The Effects of a Classic Spartathlon Race on Lipids and Prostanoids in Endurance Male Athletes

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Abstract: The aim of this study was to examine the effect of a classic Spartathlon race, which means continuous running for 246 km, on the concentration of 2,3-dinor 6-keto-prostaglandin (2, 3-dinor 6-keto PGF1α), the urinary metabolite of the prostacyclin (PGI2), 2, 3-dinor-thromboxane B2 (2, 3 dinor TXB2), the urinary metabolite of thromboxane A2 (TXB2), the 2, 3 dinor 6-keto-PGF1α, 2, 3 dinor-TXB2 ratio, Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein-L (LDL-L) and the TC: HDL-C ratio. It was hypothesized that these parameters would be changed after the completion of the Spartathlon. For this study proposes blood and urine samples were obtained from 19 male athletes, all of which finished the Spartathlon race in <35 h, before, at the end of and 24 h after the race. After result analysis, the levels of all the substances measured were different at the end of the race compared with before the race and these altered levels remained 24 h after the race. Importantly, it was observed that metabolism of 2, 3 dinor-6-keto-PGF1α at the end of the race was fourfold than before the race (p<0.001) and the concentration of 2, 3 dinor TXB2 after the race was tenfold than before the race (p<0.001).

Key words: Sustained exercise, urine prostacyclin, urine thromboxane, lipsids

INTRODUCTION

Many research studies support the view that participation in regular aerobic activities such as walking, jogging, swimming, or cycling have a positive effect on the lipid, lipoprotein and prostaglandin profile (Stergioulias et al., 1998; Durstine et al., 2001, 2002). In healthy men and women, physical activity is associated with higher HDL-C level, higher prostacyclin level, as well as lower triglyceride and thromboxane levels, while total plasma cholesterol level (TC) is not altered (Durstine and Haskell, 1994). It has been proposed that the total serum cholesterol/high density lipoprotein cholesterol ratio (TC/HDL-C) and the prostaglandin I2/thromboxane A2 (PGI2/TXA2) ratio, may be more important indicators of cardiovascular disease risk than TC, HDL-C, PGI2 and TXA2 levels (Kokkinos and Fernhall, 1999; Stergioulias et al., 2001; Kodama et al., 2007). There is also evidence for an interaction between prostanoids and lipids in the maintenance of hemostatic balance (Akkerman, 2006).

A positive correlation has also been found between prostacyclin synthesis and the levels of HDL-C; HDL-C stimulates prostacyclin synthesis both in vivo and in vitro (Frangos et al., 1985).

Moreover, marathon running, which lasts up to 3 h, causes a dramatic increase in urine arachidonic metabolites 2, 3 dinor 6-keto PGF1α and 2, 3 dinor-TXB2 (Ronni-Sivula et al., 1993).

To present knowledge, there has not been any controlled, randomized clinical trial that investigated the effects of ultramarathon race, as the Spartathlon on prostanoid and lipid metabolism. Given this background, the present study was designed as clinical trial, to determine the effects of ultramarathon race, like Spartathlon in selected lipids and lipoproteins, particularly: (a) High-Density Lipoprotein Cholesterol (HDL-C), (b) Low-Density Lipoprotein Cholesterol (LDL-L), (c) Triglycerides (TG), (d) Total Cholesterol (TC), (e) Amounts of the urinary arachidonic acid metabolites 2, 3 dinor-6-keto-PGF1α, and 2, 3 dinor-TXB2, and (f) the ratios TC/HDL-C and 2, 3 dinor-6-keto-PGF1α/2, 3 dinor-TXB2, at the end of and 24 h after the race.

MATERIALS AND METHODS

Subjects: The study was performed by the University of Athens, Department of Physical Education and Sports Science and all aspects of the study were approved by the Athens University Medical Ethics Committee.

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The Spartathlon is an ultra-distance foot race, whereby the runners attempt to cover the distance from Athens to Sparta, 246 km; thus, the body undergoes moderate-intensity exercise for an extended period of time. The study was performed during the 2000 Spartathlon race, during which the ambient daily temperatures were at maximum 32°C and at minimum 10°C and the mean daytime relative humidity was 62-84%.

All potential participants (n = 88) were informed of all the procedures and purposes of the study and their written informed consent was obtained before participating in the study. However, 52 athletes did not complete the event; the data for the study was derived from 19 healthy male subjects (median age, 37 years; range 30-49 years), who finished the race in <40 h (mean and median running time were 33 h, 2 min and 30 h, 2 min, respectively; range, 25 h, 17 min to 34 h, 43 min).

**Samples:** Blood and urine samples for determining HDL-C, LDL-C, TC, TG, 2, 3 diino 6-keto-PGF1α, and 2, 3 diino-TXB2 were taken (i) between 8 and 10 am, after a 10 h fast and at least 24 h before the start of the race, (ii) immediately after (within 20 min of) the end of the race and (iii) 24 h after the end of the race. The subjects consumed electrolytes and carbohydrates ad libitum before, during, and after running. Five milliliter sample of blood for testing was drawn from the antecubital vein by a qualified microbiologist following standard clinical procedures and was allowed to clot at room temperature for 15-20 min. Samples were then centrifuged (at 2800 rpm) for 15 min and stored at 18°C for later analysis. The urine samples were stored at -20°C. The blood and urine samples were transferred to the Department of Biochemistry, Markopoulou National Medical Center for analysis.

**Lipids measurement:** Serum lipids and lipoproteins were measured using a technicon R-XT autoanalyser (Biosys, Karlruhe, Germany) and an automatic enzymatic technique was used to determine the TC and TG (Boehringer Mannheim GmbH, Germany). HDL-C levels were quantitated in the supernatant after precipitation of LDL and Very Low-Density Lipoprotein (VLDL) with phosphotungstic acid (Boehringer Mannheim kit) (Allain et al., 1974; Haskel, 1984; Lopes-Virella et al., 1977).

**Prostanoid measurement:** Aliquots (50 mL) of urine were acidified to pH 3.0 and endogenous prostanoids were concentrated and recovered by solid-phase extraction on octadecyl C18 silica cartridges. The efficiency and reproducibility of recovery were determined by pre and post-extraction spikes with known amounts of tetradecuterated reference standards. The recovered extracts were dissolved and converted to trimethylsilyl ether derivatives. This was followed by derivatization to their pentafluorobenzyl ester methoxyamine derivatives. The derivatives were then analysed by reversed-phase high-performance liquid chromatography. Selected reaction monitoring of the characteristic daughter ions, which were generated by collision-activated dissociation of the corresponding parent ions for endogenous prostanoids and their stable isotope-labelled analogues, were used for gas chromatography and mass spectrometry (GC/MS) to be performed (TSQ45, Finnigan MAT, San Jose, CA, USA). Urinary creatinine was determined spectrophotometrically, using an alkaline picric acid reaction and standard methods, on an automatic analyser (Bechman Galway, Ireland). Lastly, the identified prostanoid metabolites were expressed as picograms per milligram of creatinine. All analyses were done in duplicate and all samples from a given subject were analysed in the same batch. The coefficients of variation for intra and inter-assay lipid and prostanoid concentrations were ±5% (Patrono et al., 1986; Frollish et al., 1988; Stergiouls and Filippou, 2006).

**Statistical analysis:** The SPSS (version 12.0 for Windows 2000) statistical package was used the analysis of the data. After assessing the normal distribution of the data, a paired observation t-test was used to analyze time course changes. The probability level for significance was set at 0.05. All parameters are expressed as Mean±SE (Kabitsis, 2004).

**RESULTS**

The mean concentration of TC, TG and LDL-C, decreased within 20 min of the end of the race, reaching a minimum value with a tendency for an increase at 24 h after the race (Table 1). Significant differences were found when the above mentioned parameters before the race were compared with those found within 20 min of the end (p<0.01, 0.001 and 0.001, respectively and at 24 h after the race (p<0.001, 0.001 and 0.001), Table 1). Non significant

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before the race</th>
<th>Within 20 min after race finish</th>
<th>24 h after the race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol L⁻¹)</td>
<td>6.66±1.22</td>
<td>4.71±1.00</td>
<td>4.90±0.85</td>
</tr>
<tr>
<td>Triglyceride (mmol L⁻¹)</td>
<td>4.31±1.32</td>
<td>1.80±0.50</td>
<td>2.19±0.57</td>
</tr>
<tr>
<td>LDL cholesterol (mmol L⁻¹)</td>
<td>3.75±0.73</td>
<td>2.71±0.62</td>
<td>2.92±0.63</td>
</tr>
<tr>
<td>HDL cholesterol (mmol L⁻¹)</td>
<td>1.57±0.20</td>
<td>1.76±0.13</td>
<td>1.64±0.16</td>
</tr>
<tr>
<td>TC/HDL ratio</td>
<td>3.66±0.61</td>
<td>2.77±0.39</td>
<td>3.02±0.44</td>
</tr>
<tr>
<td>2, 3 diino 6-keto-PGF1α (pg/n creat)</td>
<td>75.10±13.91</td>
<td>303.10±65.28</td>
<td>155.70±30.22</td>
</tr>
<tr>
<td>2, 3 diino TXB2 (pg/n creat)</td>
<td>95.70±20.15</td>
<td>944.70±74.12</td>
<td>376.38±47.66</td>
</tr>
<tr>
<td>2, 3 diino 6-keto-PGF1α/TXB2 ratio</td>
<td>0.81±0.160</td>
<td>0.32±0.070</td>
<td>0.42±0.100</td>
</tr>
<tr>
<td>2, 3 diino TXB2 ratio</td>
<td>1.02±0.160</td>
<td>0.32±0.070</td>
<td>0.42±0.100</td>
</tr>
</tbody>
</table>
differences were observed when comparing levels of TC, TG and LDL-C, within 20 min of the end of the race, with those at 24 h after the race (Table 1).

The mean concentration of HDL-C were significantly increased within 20 min of the end of the race, compared with those before the race (p<0.001), at 24 h after the race (p<0.001) and between the end of the race and 24 h after the race (Table 1). The increase in HDL-C and the decrease in TC had a significant impact on TC:HDL-C ratio at the end of the race and 24 h after the end of the race (Table 1).

The mean concentration of the prostacyclin metabolite 2,3-dinor-6-keto-PGF1α, normalized to creatinine levels, at the end of the race was fourfold that before the race (p<0.001 and continued to be high 24 h after the end of the race (p<0.001; Table 1). The mean concentration of thromboxane metabolite 2, 3-dinor-TXB2, at the end of the race was tenfold the pre-race values and remained so 24 h after the race (p<0.001 and p<0.001, respectively. Table1). The ratio of the two metabolites (2, 3-dinor-6-keto-PGF1α:2, 3-dinor-TXB2) increased at the end of the race in favor of TXB2 (p<0.001 and continued to be high 24 h after the end of the race (p<0.001; Table 1).

**DISCUSSION**

This study was undertaken to ascertain whether, in healthy endurance adult athletes, a classical Spartathlon run has any effect on lipids, in particular, the prostanooids 2,3-dinor-6-keto-PGF1α (a urinary metabolite of prostacyclin) and 2, 3-dinor-TXB2 (a urinary metabolite of thromboxane) and the ratio of 2, 3-dinor-6-keto-PGF1α to 2, 3-dinor-TXB2.

PGI2 and TXA2 are unstable compounds in vivo, therefore, their production is best estimated by measuring the levels of their metabolites in urine. 2, 3-dinor-TXB2 levels are assumed to reflect platelet TXA2 production and 2, 3-dinor-6-keto vascular PGI2 production (Patrone et al., 1986; Frolich et al., 1988).

**Effects of physical conditioning on prostanooids prostacyclin and thromboxane:** The results of this study show that Spartathlon running has clear negative effects on the concentration of prostanooids, that is, it is significantly increased adversely in favour of thromboxane. However, this finding contradicts several other studies that have investigated the acute effect of exercise on prostanooids in adults and children. For example, Boger et al. (1995) compared the PGI2:TXB2 ratio in relation to exercise and found a 72.2% increase in prostacyclin in untrained, healthy subjects and an increase of 112% in subjects who trained on a bicycle ergometer with 60% Wmax (submaximal exercise). In addition, a finding was that exercise does not significantly influence TXA2 excretion. This increase in PGI2 and unchanged TXA2 levels significantly increases the ratio in favour of vasoprotective and vasodilator prostacyclin.

Wennmalm and Fitzgerald (1988) found a three fold increase in urinary 2, 3 dinor-6-keto-PGF1α, but no changes in 2, 3 dinor-TXB2 in healthy men who exercised 2 h on a bicycle ergometer at 50% of individual Wmax.

Lemne et al. (1992) found a 76 to 160% increase in urinary 2, 3 dinor-6-keto-PGF1α and a 23% increase in 2,3 dinor-TXB2, which was not significantly different in normotensive and hypertensive subjects after 30 min of exercise at 75% of Wmax. In a study by Rorim-Sivula et al. (1993), using a sample of 15 women and ten men, the effect of strenuous physical exercise on the balance of prostacyclin and thromboxane was investigated. Measurements were taken ten days before, during and
one, three and five days after participation in a marathon. The results showed that, during the run, 2, 3-dinor-6-keto levels increased sevenfold (p<0.05), whereas 2, 3-dinor-TXB₂ levels increased fourfold (p<0.05).

In another study by Stergioulias et al. (2001) examined the effects of acute exercise on prostacyclin and thromboxane synthesis in 12 healthy untrained children who trained on a bicycle ergometer with 75% of the PWC170. The duration of exercise was one hour. The measurements of the urinary metabolites 2, 3-dinor-6-keto-PGF₁α and 2, 3-dinor-TXB₂ were determined before the exercise, at the end of 60 min of exercise and at the end of 60 min in the recovery period. The results showed that the ratio of 2, 3 dinor-6-keto-PGF₁α to 2, 3 dinor-TXB₂ was increased by exercise (p<0.05) in the experimental group.

In this study, it was found that the concentration of the urinary metabolites of the prostanooids prostacyclin and thromboxane increased inappropriately in favor of thromboxane.

It is known that HDL-C stimulates the activity of the enzyme prostacyclin synthetase and as result more prostacyclin is produced in vitro (Frangos et al., 1985). In the case of spartathlon race it seems that HDL-C looses this effect, since produced fewer prostacyclin in comparison to thromboxane. So, although HDL-C production increases significantly during the spartathlon race do not have a stimulated effect of prostacyclin in vivo (Stergioulias et al., 1998; Stergioulias and Filippou, 2006). More, a higher concentration of thromboxane affects the PG₁₂:TXA₂ ratio in favor of thromboxane.

However this effect on the PG₁₂:TXA₂ balance induced by prolonged physical activity should be interpreted with caution. Although a major conclusion of the studies is that TX metabolites increase significantly in comparison to PGI metabolites and this is detrimental, no data are available to suggest that PG-I-M and TXM should be generated in a ratio 1:1 to translate into cardioprotection (Tulppala et al., 1991; Boger et al., 1995; Stergioulias et al., 2001). Both metabolites are an index of systemic generation and the origin is unknown. Also, supporting the view that enhanced TXA₂ in the presence of decrease LDL-L and increased HDL-C and PG₁-M it is unlikely to translate into a hazard.

An other explanation of the decreasing of prostacyclin is the following: The HDL-C stimulates the activity of the enzyme prostacyclin synthetase and as result more prostacyclin is produced in vitro (Frangos et al., 1985). In the case of spartathlon race it seems that HDL-C looses this absolutely effect, since produced fewer prostacyclin in comparison to thromboxane. So, although HDL-C production increases significantly during the spartathlon race do not have a stimulated effect of prostacyclin in vivo (Stergioulias et al., 1998; Stergioulias and Filippou, 2006).

The source of the increased TX metabolites is derived from the platelet and hence COX-1. Some recent papers have suggested COX-2 and the macrophage may play an important role in Tx increase in disorders such as cigarette smoking (MacAdam et al., 2005). This is very relevant and important if the concern is about the increase in TX being pro-atherosclerotic. However, there is no support that the effects of COX-2 inhibits the TX production in endurance runners.

CONCLUSION

In conclusion, running a Spartathlon stimulates the metabolism of lipids, in particular, prostanooids PG₁, and TXA₂ in healthy adult endurance male athletes. The stimulation of TXA₂ is clearly greater than the stimulation of PG₁ during the race. If this is a marker of cardiovascular hazard, should further investigated.

REFERENCES


