Serum sex hormones and endurance performance after a lacto-ovo vegetarian and a mixed diet

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ABSTRACT

RABEN, A., B. KIENS, E. A. RICHTER, L. B. RASMUSSEN, B. SVENSTRUP, S. MICIC, and P. BENNETT. Serum sex hormones and endurance performance after a lacto-ovo vegetarian and a mixed diet. *Med. Sci. Sports Exerc.*, Vol. 24, No. 11, pp. 1290–1297, 1992. The effect of a lacto-ovo vegetarian (V) and a mixed, meat-rich (M) diet on the level of serum sex hormones, gonadotropins, and endurance performance of eight male endurance athletes was investigated in a 2 × 6 wk cross-over study. The energy contribution from carbohydrate, fat, and protein was 58%, 27%, and 15% on the V diet and 58%, 28%, and 14 E% on the M diet. For total fasting serum testosterone (T) there was a significant interaction between diet and time ($P < 0.01$). Thus, the V diet resulted in a lower total T level (13.7, 9.8–32.4 nmol·l^{-1}) (median and range) compared with the M diet (17.4, 11.8–33.5 nmol·l^{-1}). During exercise after 6 wk on the diets total T was also significantly lower on the V than on the M diet ($P < 0.05$). Serum free testosterone, however, did not differ significantly during the 6 wk dietary intervention periods and neither did serum concentrations of sex hormone binding globulin, dihydrotestosterone, dehydroepiandrosterone sulphate, 4-androstenedione, estrone, estradiol, estrone sulphate, or gonadotropins. Endurance performance time was higher for six and lower for two after the mixed diet compared with the vegetarian diet. This was not significant, however. In conclusion, 6 wk on a lacto-ovo vegetarian diet caused a minor decrease in total testosterone and no significant changes in physical performance in male endurance athletes compared with 6 wk on a mixed, meatrich diet.

NUTRITION, EXERCISE, TESTOSTERONE, SEX STEROIDS, GONADOTROPINS

The present dietary recommendations to the elite athlete are primarily focusing on the importance of a carbohydrate-rich diet, as this has been shown to optimize body glycogen stores (7,8). A minimum daily carbohydrate intake of 500–600 g (7–9 g·kg^{-1} b.w.) or 60–70% of the daily energy intake is thus recommended (8), which means that the energy contribution from protein and fat will amount to about 10% and 20%, respectively. Therefore, the athlete must to a great extent make his/her dietary choice from vegetable food sources (e.g., bread, fruits, vegetables, legumes, and cereals) and will thus adopt a near-vegetarian dietary practice.

Although a vegetarian diet may in many respects be favorable to the sedentary population (3,28), it may be disadvantageous to the heavily training athlete. Increasing the intake of carbohydrates will increase the intake of dietary fiber, which may reduce the bioavailability of several nutrients, such as minerals, vitamins (16), and amino acids (2). Furthermore, vegetable nonheme iron is more poorly absorbed than the animal heme iron (25). This may result in reduced iron stores and increase the risk of sports anemia in the heavily training athlete (10).

Moreover, in some dietary intervention studies a vegetarian or a low fat/high fiber diet have resulted in lower blood levels of sex hormones compared with a mixed Western or a high fat/low fiber diet (3,14,18). This may be unfavourable for the endurance athlete, who in times of severe training or overtraining already may develop reduced levels of serum sex hormones (13,29,30). A decreased serum level of the anabolic steroid testosterone might influence the physical performance of the heavily training athlete. The purpose of this study was, therefore, to compare the effect of a vegetarian versus a mixed diet on serum sex hormones and physical performance of heavily training athletes. As endurance performance capacity is highly influenced

0195-9131/92/2411-1290$3.00/0
MEDICINE AND SCIENCE IN SPORTS AND EXERCISE

Submitted for publication January 1991.
Accepted for publication March 1992.

by the concentration of muscle glycogen (7) and as the body's iron stores may influence aerobic capacity (6), these parameters were also measured during the study.

MATERIALS AND METHODS

Subjects. Eight endurance-trained male athletes (four cyclists, one runner, one rower, two mixed aerobic activities) with a maximal oxygen uptake ($\dot{V}O_{2max}$) of 67 $ml \cdot kg^{-1} \cdot min^{-1}$ (61–79) (median and range), aged 22.5 yr (21–28), and weighing 70.8 kg (62.4–87.5) participated in the study. A dietary history interview (1) was performed to select subjects who had an energy intake above 15 MJ and a carbohydrate intake above 50 energy percent (E%). The subjects were nonvegetarians with an average daily intake of 200 g (46–327) meat and fish products.

Before the dietary intervention the subjects were instructed to maintain a constant training schedule throughout the entire study period. The subjects kept training diaries, noting how many minutes were spent daily on physical activities graded in five intensities. The subjects were fully informed of the nature, stresses, and possible risks associated with the study before they volunteered to participate. The study was approved by the Copenhagen Ethics Committee.

Diets. The experimental period consisted of 6 wk on a vegetarian (lacto-ovo) (V) diet (57 E% CHO, 15 E% protein, 28 E% fat) and 6 wk on a mixed diet (M) (58 E% CHO, 13 E% protein, 29 E% fat). A cross-over design was used with four subjects starting on the V diet and four on the M diet.

Four weeks on the subjects' normal diet separated the two dietary periods. The energy composition of the experimental diets was chosen in order to satisfy the carbohydrate and protein demands of heavily training athletes (8,21). Dietary fiber amounted to 2.7 $g \cdot MJ^{-1}$ on the M diet and 5.7 $g \cdot MJ^{-1}$ on the V diet. According to FAO/WHO (32) protein digestibility is reduced by about 10% in a vegetable-based (fiber-rich) diet. Therefore, an extra 10% protein was included in the vegetarian diet to make the two diets metabolically isocaloric. Dietary iron amounted to 13 $g \cdot 10\ MJ^{-1}$ on the M and to 19 $g \cdot 10\ MJ^{-1}$ on the V diet.

The V diet was based primarily on vegetable food items with an extensive use of protein-rich vegetables and legumes. Some dairy products were included in order to reach the desired protein level. Thus 83% of the protein on the V diet was supplied by vegetable sources and 17% by animal sources (dairy products). Soy protein amounted to 25% of the total protein content. The M diet was based primarily on food items typical of a Western diet including a daily amount of about 240 g of meat or fish, and with the carbohydrates supplied by low-protein containing vegetables and fruits. Animal protein thus amounted to 65% and vegetable protein to 35%. The calculated amino acid composition was not significantly different between the two diets and all essential amino acids were supplied in amounts largely exceeding the FAO/WHO recommendations (32).

Four-day cycle menus were designed at the subjects' individual energy levels determined from a 4-d dietary record (three weekdays, one weekend day—including at least two training days). Data on habitual energy and nutrient intake was calculated using computer data bases of foods from the National Food Agency of Denmark (Dankost and Ken-din-Kost). The experimental diets were prepared accurate to the gram and delivered to the subjects together with dietary diaries and a food balance. The subjects consumed their meals at home and all nonprescribed food intake and omissions from the prescribed diets were weighed and recorded in the diaries. The subjects weighed themselves regularly, and the individual energy level was adjusted according to any persistent change in body weight or deviation from the prescribed food intake during the dietary periods. Vitamin and other supplements were not allowed during the study period.

Duplicate food collections were performed from 10 MJ versions of the V and M diet. After the study, the average daily energy and nutrient intake during the two diets was calculated from the dietary diaries.

Physical testing procedures. Maximal oxygen uptake ($\dot{V}O_{2max}$), maximal voluntary contraction (MVC), and isometric endurance at 35% of MVC on quadriceps muscle and elbow flexors were determined before and after each dietary period. $\dot{V}O_{2max}$ was determined during graded ergometer cycle (Krogh) or treadmill exercise. MVC was measured with a strain-gauge sitting in an upright position with angles of the body, arm, and leg being 90°. Peak force of three trials was used in the calculations of MVC.

After 0, 3, and 6 wk on each diet, an identical aerobic endurance test was performed at the same time of the day (±1 h) on either a Krogh bicycle ergometer or a treadmill. The test commenced at a workload corresponding to a heart rate of 100–120 $beats \cdot min^{-1}$, and every 15 min workload was increased corresponding to 10–15 heart $beats \cdot min^{-1}$. This was continued until exhaustion, which was defined as the time point at which the subjects were unable to keep up with the speed of the treadmill or maintain the pedalling rate on the bike. The duration of the test was used as a measure of endurance performance capacity. During the last endurance test on each diet, blood was drawn from an arm vein after 1 h of exercise for determination of serum sex hormones. Subjects abstained from any physical activity 24 h prior to the test.

Initially, after 3 and 6 wk on each diet, subjects reported to the laboratory in the morning after an overnight fast. After 40 min rest in the supine position, blood was drawn from an arm vein. The sample was taken at the same time of the day (±0.5 h) for each subject. Initially and after 6 wk, a muscle biopsy was taken from the vastus lateralis muscle. Before each blood sample and muscle biopsy, subjects had abstained from training activity the last 24 h and from alcohol consumption for 4 d. At the end of each dietary period, subjects collected total 24-h urine on four consecutive days.

Analyses. Testosterone (T), 4-androstenedione (4-AD), and dihydrotestosterone (DHT) in serum were measured by specific radioimmunoassays (RIA) as described by Parker et al. (26) after extraction of serum aliquots (0.5 ml) with 6 ml diethyl ether. Following evaporation, the samples were redissolved in iso-octane and applied to columns (celite/ethylene glycol 2:1 by weight, and 6 cm in height). The columns were then eluted with 5 ml iso-octane (discarded), 10 ml 5% toluene in iso-octane (4-AD fraction), 10 ml 10% toluene in iso-octane (DHT fraction), 5 ml 10% toluene in iso-octane (discarded), and finally 10 ml 40% toluene in iso-octane (T fraction).

Dehydro-epiandrosterone sulphate was measured by RIA as unconjugated dehydroepiandrosterone (DHA). Serum (0.05 ml) was extracted with diethyl ether to remove the unconjugated steroids, and DHAS was twice extracted from the water phase with ethyl acetate. The pH of the combined ethyl acetate fractions was lowered to 2.0 by addition of acetic acid, and solvolysis was performed overnight at 70°C. After evaporation and redissolution in iso-octane, chromatography was performed on celite/ethylene glycol (2:1 by weight) columns. The elution pattern was 5 ml iso-octane, 10 ml 5% toluene in iso-octane (both discarded), and 20% toluene in iso-octane (DHA fraction). DHA was then measured by RIA (26).

The binding capacity of sex hormone binding globuline (SHBG) was determined as described by Hertz and Johnsen (17). Serum was incubated with a fixed amount of ^{3}H-DHT and increasing amounts of unlabeled DHT. The SHBG-DHT complex was sedimented with ammonium sulphate at 50% concentration. Part of the supernatant fraction was counted, and the binding capacity of SHBG calculated by Scatchard analysis. Free (nonprotein-bound) T was calculated as described by Bartsch (5).

Unconjugated estrone and estradiol were measured by specific RIA as described by Emment et al. (11). Two-ml aliquots of serum were extracted with 10 ml diethyl ether. After evaporation and redissolution, separation was performed on columns of Sephadex LH-20 equilibrated with hexane-methanol-ethyl acetate (85:10:5 by volume). The following fractions were collected: 2.0–3.5 ml for estrone and 4.5–7.5 ml for estradiol; each was then measured by RIA.

Estrone sulphate in serum (0.5 ml) was measured after extraction with 10 ml diethyl ether. The ether phase was discarded. After addition of 1.5 ml 3 mol·l^{-1} sodium chloride, the water phase containing estrone sulphate was twice extracted with 10 ml ethyl acetate. The ethyl acetate extracts were combined and evaporated to dryness. To the residue, 2 ml distilled water and 300 μl 3% sulphuric acid were added, and acid hydrolysis was performed for 15 min at 127°C. The resulting unconjugated estrone was then extracted with 10 ml diethyl ether. The ether phase was washed with 2 ml 8% sodium hydrogen carbonate solution and with 2 ml distilled water, and evaporated to dryness. After redissolving, chromatography and RIA were performed as described for unconjugated estrone.

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin were measured by specific RIA using the double antibody method (23,24) modified according to incubation time and volumes. Standards used were: 2nd IRP-78/549 for FSH and 1st IRP-68/40 for LLH and IRP-75/504 for prolactin.

All intra-assay coefficients of variation were less than 10%. For T, free T, SHBG, DHT, 4-AD, DHAS, E1, E2, E1-sulphate, FSH, LH, and prolactin the interassay coefficients of variation were 10.4%, 6.4%, 7.5%, 11.0%, 11.4%, 11.5%, 9.6%, 10.5%, 10.5%, 9.6%, 8.0%, and 6.5%, respectively. The assay sensitivities were 0.26 nmol·l^{-1}, 0.0007 nmol·l^{-1}, 0.26 nmol·l^{-1}, 0.34 nmol·l^{-1}, 0.20 nmol·l^{-1}, 25 nmol·l^{-1}, 40 pmol·l^{-1}, 40 pmol·l^{-1}, 200 pmol·l^{-1}, 0.4 IU·l^{-1}, 0.6 IU·l^{-1}, and 20 mIU·l^{-1}, respectively.

Hemoglobin (Hb) concentration and oxygen saturation in blood was determined with a Hemoximeter OSM II (Radiometer, Copenhagen, Denmark). Serum-iron and serum-transferrin were measured by atomic absorption spectrophotometry (Perkin Elmer 403, England).

Glycogen in muscle biopsies was assayed on freeze-dried muscle dissected free of adipose tissue, connective tissue, and blood. After hydrolysis of the muscle specimen in 1 M HCl at 100°C, glucose residues were determined fluorometrically (22). Urinary nitrogen was measured using a nitrogen analyzer (NA 1500, Carlo Erba Strumentazione, Milano, Italy). Volume of expired air was measured using a Tissot spirometer, and fractions of CO_2 and O_2 were analyzed with an infrared Beckmann LB-2 medical gas analyzer and a Servomex paramagnetic O_2 analyzer.

Each day's food from the duplicate food collection was homogenized, and a weighted representative sample of the daily menus was prepared, mixed, freeze-dried (Hetosic, Heto, Birkerød, Denmark), and ground. Energy content was measured with a bomb calorimeter (IKA Calorimeter C4000 adiabatic, Janke and Kunkel IKA Analysentechnik GmbH, Heitersheim, Germany).

Statistics. Results are given as median values with range. Data were analyzed by nonparametric statistics using a two-way analysis of variance (Friedman's test). In case of significance, data were analyzed pairwise using a one-sample rank sum test (Wilcoxon/Pratt test). Relationships between variables were examined using the Spearman correlation analysis.

RESULTS

Energy and nutrient intake. During the two dietary periods the actual daily nutrient intake, calculated from the dietary diaries, was similar to the planned nutrient intake (Table 1), and the calorimetric analysis of the 10-MJ versions of the two diets yielded energy values identical to the computer-based calculations. The energy and carbohydrate intake was similar during the two dietary periods. Compared with the subjects' habitual diet, the intake of carbohydrates was 13% higher on the M ($P < 0.05$) and 19% higher on the V diet ($P < 0.01$). The daily intake of dietary fiber was the same on the M as on the habitual diet and twice as high on the V diet. The M diet supplied the same amount of protein as the subjects' habitual diet, while the V diet supplied 10% more ($P < 0.01$), as planned.

Endurance performance. Before commencing the M and V diet, median aerobic endurance time was 80.28 min (65.07–91.17) and 79.13 min (55.67–90.07), respectively (Table 2). At the end of the 6-wk dietary periods, endurance time was lower for six and higher for two of the eight subjects on the V diet compared with the M diet; median endurance time being 76.35 min (65.00–92.32) and 77.50 min (64.45–97.02) (NS), respectively. Compared with week 0, no significant differences were observed. Due to illness and injury one subject did not complete the test at week 0 on the M diet and one at week 3 on the V diet.

TABLE 2. Endurance performance time in eight male athletes after 0, 3, and 6 wk on a mixed and a lacto-ovo vegetarian diet

	Number of Weeks on Diet					
	0		3		6	
Subject	M (min)	V	M (min)	V	M (min)	V
1	81.50	79.00	87.05	83.18	80.25	77.95
2	80.28	83.00	85.58	84.02	83.75	78.00
3	91.17	79.25	88.07	—	82.80	74.75
4	—	84.35	79.00	74.43	74.75	65.03
5	65.07	55.67	61.75	59.05	74.75	69.45
6	76.17	74.75	67.53	63.38	68.17	80.00
7	90.07	90.07	86.00	88.20	97.02	92.32
8	69.23	71.28	65.38	69.82	64.45	65.00
Median	80.28	79.13	82.29	74.40	77.50	76.35
P-value	0.41		0.38		0.25	

M = mixed diet; V = vegetarian diet.

Serum sex hormones. Median baseline concentrations of fasting serum total testosterone was 21.8 $nmol \cdot l^{-1}$ (9.3–37.9) before the M diet and 21.1 $nmol \cdot l^{-1}$ (10.1–42.2) before the V diet (NS) (Table 3). During the dietary periods two-way analysis of variance showed a significant interaction between diet and time ($P < 0.01$). After 3 wk, serum testosterone had decreased on both diets, to 17.1 $nmol \cdot l^{-1}$ (10.6–32.1) ($P < 0.05$) on the M diet and to 14.7 $nmol \cdot l^{-1}$ (10.2–36.6) on the V diet (NS). After 6 wk no significant change compared with 0 wk was observed on the M diet (17.4 $nmol \cdot l^{-1}$, [11.8–33.5]), whereas on the V diet serum testosterone had decreased to 13.7 $nmol \cdot l^{-1}$ (9.8–32.4), ($P < 0.05$ compared with 0 wk).

TABLE 1. Calculated daily intake of energy and nutrients of eight male athletes during their habitual# diet and during 6 wk on a lacto-ovo vegetarian and a mixed diet@

	Habitual Diet	Experimental Diets: Mixed Diet	Experimental Diets: Vegetarian Diet
Energy (MJ)	17.2 (13.2–20.5)	17.5 (15.4–19.1)	18.3 (15.7–20.4)
Protein			
(E%)	14.0 (11.0–17.0)	13.9 (13.1–14.4)	14.7 (14.1–15.2)*
(g)	141 (119–156)	145 (124–151)	160 (133–172)$$*
($g \cdot kg^{-1}$ b.w.)	1.95 (1.70–2.20)	2.04 (1.71–2.27)	2.22 (1.88–2.54)$$*
Animal (%)	64 (60–70)	69 (68–71)	16 (15–18)$$*
Vegetable (%)	36 (30–40)	31 (29–33)	84 (82–85)$$*
Carbohydrate			
(E%)	51.5 (46.0–62.0)	57.2 (56.0–59.9)$	57.9 (56.9–58.8)$
(g)	523 (412–589)	591 (516–642)$$	622 (539–686)$$
($g \cdot kg^{-1}$ b.w.)	7.2 (5.9–7.9)	8.4 (6.9–9.6)$	8.9 (7.1–9.8)$$
Simple sugars (E%)	20 (16–30)	32 (30–34)$$	20 (19–21)*
Dietary fibers (g)	47 (35–67)	47 (42–53)	98 (82–104)$$*
Fat			
(E%)	32.0 (21.0–42.0)	28.7 (26.9–29.3)	27.4 (26.8–27.7)*
(g)	134 (87–221)	131 9113–146)	136 (113–153)
Cholesterol (mg)	527 (296–770)	512 (444–574)	180 (150–210)$$*
Essential FA (%)	5 (3–6)	6 (5–10)$	8 (4–7)$$*
P/S-ratio	0.43 (0.30–0.86)	0.49 (0.48–0.57)	1.14 (1.07–1.20)$$*
Iron (mg)	21 (17–29)	23 (20–25)	34 (29–36)$$*

Values are median and range; FA = fatty acids; P/S-ratio = ratio between polyunsaturated and saturated fatty acids; # from a 4-d dietary record; @ from 6-wk dietary dairies.
* Difference between the M and V diet, $P < 0.01$.
$ Difference between the marked experimental and the habitual diet, $P < 0.05$; $$ $P < 0.01$.

TABLE 3. Serum sex hormone concentrations in eight male athletes after 0, 3, and 6 wk on a lacto-ovo vegetarian and a mixed diet and during exercise after 6 wk on each diet

		Number of Weeks on Diet			Endurance Test[W]
		0	3	6	After 1 h
Total T (nmol·l^{-1})[y]	M	21.8 (9.3–37.9)	17.1 (10.6–32.1)	17.4 (11.8–33.5)##	21.4 (15.6–51.0)*
	V	21.1 (10.1–42.2)	14.7 (10.2–36.6)	13.7 (9.8–32.4)	15.4 (10.4–43.6)
Free T (nmol·l^{-1})[y]	M	0.53 (0.33–0.70)	0.40 (0.31–0.60)	0.45 (0.29–0.61)	0.53 (0.39–0.48)
	V	0.53 (0.32–0.74)	0.39 (0.30–0.77)	0.41 (0.19–0.62)	0.43 (0.23–0.88)
SHBG (nmol·l^{-1})	M	34 (12–70)	33 (21–65)	32 (20–71)	37 (21–89)[e]
	V	33 (17–75)	34 (19–83)	34 (18–61)	33 (20–63)
DHT (nmol·l^{-1})	M	1.40 (0.90–2.30)	1.40 (0.88–2.00)	1.35 (0.80–1.90)$	1.85 (0.77–3.40)[d]
	V	1.55 (0.80–2.10)	1.30 (0.78–2.20)	1.10 (0.78–1.90)	1.40 (0.92–1.90)
4-AD (nmol·l^{-1})	M	5.70 (4.60–7.10)	6.24 (4.40–6.90)	6.10 (4.50–7.30)#	7.44 (3.92–9.03)
	V	7.20 (5.50–8.70)	6.08 (3.95–8.00)[c]	6.53 (3.25–8.00)	5.87 (3.49–9.36)
DHAS (μmol·l^{-1})	M	7.18 (4.80–14.50)*	8.68 (5.40–13.00)	8.10 (4.60–14.00)#	10.60 (6.30–16.80)[d]
	V	10.15 (4.75–16.50)	6.80 (4.35–16.50)[c]	8.05 (4.80–14.00)[a]	9.80 (5.80–16.10)[e]
Estrone (E1) (pmol·l^{-1})	M	130 (66–200)*	120 (81–200)	140 (110–210)#	151 (81–243)
	V	155 (130–260)	135 (84–160)[b]	145 (95–250)[a]	136 (94–216)
Estradiol (E2) (pmol·l^{-1})	M	84 (61–150)	69 (54–150)	81 (67–130)$$	91 (47–153)**
	V	94 (62–180)	67 (48–150)	65 (43–190)	59 (42–121)
Estrone sulphate (pmol·l^{-1})	M	1800 (1200–3600)*	1800 (1200–4700)	2050 (1200–3800)##	2520 (1613–5874)
	V	2750 (1200–4300)	1750 (1100–4100)[b]	1700 (1100–4600)[a]	2093 (1064–5097)
LH (IU·l^{-1})	M	3.5 (2.8–6.2)	3.2 (2.7–5.2)	2.7 (1.7–5.5)	
	V	3.9 (2.2–6.3)	3.1 (2.7–5.5)	3.0 (1.8–5.8)	
FSH (IU·l^{-1})	M	3.3 (1.6–4.4)	3.5 (1.8–4.0)	3.6 (1.5–4.7)	
	V	3.9 (1.6–5.3)	3.7 (1.9–5.1)	3.7 (1.6–5.3)	
Prolactin (mIU·l^{-1})	M	209 (98–311)	146 (108–279)	164 (94–244)	
	V	200 (140–313)	167 (83–280)	152 (82–297)	

Values are median and range; M = mixed diet; V = vegetarian diet; w = results are corrected for hemoconcentration; Y = N = 7; T = testosterone; SHBG = sex hormone-binding globulin; DHT = dihydrotestosterone; 4-AD = 4-androstenedione; DHAS = dehydroepiandrosterone sulphate; LH = luteinizing hormone; FSH = follicle-stimulating hormone.

\# Interaction time and diet, two-way analysis of variance, $P < 0.05$.

\## Interaction time and diet, two-way analysis of variance, $P < 0.01$.

$ Time effect, two-way analysis of variance, $P < 0.05$; $$ time effect, two-way analysis of variance, $P < 0.01$.

* Difference between diets, one-sample rank sum test, $P < 0.05$.

** Difference between diets, one-sample rank sum test, $P < 0.01$.

[a] Difference from 0 to 6 wk, $P < 0.05$; [b] difference from 0 to 3 wk, $P < 0.05$.

[c] Difference from 0 to 3 wk, $P < 0.01$.

[d] Difference after 6 wk on the diet between the fasting state and 1-h exercise, $P < 0.05$; [e] $P < 0.01$.

Although concentrations of free serum testosterone showed changes similar to the changes in total serum testosterone concentrations (Table 3), the changes in free testosterone were not significant. However, since the mean differences were small and the number of subjects limited ($N = 8$), there was a substantial risk of overlooking a true difference. In fact, the risk of a Type 2 error was calculated to be 88%. A significant time effect was observed for serum estradiol concentrations (E2) ($P < 0.01$), which was due to a significantly lower level of E2 at 3 wk compared with 0 and 6 wk (Table 3). There were no significant differences between the diets.

A significant interaction between diet and time was observed for serum estrone (E1) ($P < 0.05$), estrone sulphate (E1-sulphate) ($P < 0.01$), dehydroepiandrosterone sulphate (DHAS) ($P < 0.05$), and 4-androstenedione (4-AD) ($P < 0.05$). This was due to a significantly higher initial level of these hormones before the V diet compared with the M diet ($P < 0.05$); and after 3 wk on the diets, no differences remained for these hormones (Table 3). Dihydrotestosterone decreased significantly over time ($P < 0.05$), but there were no differences between the diets.

Correlations between serum concentrations of total and free testosterone and SHBG on one side and dietary fiber intake on the other side were not significant. Correlations between changes in total testosterone and free testosterone and SHBG on the one hand and changes in dietary fiber intake on the other hand were also not significant.

Gonadotropins and prolactin. Baseline levels of luteinizing hormone (LH), follicle stimulation hormone (FSH), and prolactin were similar; and there were no significant changes during the two dietary periods (Table 3).

Serum sex hormones during exercise performance. The median concentrations of serum total testosterone during exercise, measured after 1 h of exercise, were significantly higher on the M (21.4 nmol·l^{-1} [15.6–51.0]) compared with the V diet (15.4 nmol·l^{-1} [10.4–43.6]) (Table 3) and serum free testosterone was higher in six of seven subjects after 1 h of exercise on the M (0.53 nmol·l^{-1} [0.39–0.84] compared with the V diet (0.43 nmol·l^{-1} [0.23–0.88]) (NS). Serum estradiol was also significantly higher on the M diet compared with the V diet ($P < 0.01$). Compared with resting fasting levels, significantly higher levels of serum

SHBG, DHAS, and DHT ($P < 0.05$) were observed during exercise on the M diet. On the V diet only DHAS was significantly higher during exercise compared with the resting, fasting state ($P < 0.01$) (Table 3). After 6 wk diet, differences in endurance performance between the two diets were not significantly correlated to differences in either total testosterone or free testosterone.

Activity and weight diaries. There were no significant differences in the duration of daily training between the V diet (85 min [46–176]) and the M diet (92 min [53–157]). The intensity of the training did not differ significantly either. Body weight did not change significantly on either diet and weight changes were in no case greater than ±2 kg.

$\dot{V}O_{2max}$ and MVC. The initial median $\dot{V}O_{2max}$ was 4.93 $l \cdot min^{-1}$ (4.22–5.33) before commencing the M diet and 4.83 $l \cdot min^{-1}$ (3.87–5.55) before commencing the V diet. No significant changes were observed after the dietary periods, median $\dot{V}O_{2max}$ being 4.90 $l \cdot min^{-1}$ (4.23–5.11) on the M and 4.79 $l \cdot min^{-1}$ (4.04–5.17) on the V diet. There were no differences in MVC or in isometric endurance at 35% of MVC before or after the dietary periods (data not shown).

Muscle glycogen concentration. Median muscle glycogen concentrations were 450 $mmol \cdot kg^{-1}$ d.w. (337–598) and 521 $mmol \cdot kg^{-1}$ d.w. (294–685) before the V and M diet (NS), respectively. There were no changes during the two dietary regiments, thus median muscle glycogen concentrations were 495 $mmol \cdot kg^{-1}$ d.w. (277–680) and 473 $mmol \cdot kg^{-1}$ d.w. (316–636) after 6 wk on the V and M diet, respectively.

Blood hemoglobin, serum-iron, and serum-transferrin. Median fasting hemoglobin (Hb) concentrations were similar before commencing the V and M diet, being 14.6 g% (13.7–15.3) and 14.3 g% (13.6–15.2), respectively (Table 4). There were no significant changes during the dietary periods. Before onset of the M and V diets serum iron and transferrin concentrations, respectively, were similar and no significant changes were observed in either diet (Table 4).

Urinary nitrogen. No differences in the average 24-h urinary nitrogen (N) excretion were observed between the diets at the end of the 6-wk dietary periods, median concentrations being 18.2 g N (15.1–21.9) on the M diet and 18.6 g N (12.2–23.6) on the V diet. *In vitro* measures of cellular immune function have been published separately (27).

TABLE 4. Fasting blood hemoglobin, serum iron, and serum transferrin in eight male athletes after 0, 3, and 6 wk on a mixed and a lacto-ovo vegetarian diet

		Number of weeks on diet		
		0	3	6
Hemoglobin (g%)	M	14.3 (13.6–15.2)	14.1 (12.9–15.2)	13.9 (13.3–15.0)
	V	14.6 (13.7–15.3)	14.1 (12.9–14.7)	13.7 (12.6–15.2)
Iron ($\mu mol \cdot l^{-1}$)	M	18.0 (9.4–26.8)	17.6 (13.0–32.0)	19.5 (15.2–34.7)
	V	20.0 (14.0–28.9)	23.7 (12.7–31.0)	17.7 (15.3–29.7)
Transferrin	M	67.4 (56.4–76.0)	65.8 (52.4–72.4)	65.2 (58.8–76.4)
($\mu mol \cdot l^{-1}$)	V	66.4 (56.0–73.6)	66.8 (55.6–76.8)	64.8 (60.8–76.8)

Values are median and range; M = mixed diet; V = vegetarian diet.

DISCUSSION

In the present study the median concentrations of fasting serum total testosterone had decreased significantly by 35% in male endurance athletes after 6 wk on a lacto-ovo vegetarian diet. When on a mixed, meat-rich diet, serum total testosterone concentrations had decreased transiently by 22% after 3 wk but were not significantly different from initial values after 6 wk on the diet. Our findings from the lacto-ovo vegetarian diet intervention are in agreement with a study in middle-aged men, in whom a change from their habitual omnivourous diet to a vegetarian diet for 6 wk caused a decrease of 15% in mean serum total testosterone concentration (14). In cross-sectional studies, however, such a difference in serum testosterone concentration is not generally found. Thus, Deslypere and Vermeulen (9) reported no difference in testosterone between vegetarian and omnivorous men; and in a recent study of male vegans (who did not consume any animal products at all), no differences were found in serum total testosterone compared with omnivores (20). In contrast, Howie and Shultz (19) reported an 18% lower mean serum testosterone concentration in male Seventh-Day Adventist vegetarians than in omnivores, but this was on a comparatively smaller number of subjects. Thus, the cross-sectional studies may suggest that the decrease in serum testosterone concentrations found in intervention studies of 6-wk duration could be transient.

In the present study, the decrease in serum testosterone following the V diet might be explained by the large amount of dietary fiber in this diet because dietary fiber has been shown to bind steroid hormones *in vitro* (31). Thus, the vegetarian diet may have resulted in an increased fecal excretion of testosterone diminishing the enterohepatic circulation of the hormone. Furthermore, a change in the intestinal flora could be expected to affect intestinal metabolism of the steroids. However, if dietary fiber were the only explanation for the decrease in testosterone, a decrease in serum concentration of the other steroids would be expected and this was not found. Furthermore, no correlations between dietary fiber intake on the habitual and experimental diets on the one hand and testosterone on the other hand could be found. Neither were the correlations between dietary induced changes in testosterone concentrations and changes in dietary fiber intake significant. This argues against an effect of dietary fiber on serum testosterone. It is interesting to note that in the present study a significant decrease in serum total testosterone also appeared after 3 wk on the M diet, although this diet did not contain more fiber than the subjects' usual diet (Table 1). Thus, it appears that a substantial change in the diet may cause a transient fall in serum testosterone regardless of the change in dietary composition. Support for such a notion is found in the study by Hill et al. (18), where a fall in serum testosterone was observed when black South Africans changed their usual vegetarian diet to a Western diet. Thus, part of the decrease in serum total testosterone in intervention studies may in fact be due to the intervention itself. Such an effect could, for instance, be a consequence of the regular eating patterns and constant daily energy intake imposed upon the subjects during intervention. However, in the present study serum testosterone was lower after 6 wk on the V diet than on the M diet, indicating a specific effect of the vegetarian diet.

Endurance performance to exhaustion remained unchanged during the vegetarian and the mixed dietary periods, and so did isometric strength. Griggs et al. (12) noted an increase in muscle mass and muscle protein synthesis with intramuscular administration of testosterone, but there was no evidence for an increase in muscle strength. However, another study indicates that androgens in combination with muscle training increase muscle dimensions and strength (4). Thus, a decrease in serum testosterone concentrations in the physically active individual might possibly impair strength and physical performance. The present study, however, revealed no evidence for such a contention since a 26% decrease in serum total testosterone did not influence endurance performance or isometric strength, and since differences in endurance performance between the two diets did not correlate significantly with differences in testosterone concentration. It might be considered, however, that the dietary intervention period in the present study was of only 6-wk duration, which may not be long enough to detect effects of a moderately lowered testosterone concentration. On the other hand, against a long-term effect of diet on performance speak findings from a cross-sectional study of vegetarians and nonvegetarian athletes (15), which revealed no differences in measures of exercise capacity such as $\dot{V}O_{2max}$ and anaerobic capacity. Serum testosterone, however, was not measured (15).

Several other aspects of the present study are worth noting. The diets contained similar amounts of available energy derived from carbohydrates, fat, and protein. The dietary protein was 11% higher on the V diet, but according to the FAO/WHO recommendations (32) the protein bioavailability is decreased by about 10% on a vegetarian, fiber-rich diet. Our findings of identical urinary N excretion on the two diets confirms this contention. A difference in the response to the two diets could therefore not be ascribed to differences in energy delivery by the different main energy sources. Such similarity was possible to obtain only by including a substantial amount of various beans and lentils and a small amount of dairy products in the vegetarian diet. Still differences remained between the diets as to fiber content, P/S-ratio, and proportions of simple and complex carbohydrates (Table 1). The large proportion of dietary fibers and complex carbohydrates and consequently lower energy density and larger volume of the vegetarian compared with the mixed diet resulted in some gastrointestinal distress when on the vegetarian diet. Although some adaptation took place over the 6 wk, it was still difficult for the athletes to consume the large quantities of food at the end of the vegetarian dietary period.

In conclusion, the present study showed a significant decrease in serum total testosterone concentration in male endurance athletes when on a lacto-ovo vegetarian diet for 6 wk. Part of this decrease could be ascribed to a "sudden dietary change" effect. This decrease in serum testosterone did not influence strength or endurance performance, neither compared with when the subjects were on their usual diet nor when they were on a meat rich mixed diet.

Anne Raben was supported by a student research fellowship from Team Danmark. Expert technical assistance was provided by Betina Bolmgreen and Irene Bech Nielsen. Merete Hippe is thanked for her great help during the experimental part of the study, and we are grateful to the subjects, who willingly and enthusiastically participated in this study.

The study was supported by grants from Team Danmark Research Foundation, The Danish Sports Research Council, and the Danish Medical Research Council grant 12-9535. Nutana, Denmark is thanked for generous provision of a large part of the vegetarian food items.

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