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## Acute changes in lipoprotein subclasses during exercise

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### ABSTRACT

**Objective.** Lipids are important substrates for oxidation in the basal fasting state and during exercise. Studies have demonstrated beneficial changes in lipoprotein subclass composition the day after an exercise bout. However, the acute effect of exercise on TG concentration and lipoprotein subclass composition remains unclear.

**Materials/Methods.** Sixteen lean, healthy individuals (8 men and 8 women) were recruited (age 20–30 years, BMI < 25 kg/m<sup>2</sup>). The subjects were studied during basal fasting conditions as well as during and after 90 min of cycling at 50% of VO<sub>2peak</sub>. Lipoprotein subclass composition was measured with <sup>1</sup>H NMR spectroscopy.

**Results.** During exercise, LDL and HDL particle concentration increased significantly (p < 0.05) despite lower total TG concentration. In addition, exercise resulted in a shift towards smaller VLDL particles in men (p < 0.05), but VLDL-TG concentration was unaltered.

**Conclusions.** Acute exercise induces beneficial changes in lipoprotein subclass composition. These changes are similar to the effects of exercise training.

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## 1. Introduction

Elevated plasma triglyceride (TG) concentrations are an independent risk factor of cardiovascular disease [1]. Additionally, the composition of lipoprotein subclasses predicts cardiovascular outcomes [2,3]. Many of these unfavourable alterations can be counteracted by exercise, thereby, reducing the likelihood of developing cardiovascular disease [4].

A previous study reported no change in total plasma TG 4 h after prolonged moderate intensity exercise, but lower TG concentrations the following day [5]. Moreover, studies of very low-density lipoprotein triacylglycerol (VLDL-TG) metabolism

have shown increased removal of VLDL-TG on the day after a single exercise bout [6,7]. One group reported that 45 min of moderate intensity exercise increased the fractional catabolic rate of VLDL-TG, but did not alter VLDL-TG concentration [8]. We recently studied VLDL-TG kinetics during and immediately after 90 min of moderate intensity exercise and found a decrease in total TG and unchanged levels of VLDL-TG in plasma [9].

In the report by Yu et al. [10], which is the only study examining whether a single bout of exercise alters lipoprotein subclasses, blood samples were collected before and after an Ironman Triathlon. This extreme exercise in highly trained individuals lowers plasma TG concentrations as well as those

**Abbreviations:** <sup>1</sup>H NMR, Nuclear Magnetic Resonance; BMI, Body mass index; CVD, Cardiovascular disease; DEXA, Dual X-ray absorptiometry; FFA, Free fatty acids; HDL, High density lipoprotein; IDL, Intermediate density lipoprotein; LDL, Low density lipoprotein; LPL, Lipoprotein lipase; TG, Triglyceride; VLDL, Very low-density lipoprotein; VO<sub>2peak</sub>, Peak oxygen uptake.

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of the small LDL subfraction. This was accompanied by an increase in the larger HDL subclasses. Exercise training has been shown to have similar effects, with lowering of small LDL and VLDL, and increases in large HDL subclasses [11–14]. These beneficial effects are thought to counteract the adverse cardiovascular profile of large VLDL and small LDL particles [4].

Our hypothesis was that acute exercise would induce a beneficial effect on lipoprotein subclasses in healthy, recreationally active men and women with a shift towards smaller VLDL particles and larger LDL and HDL particles similar to exercise training. We, therefore, took the opportunity to analyze plasma samples from a previously conducted study of VLDL–TG kinetics in healthy subjects [9]. We analyzed our samples by  $^1\text{H}$  NMR spectroscopy to determine potential changes in lipoprotein subclasses, which may aid the understanding of the beneficial effects of exercise.

## 2. Methods

### 2.1. Subjects

The study was approved by the local Ethics Committee, and written informed consent was obtained from all participants. We recently reported the effect of exercise on VLDL–TG oxidation and kinetics from the same study [9]. Sixteen lean, healthy individuals (8 men and 8 women) were recruited (age 20–30 years, BMI < 25 kg/m<sup>2</sup>). Subjects had normal blood count and chemistry panel including lipid profile (fasting total cholesterol < 6.0 mmol/L, HDL-cholesterol > 0.9 mmol/L, LDL-cholesterol < 5.0 mmol/L, TG < 1.7 mmol/L) documented before participation. They were recruited through posters at local educational institutions.

Potentially eligible volunteers visited the Clinical Research Laboratory after an overnight fast of 10–12 h. Blood was obtained for determination of a lipid profile, HbA1c, liver, kidney and thyroid function, and complete blood count. Medical history was taken and a physical examination performed including evaluation of inclusion and exclusion criteria.

Seven days prior to the metabolic study day subjects who met the eligibility criteria visited the Clinical Research Laboratory after an overnight fast of 10–12 h. Peak oxygen uptake ( $\text{VO}_{2\text{peak}}$ ) was determined by an incremental (20 W/min) cycling protocol until exhaustion (Oxycon Pro, Erich Jaeger, Hoechberg, Germany) [15]. At the same visit, a dual X-ray absorptiometry (DEXA) scan was performed to measure body composition [16].

Subjects were interviewed by a dietician, who estimated their daily caloric intake. Based on calculations made by the dietician, subjects consumed a weight maintaining diet (55% carbohydrate, 15% protein, and 30% fat) provided by the hospital kitchen during the 3 days preceding the metabolic study.

### 2.2. Metabolic study day

Subjects were admitted to the Clinical Research Laboratory at 10 p.m. the evening before the study. From this point and until the end of the study only ingestion of tap water was allowed. The study day included a 5 h basal period (0–300 min), a 1½ h bicycle exercise period at 50% of  $\text{VO}_{2\text{peak}}$  (300–390 min) and a

2 h recovery period (390–510 min). On the morning of the study day, a catheter was placed in a dorsal hand vein, which was kept heated in order to obtain arterialized blood. At  $t = 0, 180, 210, 240, 270, 300, 315, 330, 345, 360, 375, 390, 405, 420, 450, 480,$  and 510 min blood samples were drawn for measurement of total TG and VLDL–TG concentrations. At  $t = 270, 375$  and 510 min blood samples for  $^1\text{H}$  NMR spectroscopy were drawn.

#### 2.2.1. $^1\text{H}$ NMR spectroscopy

$^1\text{H}$  NMR spectroscopic assay of prepared plasma was performed at LipoScience, Raleigh, NC, as previously described [17,18]. In brief, the particle concentration and size of the different lipoprotein subclasses were derived from the measured amplitudes of the spectroscopically distinct lipid methyl group  $^1\text{H}$  NMR signals, they emit.  $^1\text{H}$  NMR spectroscopy gives quantitative measures of lipoprotein subclasses. Measurements are based on the distinctive NMR signals the individual subclasses emit. A strong correlation has been shown to other measures of lipoprotein subclasses (apoB100 ( $r = 0.928$ ), electrophoresis ( $r = 0.946$ )). Furthermore, low intra- and inter-assay variation coefficients were observed, in general below 6% [19]. The concentrations (nanomoles of particles/L or nmol/L) of the following subclasses were measured: small HDL (7.3–8.2 nm), medium HDL (8.2–8.8 nm), large HDL (8.8–13.0 nm), small LDL (18.0–21.2 nm), large LDL (21.2–23.0 nm), IDL (23.0–27.0 nm), small VLDL (27.0–35.0 nm), medium VLDL (35.0–60.0 nm) and large VLDL (>60 nm). Weighted average lipoprotein particle sizes in nanometres (nm) were calculated from the subclass levels and the diameters assigned to each subclass.

#### 2.2.2. Body composition

Total body fat, leg fat, fat percentage, and fat free mass were measured by DEXA scanning (QDR-2000).

### 2.3. Statistics

Data were analyzed with SPSS 17.0. Normality was tested with Shapiro–Wilk’s Normality test. Data are expressed as mean  $\pm$  SD or median (range) as appropriate. Differences between groups were evaluated using Student’s t-test or Mann–Whitney test. Analyses of the effects of exercise and recovery on TG concentrations were performed with two way repeated measurements ANOVA with factors for sex and time (basal, exercise and recovery). Lipoprotein particle concentrations and particle size over the study day were tested for each sex using Friedman’s test for repeated measurements. In the case of effect over time, comparison between the different periods was performed with Wilcoxon Signed Ranks test.

## 3. Results

### 3.1. Subject characteristics

Subject characteristics are presented in Table 1. The men and women had similar age and BMI. Total EE increased during exercise in both men [basal:  $1.3 \pm 0.1$  kcal/min; exercise:  $8.8 \pm 1.3$  kcal/min ( $p < 0.001$ )] and women [basal:  $1.0 \pm 0.1$  kcal/min; exercise:  $5.2 \pm 1.0$  kcal/min ( $p < 0.001$ )].

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