

The Influence of Plant- and Animal-Based Diets on Circulating Testosterone and Body Composition of Young Male Rats

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Abstract

Within the fitness community there is an unsubstantiated concern that adherence to a plant-based diet results in low circulating testosterone in men, with minimal potential for gains in Lean Body Mass (LBM). We addressed this concern using Long-Evans rats ($N=28$) assigned to experimental diets of plant (PD) or animal (AD) origin and fed *ad libitum* for 12 weeks. Animals were further divided into two additional conditions with (E) and without exercise (S). We measured total circulating testosterone and estradiol, and body composition was assessed using dual energy x-ray absorptiometry. Neither total testosterone (1.7 ± 0.2 vs. 1.6 ± 0.2 ng·mL $^{-1}$) nor LBM (384 ± 6 vs. 377 ± 6 g) differed between rats fed the PD or AD diets, respectively, apart from PD+E having higher LBM as compared to AD+E ($p=0.03$). PD rats were significantly leaner than AD rats, based on body fat ($22.4 \pm 1.0\%$ vs $32.1 \pm 0.8\%$; $p<0.0001$). Estradiol concentrations did not differ between diets or exercise regimens. These findings demonstrate a plant-based diet maintains circulating testosterone, does not increase estradiol, supports LBM gains, and minimizes fat accumulation—all while providing adequate nutrition to fuel exercise training.

Keywords: Animal Protein; Body Composition; Exercise; Hormones; Plant Protein; Vegan; Western Diet

Introduction

There has been a longstanding interest within the fitness and bodybuilding community regarding the relationship between nutritional intake and endocrine function because of the potential to impact training and performance [1]. The principal focus has been on Testosterone (T) because of the many roles in human physiology, not the least of which is facilitation of growth and Lean Body Mass (LBM) accumulation [2]. Of interest is whether plant-based diets (PD) will maintain circulating testosterone concentrations and adequately support skeletal muscle tissue compared to diets that include Animal Products (AD) [3].

The levels and sources of dietary fat, carbohydrate, and protein can influence T levels [4,5,6] and thereby have the potential to influence body composition and athletic performance. For example, individuals consuming a lower percentage of dietary fat (~20%) have lower concentrations of circulating T compared to those consuming a higher percentage (~40%) [7,8]. The source

of fat (i.e., saturated, monounsaturated, and polyunsaturated) also impacts T concentrations. Specifically, saturated and monounsaturated fats increase total blood T concentrations compared to polyunsaturated fats [9].

When dietary fat is constant, higher carbohydrate intake increases T concentrations in healthy men (22-43 years of age) more than a high protein diet. This may be related to changes in hepatic enzymes involved with the metabolism of T and changes in circulating concentrations of T binding protein (sex hormone binding globulin; SHBG), cortisol and corticosteroid binding globulin that can influence blood T concentrations. The higher amount of dietary fiber provided by a PD has been related to lower free T concentrations compared with consumption of a diet that includes animal proteins [10]. This may be attributed to the direct relationship between dietary fiber and concentrations of sex hormone binding globulin; however, the changes in circulating free T concentration perhaps are the result of the combined differences in macronutrient composition, not just fiber. Both the quantity and origin of dietary protein influences T, thus having an impact on anabolism [3]. Low protein consumption has been shown to increase SHBG in humans

and animals [11]. Therefore, decreasing circulating concentrations of T may be attributed to the increase of SHBG. Protein quality refers to the ability of a protein source to support the increase in muscle protein synthesis (MPS) post prandial and is often evaluated based on Protein Digestibility Corrected Amino Acid Score (PDCAAS) and the Digestible Indispensable Amino Acid Score [3,12,13]. Concerns about PDs generally involve the lower quantity and essential amino acid availability of plant proteins [3] and the potential detrimental impact on body composition and performance.

The present study investigated the effects of feeding a diet mimicking the macronutrient composition of a PD on endocrine parameters and body composition of male rats. The responses to the PD were compared to rats fed an AD, which mimicked the macronutrient composition of the typical Western diet. Exercise was included in the design since fitness enthusiasts engage in regular exercise and may have the desire to follow a PD, in addition to the finding that endurance exercise is associated with declines in circulating sex steroid concentrations [14]. Thus, half of the rats in each diet group were assigned to treadmill running three days per week. The objective was to determine if the PD would support endogenous T production, prevent a possible decline in T due to physical exercise, and allow for normal growth, including LBM. Circulating estradiol concentrations, fat mass, and body fat percentage were also measured.

Methods

Overview of Experimental Design

Male Long-Evans rats (N= 28) 3-4 weeks of age were individually housed in standard shoebox caging in a climate controlled room (21°C) employing a standard 12:12-h light-dark cycle (lights on 0800 hr). Rats were initially fed a standard rat chow (Harlan 1018) and then transitioned to the assigned diet over a two-week period by gradually replacing the standard chow diet with an increasing proportion of the experimental diet. During the two-week acclimation period the rats were familiarized with the treadmill on three separate days for 5 minutes at 15-20 m·min⁻¹ and the 12:12-h light-dark cycle was progressively shifted to lights on at 0300 h. All housing and experimental procedures were approved by The University of Memphis Institutional Animal Care and Use Com-

mittee and were in accordance with the 8th edition of the Guide for the Care and Use of Laboratory Animals.

Dietary and Exercise Interventions

The rats were randomly assigned to one of four intervention groups: Animal Protein Diet with exercise (AD+E; n= 7); Animal Protein Diet without exercise (AD-S; n= 7); Plant Protein Diet with exercise (PD+E; n= 7); Plant Protein Diet without exercise (PD-S; n= 7). Both diets (Table 1) were provided as pellets by Research Diets, Inc. (New Brunswick, NJ). The AD was formulated to mimic a typical human Western diet [15], with 17% of the digestible calories from protein, 43% from carbohydrates, and 40% from fat. The PD was a higher carbohydrate plan as discussed previously [16], consisting of 15% of the calories from protein, 60% from carbohydrates, and 25% from fat. The PD was formulated to mimic what human subjects consumed in terms of macronutrient types and percentages, fiber, and micronutrients (i.e., antioxidants) as noted in our prior work using a vegan diet plan [17]. The percentages and sources of macronutrients differed between the two diets, as did the amount of dietary fiber. Due to this, the density of the diets was different and this was not controlled for in the experiment, which should be considered a limitation of this work. The dietary intervention period was 12 weeks in duration, beginning after the two-week acclimation period and the transition to the assigned diets. The diets and water were provided *ad libitum* throughout the study.

Equal numbers of rats in each diet group were randomly assigned to either exercise or no exercise. Animals in the no exercise group were placed on the treadmill three days per week for a period of 5 minutes while it was turned off (+S). Animals in the exercise groups (+E) performed exercise on the treadmill three days per week (i.e., Monday-Wednesday-Friday) for the 12-week intervention. The speed of the treadmill and duration of exercise were progressively increased. Specifically, the animals began training at 20 m·min⁻¹ for 15 min·day⁻¹(week 1), progressing to 25 m·min⁻¹ for 30 min·day⁻¹(week 2), and to 25 m·min⁻¹ for 35 min·day⁻¹(weeks 3-12). This progressive increase in intensity and duration of exercise is typical for animal training studies (Huang et al., 2008) [18]. The final intensity and duration of exercise is relevant to individuals running at moderate intensity. The exercise training was performed in the morning to early afternoon hours (between 0830 - 1400 hr).

Nutrient	AD	PD
	gm%	gm%
Protein	20	15
Carbohydrate	50	58
Fat	21	11
Fiber	5	13
Total kcal/gm	4.7	3.9
Casein	195	0
Soy Protein	0	170
DL-Methionine	3	3
Corn Starch	50	0
Corn Starch-Hi Maize 260 (70 % Amylose and 30% Amylopectin)	0	533.5
Maltodextrin 10	100	150
Sucrose	341	0
Cellulose, BW200	50	100
Inulin	0	50
Milk Fat, Anhydrous	200	0
Corn Oil	10	0
Flaxseed Oil	0	130
Ethoxyquin	0.04	0.04
Mineral Mix S1001	35	35
Calcium Carbonate	4	4
Vitamin Mix V1001	10	10
Choline Carbonate	2	2
Ascorbic Acid Phosphate, 33% active	0	0.41
Cholesterol	1.5	0
Total	1001.54	1187.95
Saturated g/kg	122.6	7.8
Monounsaturated g/kg	60.2	19.7
Polyunsaturated g/kg	13.5	77.7
Cholesterol mg/kg	2048	0
Saturated % Fat	62.4	7.4
Monounsaturated %Fat	30.7	18.7
Polyunsaturated %Fat	6.9	73.9
Ascorbic Acid mg/kg	0	114

Table 1: Dietary composition of the Animal-Based Diet (AD) and Plant-Based Diet (PD).

Growth and Body Composition Assessments

At the end of the study period, all animals underwent a Dual Energy X-Ray Absorptiometry (DXA) exam (Discovery QDR series, Hologic Inc., Bedford, MA) to assess LBM, fat mass, and percent body fat. The rats were anesthetized while the scan was performed, using isoflurane. All experimental animals were scanned at least twice. If the percent body fat data varied by more than 1.5%, a third scan was performed. The two scans that were closest in agreement were averaged and the mean value was included in the data analysis. Daily body weights for the animals are not available and this may be considered a limitation of this work.

Plasma Testosterone and Estrogen Analysis

After body composition was measured, the rats were euthanized between the hours of 0800-1100 via CO₂ inhalation. The abdominal cavity of each rat was exposed and blood was collected by syringe from the inferior vena cava, placed into Vacutainer tubes containing EDTA, and centrifuged for 15 minutes at 2000×g. Plasma was collected and stored at -70°C in multiple aliquots. Total plasma T and estradiol concentrations were measured in duplicate using ELISA procedures (ALPCO Diagnostics, Salem, NH USA; 55-TESMS-E01 and 55-ESTRT-E01) per the manufacturer's instructions. Sensitivity, intra- and inter-assay coefficients of variation of the testosterone kit are reported as 0.06 pg ml⁻¹, 6.50% and 11.3%, respectively, with values for the estradiol kit as 2.5 pg ml⁻¹, 6.1%, and 7.0%, respectively.

Statistical Analysis

Outcome measures were analyzed using a two-way (diet x exercise) Analysis of Variance (ANOVA). Tukey posthoc tests and single degree of freedom contrasts were used to determine post-hoc significance. All analyses were performed using JMP statistical software (version 4.0.3; SAS Institute; Cary, NC). Statistical significance was set at p≤ 0.05. All data are expressed as the mean±SE.

Results

One animal in the AD+E group died during week two of the intervention approximately 30 minutes following the exercise training session. The necropsy revealed the abdomen was filled with blood, due to either an aneurism or tear in liver. All remaining animals completed the study through week 12 and appeared healthy and otherwise normal.

Growth and Body Composition

After the 12-week period total body mass was greater for AD vs. PD rats (P<0.0001) and for S vs E rats (P=0.006; Figure 1). There was no interaction for diet and exercise on final body mass

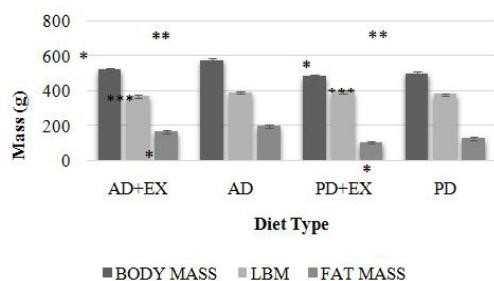


Figure 1: Body composition data of male rats assigned to two different diets with and without exercise. Values are mean \pm SE. Body Mass: Interaction ($p=0.16$); **Diet ($p<0.0001$), greater for AD vs. PD; *Exercise ($p=0.006$), lower for exercise vs. no exercise. Lean Body Mass: ***Interaction ($p=0.03$), PD+EX greater than AD+EX ($p=0.03$); Diet ($p=0.36$); Exercise ($p=0.72$); Fat Mass: Interaction ($p=0.55$); **Diet ($p<0.0001$), greater for AD vs. PD; Exercise ($p=0.001$), lower for exercise vs. no exercise.

Similar to body mass, fat mass and percent body fat were greater for AD vs PD rats ($P<0.0001$ for both; Figure 1) and for S vs E rats ($p=0.001$ and $p=0.006$, respectively), without an interaction for diet and exercise ($p=0.55$ and $p=0.59$).

Although there were no differences in LBM between diet ($p=0.36$) and exercise ($p=0.72$), there was a significant interaction ($p=0.03$) with PD+EX having a higher LBM compared to AD+EX ($p=0.03$).

Testosterone & Estradiol (Figure 2)

Values for T did not vary as a function of diet ($p=0.54$), or exercise ($p=0.14$), nor was there a significant interaction ($p=0.18$). Pooled data revealed blood testosterone was similar for PD ($1.7\pm0.2 \text{ ng}\cdot\text{mL}^{-1}$) and AD ($1.6\pm0.2 \text{ ng}\cdot\text{mL}^{-1}$) rats. Similarly, there were no diet ($p=0.31$) or exercise ($p=0.28$) effects or interactions ($p=0.22$) detected for estradiol. Pooled estradiol for the two diets were similar for the estradiol mean values ($\text{PD}=2.7\pm0.5 \text{ pg}\cdot\text{mL}^{-1}$; $\text{AD}=1.9\pm0.4 \text{ pg}\cdot\text{mL}^{-1}$). However, of the samples available for the estradiol analysis, 9 had values below the level of detection for the standard curve (AD=4 and PD=5). The sensitivity of the assay may not be adequate to detect a potential dietary influence. Future studies may increase the sample size in order to maximize the power needed to detect differences in estradiol.

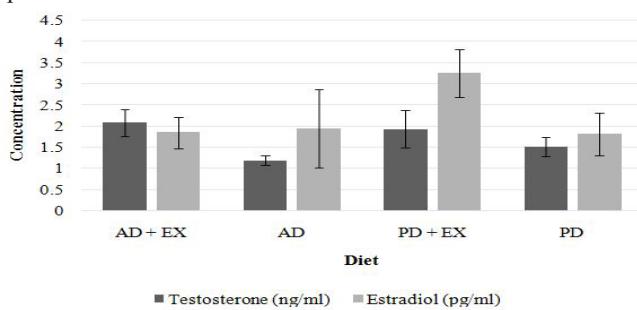


Figure 2: Testosterone and estradiol concentrations of male rats assigned to two different diets with and without exercise. Values are mean \pm SE. Testosterone: Interaction ($p=0.18$); Diet ($p=0.54$); Exercise ($p=0.14$). Estradiol: Interaction ($p=0.22$); Diet ($p=0.31$); Exercise ($p=0.28$).

Discussion

Athletes, whether competitive or recreational, are interested in diet regimens that will improve performance and enhance health. Importantly, consumption of the PD by young male rats for 12 weeks did not result in lower circulating testosterone or decreased LBM accumulation after the 12-week period compared to the AD, but did result in a significantly lower fat mass. Previous experiments revealed a decline in testosterone in male endurance athletes, especially when energy was restricted [19]. In the present experiment, T concentrations of the sedentary and exercised rats did not differ, suggesting that the relatively low-volume and moderate-intensity of training imposed was not sufficiently intense to induce a decline in T and LBM, presumably because the increased caloric and nutrient needs related to training were met by both diets [20].

Growth and Body Composition

Individuals consuming a PD typically have lower body weights compared with individuals following other dietary patterns [21,22], possibly because of lower intake of dietary fat, simple sugars, and total digestible calories. With *ad libitum* feeding, the PD supported adequate weight gain, but final body mass was slightly greater in the AD groups, and especially for the AD rats without exercise. However, this was due to increased accumulation of fat, with no increase in LBM. The retention of LBM without accumulating fat is why some athletes adopt vegan or vegetarian diets that provide adequate nutrition to fuel exercise[20] and improve biomarkers of cardiovascular health [14]. These findings highlight that development and maintenance of LBM does not require animal protein and can be supported by a moderate quantity of plant-based proteins. Exercise and the increased energy expenditure modifies the responses to different diets, mostly by altering the accumulation of fat.

Testosterone

Historically, a PD has been associated with a decrease in endogenous T [23,24], leading many away from such a plan for concern over lowered T. However, the results vary among studies and are confounded by the use of human subjects living in a free environment. A meta-analysis revealed that diets comprised primarily of soy do not negatively impact circulating T concentrations in men [25]. Similarly, total/free T and LBM did not differ among human subjects after 12 weeks of supplementing their diet with 50 g of protein per day derived from four different protein sources (soy concentrate, soy isolate, a soy and whey mixture, and a whey blend [26]. The slight, though insignificant 6.3% higher total T for the PD rats is consistent with the 7% higher total T measured in vegans compared with omnivores [10]. These findings do not support isoflavones altering activities of enzymes associated with T synthesis [2,27,28], hepatic clearance, and binding to T associated binding proteins [2,28].

Rats in the earlier stages of maturation possess higher T levels compared to later in life [29,30] and may be more sensitive to dietary influences. The lack of a significant difference in T concentrations in the young, rapidly growing male rats used for this study demonstrate that T is not negatively impacted by the exclusive consumption of a PD during maturation and after sexual maturity. These findings are consistent with the increase in serum androgen levels of rats exposed to soy isoflavones over a lifetime [31,32]. The slight but insignificant increase in T measured in the PD rats mimics the slight increase in T concentrations of male Long-Evans rats exposed to high and low soy isoflavone levels (3.2 vs 3.0 mg ml⁻¹) [31]. Interestingly, exposure of the F1 generation rats subjected to a lifetime exposure to soy isoflavones had a significant 155% increase in serum T concentration compared to the rats fed for two generations a casein based diet with 0 mg of soy isoflavone content [32].

Estradiol

Estradiol concentrations for the four groups of rats were within the expected range for this strain and age of rat [33]. Although consumption of a vegan/vegetarian type diet has been reported to increase estradiol levels in humans and animal models [23, 24,34], this was not supported by the results of the present study or by other reports [25,31]. The conflicting results may be due to the oral bioavailability, or varying concentrations, of isoflavonoid molecules (phytoestrogens) in the PD's and/or soy supplements employed. This variable was avoided by using a purified diet. For example, equol, is a metabolite derived from soy classified as a selective estrogen receptor β agonist that acts to decrease androgen hormone actions by binding to estrogen receptors with various tissue specific actions throughout the body [31,35].

Perspectives

Exercise, endurance in particular, limits accumulation of body mass and particularly fat accumulation, but has been associated with altered sex steroid concentrations [14]. The present findings with young, growing male rats, suggest sex steroid hormone concentrations of athletes and exercise enthusiasts should not be compromised by vegetarian diets that provide adequate nutrition to fuel exercise [20]. Furthermore, a PD can support LBM, and improve overall body composition and biomarkers of cardiovascular health [14].

Many sports nutritionists and athletes use knowledge gained from studies that manipulate nutritional strategies to facilitate performance and maintenance/growth of skeletal muscle. In this regard, the protein quality of soy is considered a complete protein with sufficient quantities of essential amino acids to stimulate MPS for muscle growth. The results of this study should alleviate some concerns suggesting that athletes who adopt vegan/vegetarian diets are not able to support muscle growth. We find that while exercising at low volumes to moderate intensities, a PD will not

negatively impact LBM or T concentrations.

Although PD comprised of soy does contain estrogen-like properties, the misconception that a PD will cause feminizing effects and reduce T concentrations is unsupported. Factors such as bioavailability of the active metabolite and the amount present in the diet should be taken into consideration before making such claims. Moreover, the health status of an individual, in conjunction with guidance from a healthcare professional, should be taken in consideration.

Conclusion

To further expand upon the recent findings, future research should compare the anabolic properties of plant-based diets over a longer duration of time and with more intense exercise regimens [36] to more fully assess the impact they have on skeletal muscle growth and endocrine parameters. In such experiments, care should be taken to control for the overall energy intake, as the density of the diets is much different and this may influence findings. Data from these studies may alleviate concerns surrounding PDs, thus offering alternative options for individuals seeking a better diet in the presence of exercise. It should be noted that due to certain nutritional properties, consulting with a healthcare professional is recommended before adopting solely plant-based diets.

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