Improvements in LDL particle size and distribution by short-term alternate day modified fasting in obese adults

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Abstract
Alternate day modified fasting (ADMF) beneficially modulates several indicators of CHD risk, but its effects on LDL particle size have never been tested. Accordingly, we examined the effects of ADMF on LDL particle size and distribution in obese adults. Sixteen obese subjects participated in a 10-week trial with three consecutive phases: (1) 2-week control phase; (2) 4-week ADMF controlled feeding phase; (3) 4-week ADMF self-selected feeding phase. After 8 weeks of diet, body weight and waist circumference were reduced (P<0.05) by 5.6 (SEM 0.9) kg and 4.0 (SEM 0.9) cm, respectively. LDL-cholesterol and TAG concentrations decreased (P<0.05) by 25 (SEM 10)% and 32 (SEM 6)% respectively. Peak LDL particle size increased (P<0.05) from 266 (SEM 1) to 268 (SEM 1) Å. Additionally, the proportion of small LDL particles decreased (P<0.05) from 13 (SEM 2)% to 9 (SEM 3)% post-treatment, while the proportion of large LDL particles increased (P<0.05) from 68 (SEM 4)% to 76 (SEM 4)% post-treatment. These findings suggest that ADMF is an effective diet strategy for increasing LDL particle size and decreasing the proportion of small, dense LDL particles in obese adults.

Key words: Alternate day fasting; LDL particle size; Cholesterol; CHD; Obese adults

A preponderance of small, dense LDL is associated with a 3- to 7-fold increase in CHD risk, independent of LDL-cholesterol concentrations1. The mechanisms that link small LDL particles to atherogenesis are thought to include: long residence time in plasma, enhanced oxidisability and increased permeability through the endothelial barrier2. An increased proportion of small dense LDL is associated with moderate elevations in plasma TAG as well as low levels of HDL-cholesterol3. Carrying excess adipose tissue, particularly in the abdominal region, is also correlated to an increased proportion of small LDL5. Weight loss by means of energy restriction (ER) has been shown to increase LDL particle size, and thus reduce CHD risk in obese adults(4–6).

Alternate day modified fasting (ADMF) is a form of dietary restriction that has been shown to help overweight and obese individuals lose weight and lower CHD risk7,8. ADMF regimens consist of a ‘feed day’, where food intake ad libitum is permitted for 24 h, alternated with a ‘fast day’, where energy intake is limited to 25 % of needs for 24 h. Although the data are limited, recent results indicate that ADMF for 8 weeks reduces body weight by 6–8%, decreases waist circumference and lowers LDL-cholesterol and TAG concentrations by 10–25 and 30–40%, respectively7,8. In view of these beneficial effects on plasma lipids, it is hypothesised that this diet therapy would also improve other key CHD risk factors, such as LDL particle size.

Accordingly, the objective of the present study was to examine the effects of ADMF on LDL particle size in obese subjects and to evaluate how these modulations of LDL diameter are related to changes in visceral fat mass and plasma lipid concentrations.

Materials and methods
Subjects
The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago. A written informed consent was obtained from all the subjects. The key inclusion criteria were as follows: age 35–65 years; BMI between
30 and 39.9 kg/m²; weight stable for 3 months before the
beginning of the study (i.e. <5 kg weight loss or weight
gain); non-diabetic; no history of CVD; lightly active (i.e.
<3 h/week of light-intensity exercise at 2.5–4.0 metabolic
equivalents for 3 months before the study); non-smoker;
not taking weight loss, lipid-lowering or glucose-lowering
medications.

Study design and alternate day modified fasting protocol
Sixteen subjects participated in a 10-week trial with three
consecutive intervention phases: (1) 2-week pre-loss con-
control phase; (2) 4-week weight loss/ADMF-controlled feed-
ing phase; (3) 4-week weight loss/ADMF self-selected
feeding phase. During phase 1, all the participants main-
tained their usual eating habits in order to maintain body
weight. During phase 2, subjects consumed 25% of their
baseline energy needs on the fast day (between 12.00
and 2.00 hours) and then ate ad libitum on each alternate
feed day. Baseline energy requirements were determined
by the Mifflin equation(9). During this controlled feeding
phase, the subjects were provided with a energy-restricted
meal on each fast day (25 %kJ as protein, 25 %kJ as fat and
50 %kJ as carbohydrate), while on each feed day, subjects
ate ad libitum at home. During phase 3, the subjects con-
tinued the same ADMF regimen but were required to pre-
pare all of their meals at home. During this self-selected
feeding phase, subjects met weekly with the study dietitian
to learn how to maintain the ADMF regimen on their own.
Dietary adherence on the fast day was monitored using the
‘Extra food log’. The subjects were asked to report any
extra food item consumed on the fast day that did not
comply with their prescribed plan using this log. If the
log indicated that the subject ate an extra food item on a
fast day, that day was labelled as ‘not adherent’. If the
log revealed that the subject did not eat any extra food
item, that day was labelled as ‘adherent’. Percentage adher-
ence/week was calculated as follows:

\[
\% \text{ Adherence} = \frac{\text{number of fast days adherent}}{\text{number of fast days in the week}} \times 100.
\]

In addition, average energy intake was determined by
collecting feed day food records (2 weeks and 1 week-
end day) during each phase. Fast day food records were
collected during phase 3. Nutritionist Pro Software (version
4.3; Axxya Systems, Stafford, TX, USA) was used to calcu-
late total daily intake of energy. Alcohol consumption
was restricted to no more than one drink/d for women
and no more than two drinks/d for men. Body weight
was assessed at the research centre at the beginning of
every week in light clothing without shoes. BMI was
assessed as kg/m². Twelve hour fasting blood samples
were collected between 07.00 and 09.00 hours at baseline
(day 1), at the end of phase 1 (day 14), at the end of
phase 2 (day 41: feed day and day 42: fast day) and at
the end of phase 3 (day 69: feed day and day 70: fast day).

Plasma lipids and LDL particle size
Plasma total cholesterol, HDL-cholesterol and TAG con-
centrations were measured in duplicate using enzymatic
kits (Biovision, Inc., Moutainview, CA, USA). The con-
centration of LDL-cholesterol was calculated using the
Friedewald equation(10). LDL particle size analysis was per-
formed on whole plasma using non-denaturing 2–16 %
polyacrylamide gradient gel electrophoresis, as described
previously(11,12). Peak and integrated LDL particle diameter
was computed using the approach described by Tchernof
et al.(12). The relative proportion of small (LDL<255 Å %),
medium (LDL255–260 Å %) and large (LDL>260 Å %) particles
was obtained by computing the relative area of the densi-
ometric scan (<255, 255–260 and >260 Å, respectively)(12).
The absolute concentration of cholesterol among particles
was estimated by multiplying the total plasma LDL-
cholesterol level by the relative proportion of small,
medium and large particles.

Statistics
The results are presented as means with their standard
errors. One-factor ANOVA was performed to determine
an overall P value for each variable, and the Bonferroni
correction was used to assess the significance. Relation-
ships between continuous variables were assessed by
simple regression analyses as appropriate. Tests for nor-
mality were included in the model. Data were analysed
by using SPSS software (version 17.0 for Mac OS X; SPSS,
Inc., Chicago, IL, USA).

Results
Baseline characteristics and weight loss
Sixteen subjects (four men and twelve women) completed
the entire 10-week trial. Four women were post-menopa-
sal, while eight women were pre-menopausal. Six of the
pre-menopausal women were taking oral contraceptives
as a birth control method. The other two pre-menopausal
women were using barrier methods as birth control.
Completers were middle aged (46 ( SEM 3) years), obese
(BMI 34 ( SEM 1) kg/m²), sedentary (2.4 ( SEM 0.3) h/week
of physical activity) and borderline hypercholesterolaemic
(LDL-cholesterol concentration 106 ( SEM 100) mg/l). Diet-
ary adherence on the fast day was high and stable over
the course of the study (phase 2: 86 ( SEM 4) % adherence
and phase 3: 89 ( SEM 5) % adherence). Average energy
intake was as follows: phase 1, 8104 ( SEM 748) kJ/d;
phase 2 feed day, 7497 ( SEM 953) kJ/d; phase 2 fast day,
1725 ( SEM 351) kJ/d; phase 3 feed day, 6882 ( SEM 782) kJ/d;
phase 3 fast day, 2460 ( SEM 192) kJ/d. Body weight
increased ($P<0.05$), while the proportion of small ($<255$ Å) and medium ($255–260$ Å) particles decreased ($P<0.05$). The estimated concentration of cholesterol in large particles did not change, while the concentration of cholesterol within small-sized and medium-sized LDL subfractions was reduced post-treatment ($P<0.05$). LDL particle size was associated with reduced body weight ($r=-0.32$, $P=0.04$) and smaller waist circumference post-treatment ($r=-0.36$, $P=0.03$). No relationship between post-treatment LDL particle size and TAG ($r=0.28$, $P=0.11$) or HDL-cholesterol concentrations ($r=0.18$, $P=0.65$) was observed.

**Discussion**

ER-induced weight loss is an effective means of increasing LDL particle size in obese adults. Short-term studies in this area (12–24 weeks in duration) generally report increases in peak LDL particle size (0.5–3 Å increase from baseline) with 5–9 kg of weight loss in obese men and women ($r=0.65$), with some exceptions ($r=0.15$). The ability of fasting regimens, such as ADMF, to exert similar beneficial effects as ER, had never been tested. We show here, for the first time, that ADMF is able to increase peak LDL particle size (2 Å increase from baseline) to a similar extent as ER. Whether one of these dietary restriction regimens is more effective in increasing LDL particle size is not clear at present, due to the limited amount of ADMF data presently available. Future studies are needed to clarify which dietary restriction regimen is superior with regard to increasing LDL particle size. In addition to these changes in peak particle size, we also observed a distinct decrease in the proportion of small particles ($13–9\%$) and a distinct increase in the proportion of large LDL particles ($68–76\%$) after 8 weeks of treatment. Reductions in plasma LDL-cholesterol and TAG concentrations were also observed.

**Table 1.** LDL particle size and distribution over the course of the trial

<table>
<thead>
<tr>
<th></th>
<th>Pre-loss control phase</th>
<th>ADMF controlled feeding phase</th>
<th>ADMF self-selected feeding phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 14</td>
<td>Days 41, 42</td>
</tr>
<tr>
<td></td>
<td>Mean SEM</td>
<td>Mean SEM</td>
<td>Mean SEM</td>
</tr>
<tr>
<td>LDL peak particle size (Å)</td>
<td>266(^a) 1</td>
<td>266(^a,b) 1</td>
<td>267(^a,b) 1</td>
</tr>
<tr>
<td>LDL integrated particle size (Å)</td>
<td>267(^a) 2</td>
<td>268(^a,b) 2</td>
<td>267(^a,b) 1</td>
</tr>
<tr>
<td>LDL(_{-260}) Å (%)</td>
<td>68(^a) 4</td>
<td>71(^a,b) 5</td>
<td>73(^a,b) 4</td>
</tr>
<tr>
<td>LDL(_{255–260}) Å (%)</td>
<td>19(^a) 2</td>
<td>17(^a,b) 2</td>
<td>16(^a,b) 2</td>
</tr>
<tr>
<td>LDL(_{260–265}) Å (%)</td>
<td>13(^a) 2</td>
<td>12(^a,b) 3</td>
<td>11(^a,b) 2</td>
</tr>
<tr>
<td>LDL(_{-260}) Â (mg/l)</td>
<td>700 90</td>
<td>780 10</td>
<td>520 70</td>
</tr>
<tr>
<td>LDL(_{255–265}) Â (mg/l)</td>
<td>190(^a) 30</td>
<td>170(^a) 20</td>
<td>120(^a,b) 20</td>
</tr>
<tr>
<td>LDL(_{260–265}) Â (mg/l)</td>
<td>130(^a) 20</td>
<td>110(^a,b) 30</td>
<td>80(^b) 20</td>
</tr>
</tbody>
</table>

ADMF, alternate day modified fasting; LDL-C, LDL-cholesterol.

\(^{a,b}\) Mean values within a row (for each LDL particle size parameter) with unlike superscript letters were significantly different (one-factor ANOVA with Bonferroni analysis, $P<0.05$).

\(^*\) Days 41 and 42 values combined; days 69 and 70 values combined.
However, the present study is limited in that the effect of weight loss on LDL particle size was not evaluated separately from the effect of fasting. Therefore, it is not clear whether these cardioprotective effects were mediated by reduced body weight or by the prolonged fasting period on the fast day. Future studies in this area, which administer the ADMF regimen with and without a prolonged fasting period, are necessary to tease apart the effects of these variables on changes in lipoprotein subfractions.

A secondary aim of the present study was to evaluate how modulations in LDL diameter by ADMF are related to changes in abdominal fat mass and plasma lipid concentrations. Increased waist circumference is associated with augmented plasma TAG, which in turn is associated with decreases in LDL particle size\(^\text{(16)}\). We show here that 8 weeks of ADMF is able to decrease the waist circumference by 4-0 (SEM 0-9) cm, and that this smaller waist circumference is associated with increased LDL particle size. Similar relationships have also been reported in short-term ER trials\(^\text{(3,17)}\). However, no relationship was noted between post-treatment TAG concentrations and waist circumference. Moreover, no association was observed between post-treatment TAG concentrations and LDL particle size. A possible reason for this lack of relationship may be the small sample size used here. If a larger number of subjects had been recruited, it is probable that these relationships might have been statistically significant.

In summary, these findings suggest that short-term ADMF may be an effective strategy for increasing LDL particle size and decreasing the proportion of small, dense LDL. These data support the growing body of evidence advocating ADMF as a suitable alternative to traditional ER for lowering heart disease risk in obese adults.

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**References**