concentration per se, but on the proportion of LDL-c that exists in small dense LDL-c (LDL-c III) particles. There is an increased risk of CHD in subjects with a predominance of LDL-c III particles as shown in both case-control studies and prospective studies of coronary heart disease. Despite a predominance of
LDL-c \( III \) particles, or what is regarded as a pattern B phenotype, it is unclear to what extent LDL-c \( III \) particles are independently atherogenic or predictive of CHD. They are simultaneously associated with other lipid risk factors for CHD, such as moderately raised fasting levels of very low density lipoprotein cholesterol and triglyceride (VLDL-c and VLDL-tg respectively), apo B and lower levels of high density lipoprotein cholesterol (HDL-c) in children\(^{11}\).

The prevalence of a pattern B phenotype is low in both young males and pre-menopausal females\(^{12, 13}\). This is consistent with data showing the prevalence of pattern B in samples of children to be 9.3%, 7.5%, 8.2%, 11%, 7.8\(^{14-18}\). In these studies there was a propensity of those with pattern B to be more overweight than those categorised to pattern A. In support of this finding, 54% of obese children were characterised as pattern B in a cross sectional study\(^{11}\).

The relationship of obesity to increased incidence rates of the metabolic syndrome in adolescents\(^{19}\) and the associated pattern B phenotype\(^{20}\), provides a coherent rationale for engaging children with appropriate strategies for successful weight management, and CHD risk reduction. No study to the best of our knowledge has investigated the effects of dietary restriction/modification and physical activity, of which the National Cholesterol Education Programme\(^{21}\) recommend, on LDL-c heterogeneity in a well controlled environment in overweight and obese children.

Although a phenotypic and qualitative classification of LDL peak particle size to either a pattern A or B has been undertaken in previous studies, its value has limitations; it cannot address quantitatively the absolute amount of cholesterol within certain sub-fractions or the relative proportions that reside within particular sub-fractions of LDL. Therefore, our objectives were three fold; to investigate traditional lipid classes, LDL peak particle size and LDL composition before and after weight loss. We hypothesised that the children would lose a significant amount of weight and achieve decreases in not only absolute LDL-c, and LDL-c \( III \) concentrations, but lower the proportion of LDL-c \( III \) to total LDL-c and increase LDL peak particle size. Furthermore we hypothesised that changes in LDL-c \( III \) would be positively correlated to changes in VLDL-c and VLDL-tg.

### Subjects

The Carnegie International Camp (CIC) programme is a multi-factorial weight loss programme consisting of dietary restriction and modification, fun based physical activity and lifestyle education. The programme is run by Leeds Metropolitan University, Leeds, United Kingdom, and precise details have been published elsewhere\(^{22}\). Acceptance onto the programme was contingent on having a BMI above cut-offs for overweight\(^{23}\) and a health screen by the family physician. Children were recruited from all over the United Kingdom and a few from Europe, through a range of sources including self/parental-referral, medical and social service referral or educational organizations.

In brief, these data were collated in the summer (July and August) of 2000. The duration of stay on the programme for each child varied and was contingent on factors such as the financial constraints of the family, and the willingness of the individual children to engage effectively in the programme. Sixty-five children were enrolled onto the CIC programme, however, only nine (5 boys and 4 girls) children and parents provided informed written consent for blood withdrawal and completed both pre and post blood sampling. No data were collected on socio-economic status or ethnicity. Ethical approval was granted by Leeds West National Health Service Research Ethics

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**Table 1.** Weight, BMI, standardised BMI, waist circumference, percent body fat, age and duration of stay in children who attended the camp programme

<table>
<thead>
<tr>
<th>Duration of stay (days)</th>
<th>Pre Mean ± SD ((n=9))</th>
<th>Post Mean ± SD ((n=9))</th>
<th>Change Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>15.1 ± 2.9 (7)</td>
<td>15.2 ± 2.9</td>
<td>28.5 ± 11.4 (28)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94.4 ± 35.3 (120)</td>
<td>87.6 ± 31.5</td>
<td>-6.8 ± 4.9 **</td>
</tr>
<tr>
<td>BMI (kg.m(^{-2}))</td>
<td>33.4 ± 8.4 (26.5)</td>
<td>30.9 ± 7.6</td>
<td>-2.5 ± 1.4 ***</td>
</tr>
<tr>
<td>Standardised BMI</td>
<td>3.03 ± 1.01 (3.4)</td>
<td>2.75 ± 1.06</td>
<td>-0.28 ± 0.14 ***</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>99.3 ± 17.7 (58.2)</td>
<td>93.1 ± 16.3</td>
<td>-6.2 ± 6.3 ***</td>
</tr>
<tr>
<td>Body fat %</td>
<td>43.0 ± 8 (24.7)</td>
<td>40.0 ± 8</td>
<td>-3.0 ± 3 *</td>
</tr>
</tbody>
</table>

Data is presented for all 9 children as means and ± SD (Range in brackets).

\(*p < 0.05\), \(**p < 0.01\), \(***p < 0.001\)
Post Mean \( \pm \) SD \( (n=9) \)  | Change Mean \( \pm \) SD
---|---
TC (mg/dL) | 148.1 \( \pm \) 18.0 | 128.5 \( \pm \) 10.9 | \(-\) 19.6 \( \pm \) 11.7**
LDL-c (mg/dL) | 106.2 \( \pm \) 18.4 | 88.3 \( \pm \) 11.7 | \(-\) 17.9 \( \pm \) 10.5**
HDL-c (mg/dL) | 36.3 \( \pm \) 5.1 | 40.2 \( \pm \) 8.6 | \(+\) 3.9 \( \pm \) 6.6
TG (mg/dL) | 128.4 \( \pm \) 46.1 | 105.4 \( \pm \) 36.3 | \(-\) 23.0 \( \pm \) 42.5*
VLDL-c (mg/dL) | 13.9 \( \pm \) 5.0 | 10.1 \( \pm \) 4.2 | \(-\) 3.8 \( \pm \) 4.3*
VLDL-tg (mg/dL) | 67.3 \( \pm \) 28.3 | 46.1 \( \pm \) 27.5 | \(-\) 21.2 \( \pm \) 27.5*
Glucose (mg/dL) | 82.2 \( \pm \) 10.3 | 82.3 \( \pm \) 5.6 | \(+\) 0.0 \( \pm \) 9.5
LDL-c I (mg/dL) | 21.9 \( \pm \) 1.2 | 18.8 \( \pm \) 1.2 | \(-\) 3.1 \( \pm \) 0.4
LDL-c II (mg/dL) | 30.1 \( \pm \) 1.2 | 29.3 \( \pm \) 1.2 | \(-\) 0.8 \( \pm \) 0.4
LDL-c III (mg/dL) | 54.1 \( \pm \) 2.7 | 40.4 \( \pm \) 1.9 | \(-\) 13.7 \( \pm \) 3.9**
LDL-I % | 20.6 \( \pm \) 2.00 | 20.8 \( \pm \) 2.19 | \(+\) 0.24 \( \pm \) 1.39
LDL-II % | 28.3 \( \pm \) 3.71 | 33.6 \( \pm \) 3.23 | \(+\) 3.76 \( \pm \) 4.51*
LDL-III % | 50.9 \( \pm \) 3.55 | 46.2 \( \pm \) 4.75 | \(-\) 4.7 \( \pm \) 4.90*

In addition, both qualitative and quantitative data is presented detailing the absolute concentrations of cholesterol within the three LDL sub-fractions and also the relative cholesterol concentrations within the three sub-fractions. Data is for all 9 children and presented as mean and \( \pm \) SD. 

*\( p \leq 0.05 \), **\( p \leq 0.01 \)

Committee and all children and parents provided informed written consent to be included in the study. Children taking medication known to effect lipid metabolism were excluded. Table 1 provides additional information of the participants involved in the study.

**Programme and Procedures**

Children 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. The aims of the physical activity sessions were to develop skills and competence in a range of activities with enjoyment and choice being fundamental components of this educational process. In addition there was modest dietary restriction and modification. Energy intake (kcal.day\(^{-1}\)) was provided as three meals and a snack each day and based on an approximation of basal metabolic rate, using the following equations of Schofield\(^{20}\); boys 17.7 \( \times \) body weight + 657 and girls 13.4 \( \times \) body weight + 692.

Energy intake was composed of carbohydrate, fat and protein, 55%, 30%, and 15% respectively. Saturated fat was 10% of total energy intake. Energy intake was designed to be similar to the food children would be exposed to in their home environment including foods such as salad, pasta and pizza. Children also took part in four one-hour educational sessions per week, conducted by the education team at the camp. These covered issues such as changing food choices, portion control, maintaining behaviour change and bullying.

**Measures**

Body mass was measured to the nearest 0.01 kg and height to the nearest cm. BMI (kg.m\(^{-2}\)) were calculated for each child. Waist and hip circumferences were assessed using protocols from the anthropometric standardization manual\(^{25}\). In addition, %body fat was assessed using air displacement plethysmography\(^{26}\). This method is quick to use, non-invasive, and suitable for assessing body composition in children\(^{27}\).

Blood was drawn by venepuncture in the fasted state (12-14 hours) into tubes containing EDTA and spun to separate plasma from red cells on the morning of their initial arrival and departure. Plasma was maintained at a cool temperature (3-7 degrees centigrade) and analysed within 24 hours for lipoproteins and glucose. Chylomicrons and other material of similar density were removed by centrifugation at 13,000 g for 10 minutes before separation of VLDL, LDL, and HDL\(^{28}\). We cannot guarantee that all chylomicron remnants were removed, if at all present\(^{28}\). However, if present it is likely that they would have been accounted for in the VLDL fractions\(^{28}\). In addition, given the fact that our subjects had fasted for 12-14 hours, were non diabetic and not type III hyperlipidemic, the contribution of chylomicron remnants to our findings may be negligible.

Lipoproteins were separated using rapid self generating gradients of iodixanol (Lipotek Ltd, Merseyside, UK)\(^{28}\) after 2.5 hours of centrifugation at 16 degrees centigrade and 100,000 rpm in a Beckman Optima bench top ultracentrifuge TLX-100. Deceleration was set at slow.
Two plasma samples were analysed simultaneously into 44 (3 drops) fractions using a Beckman gradient unloader which pierces the bottom of the tube and a Gilson fraction collector. Using a multipipette, 10 μL aliquots of each fraction were transferred from the wells of the microtitre plates used for collecting the gradients into new microtitre plates in duplicate, which were then used for assay of cholesterol and TG. 200 μL of cholesterol assay reagents (Boehringer, Ingelheim Pharmaceutical, Inc. Germany) or TG assay reagents (Alpha labs, Bio-stat Diagnostics, Stockport, UK) were added to each well and the plates incubated according to the manufacturer’s instructions. Absorbance was read at 450 nm for cholesterol and 570 nm for TG in a Multiscan ascent plate reader. To analyze the data, a Microsoft Excel template was set up to calculate the cholesterol and TG (μmol) in each gradient fraction. The absorbances from the plate reader were transferred directly into the template, which calculated the amount (μmol) of cholesterol or TG in each gradient fraction using the fraction volume, the absorbance for the sample, and the absorbance for the standard cholesterol or TG sample. Cut off points for HDL, LDL, and VLDL were selected for individual plasma after examining the cholesterol profiles. Cholesterol (mmol/L) and TG (mmol/L) concentrations in chylomicron free plasma were then calculated for HDL, LDL, and VLDL (and subsequently for total cholesterol and total triglycerides, see Table 2). The recoveries of cholesterol and TG were also calculated from the lipids in the appropriate gradient fractions taking into account the volume of chylomicron remnant free plasma used, and was very close to 100%. The gradient distribution profiles for cholesterol, TG, and histograms showing the lipid content of total HDL, LDL, and VLDL were then plotted. The coefficient of variation for LDL-c was 4% in a series of identical samples subject to sub-fractionation.

The density of the LDL-c particles in the gradient is known to exhibit a linear relationship with the diameter of the gradient fractions indicating that LDL-c particles are resolved on the basis of size and density as confirmed by gradient gel electrophoresis. The LDL-c profile consisted of three major sub-fractions, small dense (LDL-c III), intermediate (LDL-c II) and light (LDL-c I), and these corresponded to fractions 15-22 density (1.060-1.038 g/mL), 23-26 density (1.038-1.019 g/mL) and 27-33 density 1.019-1.011 g/mL) respectively. LDL pattern B was defined as having a peak LDL particle density between fractions 15-22 and a pattern A between fractions 23-33.

### Statistical Analysis

Paired t-tests was used to investigate the pre-post differences, and Pearson moment correlation was used to determine the relationships between variables. We calculated densities from the known linear relationship between density and fraction numbers and calculated statistical significance by a paired t-test. Compositional analysis was calculated by dividing concentration of cholesterol in each discrete sub-fraction by the absolute LDL-c concentration. Differences and conclusions resulting in $p<0.05$ were considered statistically significant.

### Results

Data for anthropomorphic and biochemical variables are shown in Table 1 and 2, respectively. During the intervention the campers significantly decreased body weight from 94.4 kg to 87.6 kg ($p<0.01$), waist circumference from 99.3 cm to 93.1 cm ($p<0.001$), BMI from 33.4 kg.m$^{-2}$ to 30.9 kg.m$^{-2}$ ($p<0.001$), and %body fat from 43% to 40% ($p<0.01$). This period of weight change was not associated with a change in stature (data not shown) but was associated with favourable changes in traditional lipid classes with mean TC and LDL-c concentrations showing a significant decrease from 148.1 mg/dL to 128.5 mg/dL and 106.2 mg/dL to 88.3 mg/dL respectively ($p<0.01$). A decrease was also observed in total TG from 128.4 mg/dL to 105.4 mg/dL ($p<0.05$), VLDL-c from 13.9 mg/dL to 10.1 mg/dL ($p<0.05$), and a decrease in VLDL-tg from 67.3 mg/dL to 46.1 mg/dL ($p<0.05$). There was an increase in HDL-c from 36.3 mg/dL to 40.2 mg/dL however this was not statistically significant.

Further analysis of LDL-c showed clearly that all the campers were defined as having a predominance of LDL-c III particles or a pattern B phenotype at pre intervention with a peak LDL particle size between fractions 15-22. At post intervention, only three of the nine campers remained pattern B. Pre mean LDL peak particle density of the campers was 1.041 g/mL and this reduced significantly to 1.035 g/mL post intervention ($p<0.01$) (Fig. 1). Cholesterol concentrations of LDL-c III particles reduced from 54.1 mg/dL to 40.4 mg/dL ($p<0.01$). Cholesterol concentrations in LDL-c II particles reduced from 30.1 mg/dL to 29.3 mg/dL (non-significant, NS), and cholesterol concentrations in LDL-c I particles reduced from 21.9 mg/dL to 18.5 mg/dL (NS). Compositional analysis of the cholesterol distribution showed that the campers had 50.9% of cholesterol concentration within LDL III particles at pre intervention. At post intervention the
Campers had significantly shifted the relative cholesterol concentration of LDL \( \text{اوي} \) particles to 46.2\% \( (p < 0.05) \). LDL-\( \text{اوي} \) constituted 28.5\% of total LDL-\( \text{اوي} \) at pre intervention and this increased at post intervention to 32.3\% \( (p < 0.05) \). LDL-\( \text{أتي} \) constituted 20.6\% of total LDL-\( \text{أتي} \) at pre intervention and this increased slightly to 20.8\% at post intervention (NS). There were significant positive correlations between change in LDL-\( \text{اوي} \) and change in both VLDL-\( \text{أتي} \) \( (r = 0.837, p < 0.01) \) and VLDL-tg \( (r = 0.739, p < 0.05) \). No anthropometric variable correlated with a change in LDL-\( \text{أتي} \). We were unable to investigate gender differences, the independent effects of VLDL-\( \text{اوي} \) or VLDL-tg reductions, weight loss, or insulin resistance on LDL-\( \text{أتي} \) \( \text{اوي} \) or LDL-\( \text{أتي} \) peak particle size given the small sample of non homogenous children (see Table 1).

**Discussion**

An increasing prevalence of obesity in children is a major concern as 80\% of overweight and obese children will probably become overweight and obese as adults\(^{29}\). We have shown that our residential programme of dietary restriction/modification and fun-based physical activity in children can significantly and favourably modify a number of anthropometric and lipid classes, such as absolute TC, LDL-\( \text{أتي} \), HDL-\( \text{أتي} \), and TG, which are associated with a reduction in CHD risk\(^{30}\). Despite favourably modifying these traditional risk factors through changes in lifestyle, the emergence of lipoprotein heterogeneity as an important measure of risk associated with LDL-\( \text{أتي} \), has not been widely studied. This is the first study to our knowledge to investigate changes in particular LDL sub-fractions that occur during a weight loss intervention for children.

In this study, all children were classified as having pattern B at pre intervention, of which 6 of the 9 children shifted to pattern A post intervention. Of those children who remained pattern B, one child had the most dense LDL-\( \text{أتي} \) peak particle size at pre intervention, and despite a shift in LDL-\( \text{أتي} \) peak density (1.045 g/mL to 1.038 g/mL) it was insufficient to change
to pattern A. The other two children who remained pattern B showed no change in peak LDL-c density/size and this was associated with no change in either VLDL-c or VLDL-tg concentrations. Interestingly, these two children did lose weight −5.6 kg (5% of body mass) and −1.7 kg (2.8% of body mass) and reduced waist circumference by 4.5 cm and 3.7 cm respectively. One possible explanation is the genetic control of LDL subclasses, as described by Austin\textsuperscript{12}.

Despite these two cases, a favourable shift in mean LDL-c peak particle density occurred from 1.041 g/mL to 1.035 g/mL, and a highly significant decrease in LDL-c III was observed from 54.1 mg/dL to 40.4 mg/dL (p<0.01). In addition, there was a relative shift in the distributions of cholesterol within the subfractions. At pre intervention the percentage of cholesterol within LDL-c III particles was 50.9% and this reduced to 46.2% at post intervention, indicating that some degree of lipoprotein remodelling had occurred.

There has been a small amount of inconsistent data regarding LDL-c heterogeneity in obese children. For example, a longitudinal study incorporating physical activity/exercise for 40 minutes per day, 5 days per week, (mean attendance 4 days per week) for four months showed no significant improvements in peak LDL-c size, pre 25.5 nm to post 25.7 nm, or apo B concentration pre 78 mg/dL to post 75 mg/dL\textsuperscript{31}. However a study that assigned free living obese girls to either lifestyle education (n=10) or structured physical activity sessions (n=11) run 5 days per week for 30 minutes, showed significant increases in the LDL-c/apo B ratio, which indicates a decrease in small dense LDL. In the lifestyle group the LDL-c/apo B ratio increased from 1.25 to 1.33 and in the physical activity group the ratio increased from 1.19 to 1.26\textsuperscript{32}. In a study by Kang et al.\textsuperscript{33} obese children were randomly assigned to either lifestyle education only or exercise and lifestyle education. LDL-c peak particle size decreased in the lifestyle education group by −1.92Å (angstroms) whereas LDL-c peak particle size increased in the combined intervention by 4.18Å. It appeared that the children who increased the most in cardio-respiratory fitness and reduced the most in percentage body fat showed the greatest increase in LDL-size. It is likely that the contribution of diet, physical activity and lifestyle education in our programme, which was not simultaneously part of previous studies, could result in separate and specific effects but when integrated may have been synergistic and more cumulative. The advantage of the current study is that unlike those aforementioned studies, the children were not free living. Factors such as diet were monitored and children were supervised with regards to the amount of physical activity that was undertaken. A particular limitation however is the inability to establish independent effect of exercise or diet on LDL-c III as all children were exposed to the same dietary and physical activity programme.

We have shown quite clearly a reduction in absolute LDL-c, LDL-c III, LDL-c peak particle size and a lower proportion of LDL-c III to LDL-c (Table 2). Changes in LDL-c III were positively and significantly correlated to changes in VLDL-c and VLDL-tg, and this is consistent with the findings that LDL-c III particles are regulated through mechanisms related to VLDL metabolism and lipid transfers\textsuperscript{8}. Despite the simultaneous improvement in VLDL and HDL concentrations, there is evidence that LDL-c III particles have direct atherogenic potential, for example they express increased susceptibility to oxidation, which is required for the deposition of cholesterol in the arterial wall\textsuperscript{34}, reduced binding ability to LDL receptors\textsuperscript{35} and greater residence time in plasma. Thus, the balance of absolute LDL-c concentration and relative proportion of LDL-c III may be important in the pathological interaction with arterial proteoglycans and non-LDL receptor catabolism for atherogenic susceptibility and progression. For example, adjustment for LDL-c concentration did not reduce the relationship between LDL-c particle size and risk of CHD in two prospective studies\textsuperscript{9, 10}. These results emphasise that absolute LDL-c concentrations and LDL-c particle size and density represent two quite distinctive measures CHD risk.

However, there is evidence that the number of LDL particles should be considered when assessing the LDL-c related CHD risk\textsuperscript{36}. Unlike cholesterol concentration that can vary between LDL-c particles, there is only one apo B molecule per LDL particle and as shown in a recent study apo B concentrations was more critical than LDL-c to characterise a broad range of cardiovascular risk factors in a multi-ethnic and multi-centre study\textsuperscript{7}. In the Quebec Cardiovascular study, men who had a pattern B phenotype, and with elevated plasma apo B levels (>120 mg/dL) had >6-fold increase in CHD risk than men with pattern B and low apo B levels <120 mg/dL\textsuperscript{10}. Therefore a particular limitation to our study is the inability to accurately quantify a reduction in LDL particle numbers, as apo B was not measured.

In conclusion, we have shown reductions in absolute LDL-c, LDL-c III, LDL-c peak particle size density and a lower proportion of LDL-c III to LDL-c. In addition, we have shown an increase in HDL-c concentration and a reduction in VLDL-c and VLDL-tg. All these favourable adaptations, accrued in a matter
of 4 weeks, are considered to be associated with a reduction in CHD risk. The long term clinical implications in altering the proportion of LDL-c III versus the absolute concentration of LDL-c is unknown, as is the durability or persistence of these favourable changes and the relationship to weight change. Further studies are currently underway assessing the long term persistence of these changes in a larger group of children.

Declaration of Conflicting Interests

The authors declare that they have no competing interests.

Authors Contribution

All authors had full access to all data in the study and take responsibility for the integrity of data and the accuracy of data analysis.

Conception and design – RFGJ King, PJ Gately, CB Cooke, J Higgins.
Acquisition of data – P Gately, J Higgins.
Drafting of manuscript – JP Hobkirk.
Critical revision of the manuscript – RFGJ King, JP Hobkirk, CB Cooke, PJ Gately, D Radley.
Funding obtained – CB Cooke, PJ Gately, J Higgins.
Administrative, technical and material support – RFGJ King, JP Hobkirk J Higgins.
Final approval – RFGJ King, PJ Gately.

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