http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (17): 2139-2143, 2008
ISSN 1028-8880

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

[1]A. Stergioulas, [1]A. Tripolitsioti and [2]A. Nicolaou
[1]Faculty of Human Movement and Quality of Life, University of Peloponnese, Greece
[2]Chair in Biological Chemistry, School of Pharmacy, University of Bradford, UK

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 PGF1a), the urinary metabolite of the prostacyclin (PGI_2), 2, 3-dinor-thromboxane B2 (2, 3 dinor TXB_2), the urinary metabolite of thromboxane A_2 (TXB_2), the 2, 3 dinor 6-keto-PGF1a:2, 3 dinor-TXB_2 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 Sparthathlon. 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-PGF1a at the end of the race was fourfold than before the race ($p<0.001$) and the concentration of 2, 3 dinor TXB_2 after the race was tenfold than before the race ($p<0.001$).

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

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 (Stergioulas *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 I_2/thromboxane A_2 (PGI_2/TXA_2) ratio, may be more important indicators of cardiovascular disease risk than TC, HDL-C, PGI_2 and TXA_2 levels (Kokkinos and Fernhall, 1999; Stergioulas *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 $PGF_{1\alpha}$ and 2, 3 dinor-TXB_2 (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-$PGF_{1\alpha}$ and 2, 3-dinor-TXB_2 and (f) the ratios TC/HDL-C and 2, 3-dinor-6-keto-$PGF_{1\alpha}$/2, 3-dinor-TXB_2. before, 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.

Corresponding Author: Apostolos Stergioulas, 3 Lyssandrou Street, Sparta 23100, Laconia, Greece

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 times 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 dinor 6-keto-PGF1a and 2, 3 dinor-TXB_2 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, Markopoulon National Medical Center for analysis.

Lipids measurement: Serum lipids and lipoproteins were measured using a technicon R-XT autoanalyser (Biosis, 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 (C_{18}) silica cartridges. The efficiency and reproducibility of recovery were determined by pre and post-extraction spikes with known amounts of tetradeuterated 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 (TSG45, 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; Frolish *et al*., 1988; Stergioulas 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 1: Measurement parameters (Mean±SD) examined in athletes that successfully completed the 2000 Spartathlon

Variables	Before the race	Within 20 min after race finish	24 h after the race
Total cholesterol (mmol L^{-1})	6.06±1.22	4.71±1.00	4.90±0.85
Triglyceride (mmol L^{-1})	4.31±1.32	1.80±0.50	2.12±0.57
LDL cholesterol (mmol L^{-1})	3.75±0.73	2.71±0.62	2.92±0.63
HDL cholesterol (mmol L^{-1})	1.57± 0.20	1.76±0.13	1.64±0.16
TC/HDL ratio	3.60±0.610	2.77±0.39	3.02±0.44
2, 3 dinor 6-keto-PGF1a (pg/n creat)	75.10±13.91	303.10±65.28	155.70±30.22
2,3 dinor TXB_2 (pg/n creat)	95.70±20.15	944.70±74.12	376.38±47.66
2,3 dinor 6-keto-PGF1a/2,3 dinor TXB_2 ratio	0.81±0.160	0.32±0.070	0.42±0.100

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-PGF1a 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-TXB_2 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; Table 1). The ratio of the two metabolites (2, 3-dinor-6-keto-$PGF1^{\alpha}$:2, 3-dinor-TXB_2) increased at the end of the race in favor of TXB_2 ($p<0.001$ 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 prostanoids 2,3-dinor-6-keto-PGF1a (a urinary metabolite of prostacyclin) and 2, 3-dinor-TXB2 (a urinary metabolite of thromboxane) and the ratio of 2, 3-dinor-6-keto-PGF1a to 2, 3-dinor-TXB_2.

PGI_2 and TXA_2 are unstable compounds *in vivo*, therefore, their production is best estimated by measuring the levels of their metabolites in urine. 2, 3-dinor-TXB_2 levels are assumed to reflect platelet TXA_2 production and 2, 3-dinor-6-keto vascular PGI_2 production (Patrono *et al*., 1986; Frolish *et al*., 1988).

Effects of physical conditioning on lipids: The changes in lipid concentration observed in this study are similar to those of previous studies. For example, Ferrauti *et al*. (1997) investigated the effects of intensive tennis training on lipid and lipoprotein metabolism in 22 healthy adults. The results showed that the subjects of the experimental group had higher HDL levels than the control subjects. The researchers concluded that regular tennis training influences cardiovascular risk factors in a positive manner.

Hsieh *et al*. (1998) investigated the effects of a continuous physical activity for 30 min or more, three times per week in 3331 adult Japanese men. After analysis were founded that the subjects that participated in the exercise had higher HDL cholesterol values in comparison to control group and lowest triglyceride.

Wirth (1993) investigated the changes in lipids in subjects that participated in a soccer game that lasts up to 2 h and observed a significant increase in HDL concentration in comparison with values before the game. Durstine *et al*. (2002) pointed out that participation in sub maximal exercise increases HDL levels and decreases TG levels significantly. Marti *et al*. (1991) investigated the relationship of HDL with training in two groups: one that constituted high-level athletes and a second that were sedentary controls. A strong relationship between HDL levels and the number of training hours of the athletes was the outcome of their study.

Several other investigations support the view that participation in regular athletic activities increases the blood concentration of HDL-C, decreases LDL-L and TG concentration, while the concentration of TC is unchanged (Tsopanakis *et al*., 1986; Tamai *et al*., 1992; Sharma, 1993; Fallon, 2001).

Effects of physical conditioning on prostanoids prostacyclin and thromboxane: The results of this study show that Spartathlon running has clear negative effects on the concentration of prostanoids, 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 prostanoids in adults and children. For example, Boger *et al*. (1995) compared the PGI_2:TXB_2 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% W_{max} (submaximal exercise). In addition, a finding was that exercise does not significantly influence TXA_2 excretion. This increase in PGI_2 and unchanged TXA_2 levels significantly increases the ratio in favour of vasoprotective and vasolidalator prostacyclin.

Wennmalm and Fitzgerland (1988) found a three fold increase in urinary 2, 3 dinor-6-keto-PGF1a, but no changes in 2, 3 dinor-TXB_2 in healthy men who exercised 2 h on a bicycle ergometer at 50% of individual W_{max}.

Lemne *et al*. (1992) found a 76 to 160% increase in urinary 2, 3 dinor-6-keto-PGF1a and an 23% increase in 2,3 dinor-TXB_2, which was not significantly different in normotensive and hypertensive subjects after 30 min of exercise at 75% of W_{max}. In a study by Ronni-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-TXB_2 levels increased fourfold ($p<0.05$).

In another study by Stergioulas *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-PGF1a and 2, 3-dinor-TXB_2 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-PGF1a to 2, 3 dinor-TXB_2 was increased by exercise ($p<0.05$) in the experimental group.

In this study, it was founded that the concentration of the urinary metabolites of the prostanoids 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 sparthathlon race do not have a stimulated effect of prostacyclin *in vivo* (Stregioulas *et al*., 1998, 2001; Stergioulas and Phillipou, 2006). More, a higher concentration of thromboxane affects the PGI_2:TXA_2 ratio in favor of thromboxane.

However this effect on the PGI_2-TXA_2 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 PGI-M and TXM should be generated in a ratio 1:1 to translate into cardioprotection (Tulppala *et al*., 1991; Boger *et al*., 1995; Stergioulas *et al*., 2001). Both metabolites are an index of systemic generation and the origin is unknown. Also, supporting the view that enhanced TXA_2 in the presence of decrease LDL-L and increased HDL-C and PGI-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 sparthathlon race do not have a stimulated effect of prostacyclin *in vivo* (Stergioulas *et al*., 1998; Stergioulas 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, prostanoids PGI_2 and TXA_2 in healthy adult endurance male athletes. The stimulation of TXA_2 is clearly greater than the stimulation of PGI_2 during the race. If this is a marker of cardiovascular hazard, should further investigated.

REFERENCES

Akkerman, J.W.N., 2006. The Interaction between lipoproteins and platelets. Pathophysiol. Haemos. Thromb. 35: 265-267.

Allain, C.C., L.S. Poom, C.S. Chan, W.S. Richmonal and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Boger, R.H., S.M. Bode-Boger, E.P. Schroder, D. Tsikas and J.C. Frolich, 1995. Increased prostacyclin production during exercise in untrained and trained men: Effect of low-dose aspirin. J. Applied Physiol., 78: 1832-1838.

Durstine, J.L. and W.I. Haskell, 1994. Effects of exercise training on plasma lipids and lipoproteins. Exerc. Sport Sci. Rev., 22: 477-521.

Durstine, J.L., P.W. Grandjean, P.G. Davis, M.A. Ferguson and N.L. Alderson *et al*., 2001. Blood lipid and lipoprotein adaptations to exercise: A quantitative analysis. Sports Med., 31: 1033-1062.

Durstine, J.L., P.W. Grandjean, C.A. Cox and P.D. Thompson, 2002. Lipids, lipoproteins and exercise. J. Cardiopulm. Rehabil., 22: 385-398.

Fallon, K.E., 2001. The acute phase response and exercise: The ultramarathon as prototype exercise. Clin. J. Sport Med., 11: 38-43.

Ferrauti, A., K. Weber and H.K. Struder, 1997. Effects of tennis training on lipid metabolism and lipoproteins in recreational players. Br. J. Sports Med., 31: 322-336.

Frangos, J.A., S.E. Eskin, L.V. Mclutire and C.L. Ives, 1985. Flow effects on prostacyclin production by cultured human endothelial cells. Science, 227: 1477-1479.

Frolish, J.C., K.H. Marx, H. Bippi, J. Faouler and B. Rosencranz *et al*., 1988. Analysis of Eicosanoids by GC/MS/MS. In: Bioanalysis of Drugs and Metabolites. Methological Surveys in Biochemistry and Analysis, Reid, E., J.D. Robinson and I.D. Wilson (Eds.). Plenum Press, New York, ISBN: 0306429969, pp: 15-21.

Haskel, W.L., 1984. The influence of exercise on the concentrations of triglyceride and cholesterol in human plasma. Exerc. Sports Sci. Rev., 12: 205-244.

Hsieh, S.D., H. Yoshinaga, T. Muto and Y. Sakurai, 1998. Regular physical activity and coronary risk factors in Japanese men. Circulation, 97: 661-665.

Kabitsis, C., 2004. Research Methods in Sport Sciences. 1st Edn., Tsirtsianis Publ., Thessaloniki, ISBN: 960-8237-27-0.

Kodama, S., S. Tanaka and K. Saito, 2007. Effect of aerobic exercise training on serum levels of high-density lipoprotein cholesterol: A meta-analysis. Arch. Int. Med., 167: 999-1008.

Kokkinos, P.F. and B. Fernhall, 1999. Physical activity and high density lipoprotein cholesterol levels: What is the relationship. Sports Med., 28: 307-314.

Lemne, C., O. Vesterqvist, N. Egberg, K. Green and T. Jogestrand *et al*., 1992. Platelet activation and prostacyclin release in essential hypertension. Prostaglandins, 44: 219-235.

Lopes-Virella, M.F., P. Stone, S. Ellis and J.A. Colwell, 1977. Cholesterol determination of high density lipoprotein separated by three different methods. Clin. Chem., 23: 882-884.

MacAdam, B.F., D. Byrne, J.D. Morrow and J.A. Oates, 2005. Contribution of cyclooxygenase-2 to elevated biosynthesis of thromboxane A2 and prostacyclin in cigarette smokers. Circulation, 16: 941-945.

Marti, B., M. Knobloch, W.F. Riesen and H. Howald, 1991. Fifteen year changes in exercise, aerobic power, abdominal fat and serum lipids and controls. Med. Sci. Sports Exerc., 23: 115-122.

Patrono, C., G. Ciabatoni, F. Pugliese, A. Pierucci, I.A. Blair and G.A. FitzGerald, 1986. Estimated rate of thromboxane secretion into circulation in normal humans. J. Clin. Invest., 77: 590-594.

Ronni-Sivula, H., H. Malm, N. Ylikorkala and L. Viinikka, 1993. Marathon run stimulates more prostacyclin than thromboxane synthesis and differently in men and women. Prostaglandins, 46: 75-79.

Sharma, A.M., 1993. Effects of exercise on plasma lipoproteins. N. Engl. J. Med., 348: 1494-1496.

Stergioulas, A., A. Tripolitsioti, D. Messinis, A. Bouloukos and C.H. Nounopoulos, 1998. The effects of endurance training on selected coronary risk factors in children. Acta Paed., 87: 401-404.

Stergioulas, A., P. Baltopoulos and T.H. Papadopoulou, 2001. Prostacyclin and thromboxane urinary metabolites in children during acute exercise. Acta Med. Bulg., 28: 125-134.

Stergioulas, A. and D. Filippou, 2006. Effects of physical conditioning on lipids and arachidonic acid metabolites in untrained boys: A longitudinal study. Applied Phys. Nutr. Metabol., 32: 1-10.

Tanıai, T., M. Huguchi, K. Oida, T. Nakai and S. Miyabo *et al*., 1992. Effects of Exercise on Plasma Lipids and Lipoprotein Metabolism. In: Integration of Medical and Sports Sciences, Sato, Y., J. Poortmans, I. Çashimoto and Y. Oshida, (Eds.). Basel, Karger, Germany, ISBN: 3805555792, pp: 430–438.

Tsopanakis, C., D. Kotsarellis and A. Tsopanakis, 1986. Lipoprotein profile of elite athletes in olympic sports. Int. J. Sports Med., 7: 316-321.

Tulppala, M., L. Viinikka and O. Ylikorkala, 1991. Thromboxane dominance and prostacyclin deficiency in habitual abortion. Lancet, 13: 879-881.

Wennmalm, A. and G.A. Fitzgerald, 1988. Excretion of PGI_2 and TXA_2 metabolites during leg exercise in humans. Heart Circ. Physiol., 225: H15-H18.