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The effect of exercise on the production and clearance of testosterone in well trained young men

T. A. Cadoux-Hudson[1], J. D. Few[1], and F. J. Imms[2]

[1] Department of Chemical Pathology, St. Mary's Hospital Medical School, London W2 1PG, Great Britain
[2] Department of Physiology, Guy's Hospital Medical School, London SE1, Great Britain

Summary. Tritium-labelled testosterone was infused into four well-trained subjects at rest and during one hour of exercise at about 60% of their maximum aerobic power. This exercise regime led to a mean increase of 27% (range 10—51%) in plasma testosterone concentration. At the same time there were significant decreases in the estimated hepatic plasma flow (EHPF) (45%; range 28—67%), metabolic clearance rate of testosterone (MCR_T) (29%; range 18—37%) and plasma volume (8.2%; range 3—10%). The production rate of testosterone decreased by 10% (range 9—22%) but this was not statistically significant. The ratio MCR_T:EHPF increased in 3 out of 4 subjects in response to exercise but there was considerable inter-subject variation both at rest and during exercise.

These findings suggest that the exercise-induced elevation of testosterone level is due solely to the reduction in the rate at which testosterone is cleared from the plasma. The principal cause of the reduction in MCR_T is probably the reduction in EHPF but the variation in the ratio MCR_T:EHPF suggests that changes in the extrahepatic clearance of testosterone may also be involved.

Key words: Testosterone — Exercise — Liver circulation — Metabolic clearance rate — Adipose tissue

Introduction

Review of the literature shows that the effect of aerobic exercise upon plasma testosterone level may be variable. Long-term endurance events, such as marathon runs and longer, led to a reduction in plasma testosterone level (de Lignieres et al. 1976; Dessypris et al. 1976; Morville et al. 1979) whereas short to moderate duration exercise (up to one hour) commonly leads to an increase (Sutton et al. 1973; Metivier et al. 1980; Wilkerson et al. 1980). The mechanism causing this increase is not clear. The fact that there is no simultaneous increase in plasma luteinising hormone (LH) levels (Sutton et al. 1973; Brisson et al. 1977; Metivier et al. 1980) has led to the suggestion that the rise in plasma testosterone concentration is not due to an increase in the rate of testicular secretion of testosterone. Wilkerson et al. (1980) claimed that the exercise-induced increase in plasma testosterone level could be accounted for by haemo-concentration, but the increases observed by some other workers have been too great to be explained in this manner.

Another possibility is that the increase in testosterone level in short-term exercise might be a result of a reduced rate of clearance of testosterone from the plasma. The liver is a major site for the clearance of testosterone (Rivarola et al. 1967; Ishimaru et al. 1978) and it is well established that liver blood flow decreases during exercise (Rowell et al. 1964). Keizer et al. (1980) have shown that exercise leads to a decrease in the rate of clearance of oestradiol in young women.

In order to investigate further the effect of exercise upon the secretion and metabolism of testosterone, we have measured the rates of production and clearance using a continuous infusion of [^{3}H]testosterone in well-trained young men at rest and during exercise on a bicycle ergometer.

Materials and methods

Radioactive steroids were obtained from Amersham International and their purity was checked by thin layer chromatogra-

Offprint requests to: J. D. Few at the above address

Table 1. Anthropometric data for the 4 subjects

Age (years) 20.9 ± 1.3	
Height (cm)	187.9 ±2.5
Weight (kg)	84.2 ±2.7
$V_{O_{2max}}$ ($l \cdot min^{-1}$)	5.62 ± 0.96
($ml \cdot kg^{-1}\ min^{-1}$)	66.7 ±3.1
Years in training	7.2 ±1.1

phy (TLC). Solvents were of analytical reagent grade. The antiserum for radioimmunoassay (RIA) was raised in rabbits against testosterone-3-(O-carboxy-methyl)oximino-BSA.

Experimental

The subjects were four oarsmen training for international events whose anthropometric details are listed in Table 1. The subjects were generally tested in pairs. Left and right forearm veins were cannulated (Venflon) and PTFE-connecting tube was used to couple the infusion cannula to the syringe containing the [^{3}H]testosterone dissolved in 10% ethanol in saline. A priming dose of [^{3}H]testosterone (~2 μCi) was administered over 2 min followed by a continuous infusion of 6 $\mu Ci \cdot h^{-1}$ for 2.5 h. Samples of the infusate were taken before and after the period of infusion for estimation of the rate of infusion of [^{3}H]testosterone. Previous experience has shown that no components of the infusion system absorb significant quantities of [^{3}H]testosterone. One subject of the pair remained seated throughout the period of the infusion and the other exercised on a bicycle ergometer during the final hour. During the first 10 min of exercise the work load was gradually increased until a heart rate of 160—165 bpm was reached which corresponded to 60% of the previously determined $\dot{V}_{O_{2max}}$. All four subjects completed the exercise test but one was not available for a control experiment. Blood samples (15 ml) for radiochemical analysis were taken at 15 min intervals while at rest and at 10 min intervals during exercise. Packed cell volume was measured using a microhaematocrit technique and this data was used to calculate changes in plasma volume. The protocol is illustrated in Fig. 1.

Indocyanine green clearance

This was determined 70 and 130 min after the priming dose of [^{3}H]testosterone. Following an intra-venous injection of indocyanine green (ICG) (20 mg), blood samples (7 × 4 ml) were obtained at 2 min intervals and allowed to clot. Serum ICG was estimated spectrophotometrically at 805 nm using a standard diluted in autologous serum.

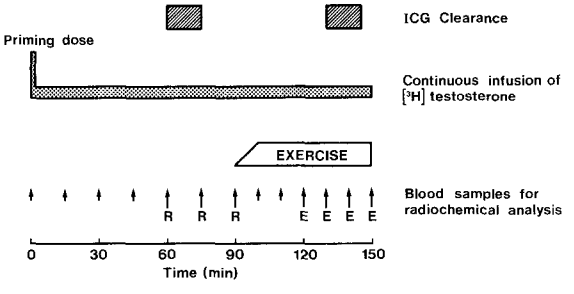

Fig. 1. Experimental protocol. Arrows indicate times at which blood samples were drawn. The samples designated R and E were used for the rest vs. exercise comparison

Radiochemical analysis

To each sample of plasma (4 ml) was added 500 cpm (2 ng) [4-^{14}C]testosterone. Steroids were extracted by gently mixing with 20 ml diethyl ether. The aqueous layer was frozen in ethanol-solid CO_2 and the ether decanted and evaporated to dryness. The residue was submitted to TLC on silica gel (Merck 5554) using dioxane-dichlormethane (4:96) in lined tanks. Each plate was scored to give twelve 1.5 cm lanes, the outer two of which were used for [^{3}H]testosterone, [^{3}H]dihydrotestosterone and [^{3}H]androstenedione markers which were located using a spark-chamber detector. The areas parallel to the marker spots were cut out and eluted with methanol. From the testosterone fractions aliquots were taken for RIA and liquid scintillation counting. The whole of the eluates of the dihydrotestosterone and androstenedione fractions were taken for liquid scintillation counting. RIA was carried out in the conventional manner using [^{3}H]testosterone as the ligand and dextran-coated charcoal to separate the free and bound fractions. The recovery of [^{14}C]testosterone (58 ± 9.2%) was used to correct the RIA and liquid scintillation data for methodological losses.

Data handling and calculations

Metabolic clearance rate of testosterone (MCR_T) was calculated from the formula

$$MCR_T = \frac{\text{Rate of infusion of [}^3\text{H]testosterone}}{\text{Plasma concentration of [}^3\text{H]testosterone}}$$

Production rate of testosterone (PR_T) was calculated as the product of MCR_T and the plasma concentration of testosterone.

The regression line of $\log_e$ ICG concentration vs. time was computed. Plasma volume was obtained by dividing the dose of ICG by the notional zero time concentration of ICG. Changes in plasma volume estimated in this manner were similar to those calculated from the packed cell volumes. Estimated hepatic plasma flow (EHPF) was calculated using the formula

$$EHPF = \frac{Vk}{E}$$

where V is the plasma volume, k is the slope of the regression line and E is the fractional extraction of ICG (Caesar et al. 1961). Because it was not possible to determine this factor in our own experiments we used the mean values reported by Rowell et al. (1964) — 0.77 for subjects at rest and 0.84 during exercise.

In making comparisons between resting and exercise states we averaged testosterone data from samples at 60, 75 and 90 min (rest) and 120, 130, 140 and 150 min (exercise). Data from the experiments in which the subjects did not exercise has been used only to demonstrate lack of spontaneous change in the parameters measured.

For each subject the means of the resting and exercise values for plasma testosterone concentration, MCR_T, PR_T and plasma volume were compared using Student's *t*-test for pooled data. The significance of changes in EHPF was assessed by comparison of the slopes of the ICG clearance regression li-

nes. When a majority of the individual changes were significant the mean values for the whole group were compared using the paired *t*-test. Differences were considered significant when $p < 0.05$.

Results

Non-exercising subjects (n = 3)

In the period from 60—150 min after the priming dose of [^{3}H]testosterone, the coefficient of variation of plasma testosterone concentration, MCR_T, and PR_T were less than 10%. Moreover, regression analysis revealed no tendency for any of those variables to change with time. We therefore believe that our protocol results in a stable baseline from which exercise-induced changes may be distinguished.

Exercising subjects (n = 4)

The principal findings are shown in Fig. 2. In all 4 subjects exercise led to a significant increase in plasma concentration of testosterone. At the same time MCR_T, EHPF and plasma volume were significantly decreased. There was a tendency for PR_T to decline slightly (~10%) but this was not statistically significant either in the group as a whole, or in any individual subject. When the data from the four exercise experiments were pooled, MCR_T and EHPF appeared to be unrelated, either at rest or during exercise. Moreover, the exercise-induced decrements in these two variables appeared to be unrelated.

Very little tritium was present in the androstenedione and dihydrotestosterone fractions, indicating that there was little conversion of testosterone into these compounds. It was therefore not possible to determine whether the rates of these conversions were influenced by exercise.

Discussion

Our finding that a 50 min period of vigorous exercise led to a modest (27%; 3 nmol·l^{-1}) increase in plasma testosterone concentration is consistent with the findings of previous investigators (Sutton et al. 1973; Métivier et al. 1980; Wilkerson et al. 1980). However, this increase is due solely to the reduction in the rate of the clearance of testosterone (MCR_T) and not to any increase in the rate of production of testosterone (PR_T). This lack of increase, or even a slight fall, in PR_T is also consistent with the failure of earlier workers to detect any exercise-related increase in plasma LH level (Sutton et al. 1973; Brisson et al. 1977; Métivier et al. 1980). Exercise of very prolonged duration leads to a decline in plasma testosterone (de Lignieres et al. 1976; Dessypris et al. 1976; Morville et al. 1979) which we may assume represents a decrease in PR_T. Although in our experiments the decrease in PR_T did not attain statistical significance, it could indicate an incipient reduction in PR_T that might have become significant if the period of exercise had been extended. Thus we may postulate that the apparently contradictory findings with respect to plasma testosterone in moderate and long-term exercise are not wholly inconsistent: exercise tends to lead to a decrease in PR_T but this is not apparent unless it is continued for a considerable period. During shorter periods of exercise any tendency to a reduction in PR_T is ob-

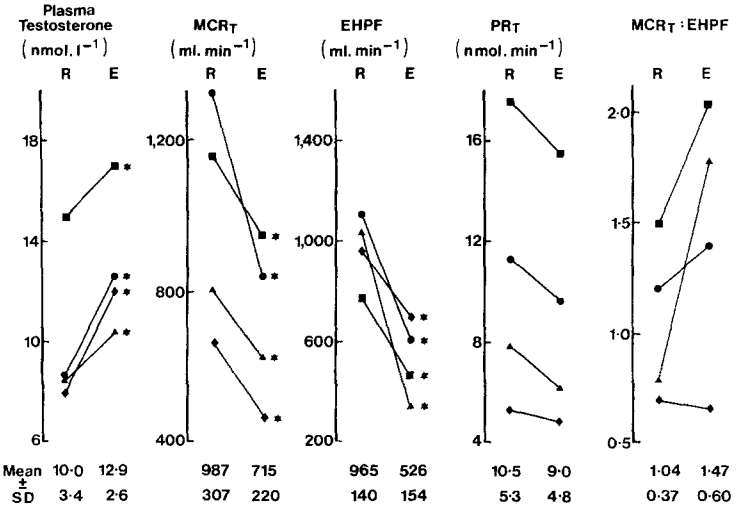

Fig. 2. The effect of exercise on some parameters of testosterone metabolism and cardiovascular function. Each data point represents the mean of 3 (rest) or 4 (exercise) blood samples drawn over a period of 30 min. * Indicates that the mean values are significantly different ($p < 0.05$). *R* = Rest; *E* = Exercise; MCR_T = Metabolic clearance rate of testosterone; *EHPF* = Estimated hepatic plasma flow; PR_T = Testosterone production rate

scured by the correspondingly greater decrease in MCR_T leading to an increase in plasma testosterone level.

In man the liver is an important site for the clearance of testosterone though only about 70% (range 60—79%) is removed from the plasma during each transit through the liver (Ishimaru et al. 1978). When we consider the mean data from our subjects at rest it is seen that MCR_T approximates to EHPF. If we assume hepatic extraction to be 70% this suggests that about 30% of testosterone is cleared by an extra-hepatic route. Exercise leads to a mean reduction in MCR_T of 272 $ml \cdot min^{-1}$ which is about 60% of the mean reduction in EHPF (439 $ml \cdot min^{-1}$). That is, the exercise-induced decrements in these two variables bear the same relationship to each other as do the resting values. This is suggestive that the reduction in MCR_T is solely a result of the decrease in EHPF. However, when we examine the data from individual subjects the situation is seen to be more complex. If we calculate the ratio MCR_T:EHPF we find considerable inter-subject variation, both at rest and during exercise (Fig. 2). In two subjects this ratio approximated to 0.70 at rest suggesting that clearance was almost entirely hepatic, whereas in the other two it was greater than 1.0 suggesting considerable extra-hepatic clearance. In 3 out of 4 subjects the ratio increased during exercise indicating an increase in the proportion of testosterone cleared by the extra-hepatic route. The apparent similarity between the MCR_T and EHPF when the data from the four subjects were averaged is thus a statistical artefact. The marked variation in MCR_T:EHPF indicates that these two variables are not directly related.

It is difficult to estimate to what extent the reduction in EHPF contributes to the reduction in MCR_T because it is not known how exercise affects the hepatic extraction or the extra-hepatic clearance of testosterone. At rest the hepatic extraction of testosterone is similar to that of ICG ($\sim$70%) and it has been shown (Rowell et al. 1964) that there is a small increase ($\sim$7%) in the extraction rate of ICG during severe exercise. It therefore seems unlikely that the hepatic extraction rate of testosterone would have changed markedly in our experiments.

The only evidence for the clearance of testosterone by an extra-hepatic route is the discrepancy between the total clearance of testosterone and that which can be attributed to the liver on the basis of hepatic extraction rate and EHPF (Ishimaru et al. 1978). Neither the mechanism nor the anatomical site of such extra-hepatic clearance have been elucidated. We suggest that sequestration in adipose tissue is a possible explanation. Support for this view is provided by the work of Longcope et al. (1976) who infused [^{14}C]testosterone and found that the concentration in plasma from a contralateral superficial vein was 12% lower than the arterial concentration.

It is difficult to evaluate the physiological significance of the effect of the exercise-induced elevation of testosterone concentration. Previous investigators have shown that the effect is transient, basal testosterone level being restored within an hour of the end of exercise. It might be argued that any elevation of plasma testosterone level must lead to increased penetration of testosterone into extra-vascular sites. However, it is possible that the mass action effect of increased plasma testosterone level during exercise might be less than that due to changes in the distribution of testosterone consequent upon changes in the blood flow pattern which occurs during exercise.

We conclude that exercise of the duration and intensity employed in our experiments does not lead to an increase in the rate of production of testosterone. However, the plasma concentration of testosterone increases due to a decrease in the rate of clearance of this hormone from the plasma. This reduced rate of clearance of testosterone may be partly accounted for by the simultaneous decrease in liver blood flow, but the lack of correlation between these two variables suggests that extra-hepatic processes may also be involved.

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