Effects of acute exercise on high density lipoprotein cholesterol and high density lipoprotein subfractions in moderately trained females

P M Gordon, S Fowler, V Warty, M Danduran, P Visich, S Keteyian

Abstract
Increases in high density lipoprotein cholesterol (HDL-C) levels have previously been reported after moderate exercise bouts lasting less than two hours in men. Little information exists, however, on HDL-C responses after moderate duration exercise in women. Post-exercise HDL-C modifications may appear differently in women because of higher baseline HDL-C concentrations and differences in lipolytic activity. To determine the influence of exercise on acute HDL-C responses in women, 12 trained premenopausal women (22 (4) years old; mean (SD)) who ran 24–48 km a week exercised on a motor driven treadmill at 75% VO₂MAX until 3.34 MJ (800 kcal) were expended (72 (9) min). Subjects were all tested during the early follicular phase of their menstrual cycle. Subjects were all tested during the early follicular phase of their menstrual cycle. Subjects were all tested during the early follicular phase of their menstrual cycle.

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Methods
SUBJECTS
Twelve women runners were recruited from Mankato State University and the surrounding community to participate in this investigation. They were selected on the basis of the following criteria: 18–35 years of age (mean (SD) age 22 (4) years); running 24–48 km a week for a minimum of six months (35.7 (9.1) km/week); stable weight for at least six months (35.7 (9.1) km/week); no use of medications or oral contraceptives; no current use of alcohol; no medical conditions that would preclude them from participation. Mean percentage body fat, determined using hydrostatic weighing and the Siri equation, was 14.3 (6.6)%. All participants reported regular menstrual cycles (27–32 days) for the previous six months. Each participant signed a written informed consent approved by the Institutional Review Board for Human Subjects. Subjects were given a health screening and oriented to running on a motor driven treadmill before participating.

EXPERIMENTAL DESIGN
On day 1 subjects underwent a graded exercise test on a motor driven treadmill to determine VO₂MAX. After a 15 minute period of rest, participants then ran on the treadmill to deter-
mine the work rate corresponding to 75% VO_{2}\text{MAX}.

One to two weeks later, on day 2, subjects ran on a motor driven treadmill at 75% VO_{2}\text{MAX} until 3.34 MJ (800 kcal) was expended. Each subject was scheduled to perform the experimental trial during the early follicular phase (days 5–10) of their menstrual cycle since minimal lipid and lipoprotein variation occurs during this phase. On day 2 subjects underwent a standard warm up run, at which time the treadmill speed was gradually increased until the appropriate power output (75% VO_{2}\text{MAX}) was reached. This was accomplished by increasing the treadmill speed to 70, 85, and 100% of the predetermined running speed every other minute. The treadmill speed was adjusted to maintain the target intensity throughout the exercise trial. This was done if VO_{2} varied ± 2 ml/kg per minute from the target intensity. During the exercise trial, cardiovascular and respiratory metabolic measures were recorded every minute. However, once steady state was ensured, subjects were allowed to remove the respiratory apparatus for seven minutes after every ten minutes of respiratory gas measurement. Respiratory gas measurements were obtained using a MedGraphics CPX metabolic system. Energy expenditure was calculated using the steady state respiratory exchange ratio and absolute VO_{2}. Cool water was provided to subjects every ten minutes during the exercise trial.

To avoid dietary interaction with lipid measurements, each subject was asked to maintain their normal dietary habits and complete a four day food diary beginning two days before the experimental trial. Daily nutrient intakes were analysed by the Food Processor II nutritional software package from ESHA Research Inc.

**BLOOD SAMPLING AND STORAGE**

Venous blood samples were obtained at the following time points: baseline and immediately (IPE), one hour (1 h PE), 24 hours (24 h PE), and 48 hours (48 h PE) after exercise. All blood samples were taken after a 12 h fast from an antecubital vein with the subject in the seated position. Subjects abstained from exercise for 48 hours before the baseline measurement, to avoid any influence on baseline. Blood samples were collected into a 10 ml heparinised tube. Plasma was analysed for total cholesterol, triglycerides, HDL-C, HDL_{2}-C, and HDL_{3}-C. The plasma samples were separated by low speed centrifugation and stored at −70°C until analysis.

**BLOOD ANALYSIS**

All blood assays were performed in duplicate in a Centers for Disease Control reference laboratory by the same technician. Interassay variation was prevented by analysing all samples from each subject in a single batch run. Cholesterol and triglycerides were measured using enzymic methods on a Cobas Bio (Roche) Centrifugal Analyzer. HDL-C was determined after precipitation of lower density lipoproteins using heparin/MnCl_{2}. The methods of Delong et al were used to calculate low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C). High density lipoprotein (HDL) subfractions were determined using HDL 2/3 precipitation methods. Packed cell volume and haemoglobin were measured using a Coulter Counter model STKS from Coulter Electronics Inc. Changes in plasma volume were determined by the methods of Dill and Costill.

**STATISTICAL ANALYSIS**

Results are all means (SD). Lipid and lipoprotein variables were analysed using one way analysis of variance with repeated measures. All values were corrected for changes in plasma volume. A Tukey HSD post hoc test was utilised to test for differences between means. Values for each time point were compared with baseline values. p<0.05 was required for statistical significance.

**Results**

On day 2 subjects exercised for 72 (9.8) min at 73.4 (2) % of VO_{2}\text{MAX} (41.1 (3.4) ml/kg per min). The subjects’ total energy expenditure was 3.35 (0.01) MJ (801.4 (3.4)) kcal, completing a total distance of 12.2 (2.1) km. The subjects’ average daily diet consisted of 3.35 (0.01) MJ (1630 (338)) kcal and was comprised as follows: 65 (10)% carbohydrate, 14 (3)% protein, and 22 (8)% fat. The mean polyunsaturated to saturated fat ratio was 0.7 (0.2). Diet composition was unchanged over the course of the experiment (table 1).

**UNCORRECTED FOR PLASMA VOLUME**

Significant differences in lipid and lipoproteins, uncorrected for changes in plasma volume, were observed over time (table 2). HDL-C at IPE was increased above baseline values (p<0.01) but returned to baseline at 1 h PE. Similarly, HDL-C was increased above baseline values at IPE (p<0.01). However, no significant increase was observed in LDL-C. Plasma triglyceride values were significantly lower than baseline at 24 h PE (p<0.05) and 48 h PE (p<0.05). As expected, VLDL-C replicated plasma triglyceride values (p<0.05). No significant differences were observed between baseline and any other sampling point for total cholesterol or LDL-C.

**CORRECTED FOR PLASMA VOLUME**

Once corrected for post–exercise plasma volume shifts, significant lipid and lipoprotein changes were observed which differed from the uncorrected values. The calculated loss in plasma volume IPE was 4 (6)% (p<0.05) (table 3). When the shift in plasma volume was...
Table 2 Uncorrected plasma lipid and lipoprotein responses after exercise in moderately trained females. Values are mean (SD)

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<th>Baseline</th>
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<tr>
<td></td>
<td>IPE</td>
<td>1 h PE</td>
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<tr>
<td>TC</td>
<td>3.88 (0.6)</td>
<td>4.13 (0.8)</td>
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<td>TG</td>
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<tr>
<td>HDL3-C</td>
<td>0.67 (0.3)</td>
<td>0.64 (0.3)</td>
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<tr>
<td>HDL2-C</td>
<td>0.8 (0.14)</td>
<td>0.98 (0.2)*</td>
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All values are given as mmol/l. To convert cholesterol to mg/dl divide by 0.02586. To convert triglycerides to mmol/l divide by 0.01129. TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; VLDL, very low density lipoprotein; HDL, high-density lipoprotein. *Significant difference from baseline value (p<0.05). **Significant difference from baseline value (p<0.01).

Table 3 Corrected plasma lipid and lipoprotein responses after exercise in moderately trained females. Values are mean (SD)

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to HDL₃-C (fig 1). We observed a non-significant decline in HDL₃-C IPE which is not fully understood. Nevertheless, other investigators have reported transient increases in HDL₃-C after shorter duration exercise (less than two hours) in women.²⁶ ²⁷ Moreover, studies involving short term exercise in men with the same training level and energy expenditure as in the present study have also reported similar post-exercise increases in HDL₃-C.²⁶ ²⁷ However, the time course for post-exercise increases in HDL₃-C appeared different. For example, both Gordon et al and Visich et al did not observe a post-exercise increase in HDL₃-C until 24 h PE.

In contrast, HDL₄-C was not significantly different across sampling points. However, a non-significant increase in HDL₄-C occurred as HDL₄-C began to decline at 1 h PE (fig 1). In fact, by 48 h PE the change in HDL₄-C was more highly associated with an increase in HDL₄-C (r = 0.917; p<0.001) than HDL₃-C (r = 0.562; p<0.05). This may exemplify the dynamic environment to which HDL subfractions exist. Berger et al first proposed that the esterification of free cholesterol by lecithin: cholesterol acyltransferase may be rate limiting, suggesting that additional time may be required for sufficient conversion of HDL₃-C to HDL₄-C to raise HDL₄-C concentrations significantly.²⁷ On the basis of the present findings, we speculate that an acute exercise induced increase in HDL₄-C may be controlled by a rate limiting lipolytic process and therefore may require more time to increase significantly above baseline.

The underlying biochemical mechanism responsible for acute increases in HDL₄-C after exercise is probably related to catabolism of triglyceride rich lipoproteins via lipoprotein lipase. Increased lipoprotein lipase activity after exercise results in chylomicron and VLDL hydrolysis and reductions in plasma triglyceride levels.²⁸ Consequently, surface remnants are converted into nascent HDL₄-C. However, while HDL₄-C levels may increase after exercise, a concomitant decline in plasma triglyceride levels has not been consistently observed. Moreover, reductions in plasma triglyceride concentrations after acute exercise appear to be related to the amount of work completed.²⁸ Exercise studies of women taking part in endurance exercise that require a high energy expenditure—for example, marathon—have reported increased plasma triglyceride levels 24 h after exercise.²⁹ In contrast, shorter durations of exercise may result in only modest decreases in plasma triglyceride or no change at all.³⁰ In the present study, once values were corrected for changes in plasma volume, triglyceride levels at 48 h PE were no longer significantly different from baseline (p = 0.06). However, the average decline in plasma triglyceride concentrations at 48 h PE was 21% and was similar to observations in men performing the same volume of exercise.²⁸ Further, the decline in plasma triglyceride observed in the present study, while not statistically significant, probably has clinical ramifications for HDL₄-C improvements.

In summary, it appears that an exercise bout of less than two hours in duration can elicit post-exercise increases in HDL₃-C in moderately trained parthenons, but in women have higher baseline HDL₃-C values than men, a similar delayed increase can be observed provided that training status and amount of exercise performed is equivalent. In addition, post-exercise modifications in HDL subfractions may be more dynamic in women than men. However, the exact mechanisms responsible for these alterations in lipolytic activity requires further investigation.

1 Miller NE. Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. Am Heart J 1987;2:589–97.
Musings of a running “agona aunt”

Although the learned researchers and clinicians who have their offerings published in the British Journal of Sports Medicine probably do not realise it, much of their material is relatively unintelligible to the general public. Yet if you scan the book stalls, sports magazines abound, and many include medical and injury columns together with the obligatory “Agony Aunt” to satisfy the need for definitive advice on sport and exercise medicine.

Although it may seem easy for a doctor writer to fill the pages of a magazine with precis of interesting research, cautionary tales, and simple common sense, pitfalls abound. In the early 1980s, when people only wanted to run marathons and any other distance was considered soft, queries came from men with broken legs who had only one ambition in life—to run a marathon—when could they? Doubts begin to surface, however, as one realises that there is a considerable responsibility, both to the reader and their medical advisers, in replying. Should the exfootballer, already missing part of a meniscus, be warned that running high mileages on tarmac could aggravate his knee condition, and what of the lady who vomits at the three mile mark of every run, then can run for ever? Is it pathological or psychological?

Having read the closely written pages of A4, often with detailed training schedules and racing programmes, sometimes with an action photo thrown in, one has to appreciate the desperation with which some of these letters are written. Most have already sought medical advice and met with varying degrees of disinterest, incomprehension, trivialisation, and sometimes—only having received a highly subjective account of the problem—what appears to be an arrogant dismissiveness by the profession that the enquirer should never have entertained hopes of competing or running after injury. While it is all too easy to criticise the obsession and introspection with which these people view their sport, to many it is not only a hobby and an escape, but their whole social life. Replying tactfully in order not to exacerbate differences between patient and doctor can require the skills of a Kissinger on occasions.

Many have received advice that is totally negative, and seek a second opinion. One has to be guarded in one’s answers, for, although the runner whose myopic retina detached in a race really is at risk of a recurrence, the MI victim may benefit his coronary arteries through a graduated training programme, although this really ought to be under the guidance of a cardiologist after full investigation and counselling, most of which is outside the scope of a written and published reply.

The postbag confirms that injured sportsmen feel that they do not receive the urgent priority care that their injuries merit. Whether they are more poorly treated than other sections of the population is another debate. What is undoubtedly true is that they feel there is neither the interest nor very often the local ability to understand and treat their problems. Much of their frustration is the result of a high degree of motivation which tempts some to overdo the therapeutic processes.

Finally, what of the letter I received from a lady about to have a reduction mammoplasty, as the offending objects were slowing her running down. Would this operation affect her centre of gravity and therefore cause injury, she wanted to know? Answers please on a plain postcard!

Patrick Milroy

Dr Milroy has been the medical advisor to Runners World since 1982.
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doi: 10.1136/bjsm.32.1.63

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