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The Influence of Plant- and Animal-Based Diets on Testosterone and Lean Body Mass is Male Rats

Damien Christopher Moore

THE INFLUENCE OF PLANT- AND ANIMAL-BASED DIETS ON TESTOSTERONE AND LEAN BODY MASS IN MALE RATS

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Damien C. Moore

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Damien C. Moore (DCM), Richard J. Bloomer (RJB), Randal K. Buddington (RKB), and David A. Freeman (DAF) wrote the manuscript. Damien C. Moore and Richard J. Bloomer had primary responsibility for the final content. All authors read and approved the final manuscript.

Abstract

Background: It is commonly believed but unproven that plant-based diets result in low circulating testosterone, with minimal gains in lean body mass (LBM). **Methods:** Long-Evans rats (N=28) were assigned to experimental diets with either plant (PD) or animal (AD) protein sources. Animals were further divided into two additional conditions without and with exercise. Animals were fed *ad libitum* for 3 months and at the end of month three blood was collected for measurement of testosterone and estradiol concentrations. Dual x-ray absorptiometry assessed body composition. **Results:** Neither blood testosterone concentrations nor LBM differed between rats fed the PD or AD. **Conclusion:** Diet did not influence blood testosterone concentrations. Moreover, LBM increased at a similar rate between PD & AD groups. These findings indicate there is no significant difference between PD and AD regarding testosterone or LBM in male rats.

Keywords: animal protein, plant protein, western diet, exercise, vegan, hormones, body composition

Conflict of Interest Statement:

The authors declare no conflict of interest related to this work

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Chapter 1

Introduction

There has been growing interest in nutrition and its relationship with endocrine function, specifically with regards to the hormone testosterone (T). Nutrient manipulation involving dietary fat, carbohydrate, and protein, as well as other components of nutrition can alter T levels[1-3]. For example, individuals consuming a ~20% fat diet compared to those consuming a diet containing ~40% fat exhibit lower concentrations of testosterone[3,4]. Furthermore, the type of fat (saturated, monounsaturated, and polyunsaturated) affects T concentrations[4]. Anderson and colleagues noted increased T concentrations after consuming a high carbohydrate diet compared to a high protein diet[1]. Similar studies suggested consuming a diet low in carbohydrate[5] and protein[6,7] resulted in either no change or reduced T concentrations. Moreover, high dietary fiber intake has been associated with low testosterone levels in vegans compared to omnivores[8-10]. Not only the amount of protein, but the origin of protein, whether it is animal- or plant-based is believed to play a role in regulating testosterone, thus priming the body for anabolism[11]. Clearly, regulating the quantity and type of macronutrients consumed influences circulating testosterone, as well as the ensuing effects related to muscle growth and associated outcomes.

Resistance exercise training is extremely effective in maximizing physiological adaptations such as increased strength[13], muscle hypertrophy[14] and fat mass reduction[15]. In addition to these physiological adaptations, acute resistance training also elicits a sizeable elevation in T[16] secretion. For example, protocols high in volume, moderate to high in intensity, using short rest intervals and stressing a large muscle mass, tend to produce the greatest acute elevations in T[17]. Tremblay et al.[18] reported elevated free T concentrations following resistance exercise. Interestingly, the acute elevation was greater in resistance-trained men than endurance-

trained men. These data are similar to the findings of Tauler et al.[19] who reported a decrease in T in ultra-endurance athletes. In addition, these data support Ahtiainen et al.[14,20] who reported an increase in total- and free-testosterone in resistance trained men compared to those who did not perform resistance training. Thus, resistance training appears to be a stimulus for acute increases in circulating T which may have a role in the increase in hypertrophy associated with chronic resistance training.

Testosterone exerts multiple effects on physiology and behavior[21]. T's influence on human physiology ranges from muscle protein synthesis[22], increased muscle mass[14], slowing down osteoporosis[23] and supporting the reproductive system[24]. Low T (hypogonadism) is linked to depression, fatigue, low energy, and reduced sex drive[25]. These symptoms are seen primarily in men and to a lesser extent in women, who are often treated with hormone replacement therapy or not treated at all[26]. In addition, T plays a key role in carbohydrate, fat, and protein metabolism[21,27]. Numerous studies have shown positive associations with endogenous T production and muscle hypertrophy[28], strength[24], and muscle protein synthesis in men[29]. However, a reduction in T can lead to increased fat mass[30], decreased LBM[29], and impaired glucose tolerance[21].

There has been considerable speculation regarding whether plant-based diets (PD) can adequately support skeletal muscle tissue compared to diets that include animal products (AD)[11]. This debate is in part due to the lower essential amino acid availability of plants[11] in conjunction with naturally occurring endocrine-disrupting chemicals (EDC's)[31,32]. EDC's are a class of phytoestrogens which mimic estrogen activity within the human body[33]. This is of concern due to the potential feminizing effects of estrogen in men[34], leading to a reduction in lean body tissue[35] and an altered testosterone/estradiol ratio[36].

The effects of a PD on endocrine and physiological parameters were investigated over a duration of three months, as compared to an AD administered *ad libitum* in rats. The primary focus was to examine the differences in circulating T concentrations between both animal- and plant based diet groups. In addition, circulating estradiol concentrations, lean body mass (LBM), total body mass, fat mass, and body fat percentage were measured. Exercise was introduced into the design as well, with 50% of the animals assigned to perform treadmill running three days per week. The hypothesis tested was that the PD would support endogenous T production and allow for normal growth (including lean body mass) in young male rats. Results from this investigation may show that plant protein can increase LBM, as well as support or increase circulating T.

Chapter 2

METHODS

Overview of Experimental Design

Male Long-Evans rats (N= 28) were purchased from Harlan Laboratories, Inc. (Indianapolis, IN) at the age of 3-4 weeks. Upon arrival, all rats were individually housed in standard shoebox caging in a climate controlled room (21ºC) employing a standard 12:12-h light-dark cycle (lights on 0800 hr). Rats were initially fed a standard rat chow (Harlan 1018) with *ad libitum* water, 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 this two-week acclimation period the rats were familiarized with the treadmill on three separate days (i.e., walking on the treadmill for 5 minutes at 15-20 $m \cdot min^{-1}$) and the 12:12-h light-dark cycle was progressively shifted to lights on at 0300 hr. All housing and experimental procedures were approved by The University of Memphis Institutional Animal Care and Use Committee and was 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; n= 7); Plant Protein Diet with exercise (PD+E; n= 7); Plant Protein Diet without exercise (PD; n= 7). Both diets (provided in pellet form) were purchased from Research Diets, Inc. (New Brunswick, NJ). The AD was formulated to mimic a typical human Western Diet[37], containing 17% protein, 43% carbohydrates, and 40% fat. The PD[38] included 15% protein, 60% carbohydrates, and 25% fat. However, it is important to note that other variables within the diet differ, specifically, the amount of dietary fiber and the source of dietary carbohydrate and fat (See Table 1 for details). The resulting 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[39]. The dietary intervention period was three months in duration, beginning after the two-week acclimation period and the transition to the assigned diets. The water and diets 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. Animals in the exercise groups (+E) performed endurance exercise on a motorized treadmill three days per week (i.e., Monday-Wednesday-Friday) for the 3-month intervention. The speed and duration of the motorized treadmill was progressively increased. Specifically, the animals began training at 20 $m \cdot min^{-1}$ for 15 $min \cdot day^{-1}$ (week 1), progressing to 25 $m \cdot min^{-1}$ for 30 $min \cdot day^{-1}$ (week 2), and 25 $m \cdot min^{-1}$ for 35 $min \cdot day^{-1}$ (weeks 3-12). This progressive increase in intensity and duration of exercise is typical for animal training studies[40]. 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).

Body Composition Assessments

At the end of month 3, all animals received a dual energy x-ray absorptiometry (DXA) exam using a Hologic device (Discovery QDR series, Hologic Inc., Bedford, MA). The rats were anesthetized using isoflurane for a total duration of approximately 10 minutes while the scan was performed. All experimental animals were scanned at least twice during each assessment period. If the first two scans provided percent body fat data that varied by more than 1.5%, a third scan was performed. The two scans that are the closest were then averaged and the mean value of the two scans were included in the data analysis. The DXA exam provided data specific to LBM, fat mass, and percent body fat.

Plasma Testosterone and Estrogen Analysis

After the three-month period, rats were euthanized between the hours of 0800-1100 via CO_2 inhalation, the abdominal cavity of each rat was exposed and blood samples were immediately collected from the inferior vena cava via syringe, placed into Vacutainer tubes containing EDTA, and centrifuged for 15 minutes at 2000g. Plasma was collected and stored at -70°C in multiple aliquots. Plasma T and estradiol concentrations were measured using an ELISA kit (ALPCO Diagnostics, Salem, NH USA) according to the manufacturer's instructions. Sensitivity, intra- and inter-assay coefficients of variation of the testosterone kit is noted as 0.06 ng/ml, 6.50% and 11.3%, respectively, with values for estradiol kit noted as 2.5 pg/mol, 6.1%, and 7.0%, respectively. Samples for all assays were analyzed in duplicate.

Statistical Analysis

Individual data obtained from the DXA scan (fat mass, lean mass, and body mass), as well as the blood-borne measures, were analyzed using a one-way analysis of variance (ANOVA). Tukey post hoc tests and simple 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 $p \leq 0.05$. All data are expressed as the mean ± SEM.

Chapter 3

Results

One animal in the AD+EX 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, with a suspected aneurism or tear in liver. All remaining animals completed the study through month 3.

Diet and Testosterone & Estradiol

Diet had no statistical effect on total T concentrations (PD 1.89 ng/ml ± 0.27 SEM vs AD 1.78 ng/ml ± 0.27 SEM; P=0.54). In addition, no difference existed between diets regarding serum estradiol concentration (2.70 pg/ml ± 0.42 SEM vs 1.88 pg/ml ± 0.42 SEM; p=0.31), respectively. Of the 27 samples available for the estradiol analysis, 11 had values outside the range of the standard curve. Hence, the estradiol mean values may not be representative of true dietary influence.

Exercise

Testosterone

Interestingly, T concentration was higher by 43% (2.07 ng/ml ± 0.24 SEM vs 1.45 ng/ml ± 0.31 SEM) in the exercise group compared the no-exercise group, respectively. Although, there was no significant interaction effect between diet and exercise on T concentrations, comparisons revealed that T concentrations were elevated for AD+E (2.07 ng/ml ± 0.77 SD; Effect Size (ES) > .18; p = 0.14) compared to all other groups. There was a 76% difference in T concentration between AD & AD+EX (1.18 ng/ml ± 0.29 vs 2.07 ng/ml ± 0.77 SD; ES=1.69; p = 0.18). PD + EX exhibited no significant differences in T concentrations (1.92 ng/ml ± 0.97 SD; ES = 0.52; p = 0.18) compared to PD (1.51 ng/ml ± 0.59 SD); however, there was a 27% difference in T concentration between groups.

Estradiol

The PD+EX group produced higher concentrations of estradiol (3.25 pg/ml ± 0.52 SEM; p = 0.22) compared to all other groups. The interaction effect between diet and exercise revealed increased estradiol concentrations for PD + EX (3.25 pg/ml ± 1.28 SD) compared to all other groups AD (1.94 pg/ml ± 1.61 SD; 67%↑; ES=.90), AD + E (1.84 pg/ml ± 0.81 SD; 76%↑; ES=1.33), and PD (1.80 pg/ml ± 0.86 SD; 81%↑; ES=1.35) with all percent differences between groups greater than 67%.

Body composition

There was no significant difference in LBM between groups (p=0.14), however, LBM accounted for ~82% and ~76% of total body mass in the PD+EX & PD groups, respectively. Whereas LBM accounted for ~71% and ~68% of total body mass in the AD+EX & AD groups,

respectively (see Figure 1). Several differences were noted for the remaining body composition measurements. For example, mean fat mass was significantly ($p<0.0001$) lower by ~36% in PD groups (124.45 g ± 9.8 SEM) compared AD groups (195.5 g ± 8.4 SEM); AD+E (161.6 ± 8.0 SEM) was ~17% lower than AD (195.5 ± 8.4 SEM) ($p<0.05$). A group effect was noted for body fat percentage ($p< 0.0001$), with PD (24.6 ± 1.4 SEM) groups ~27% lower than AD (33.5 ± 1.0 SEM) groups; AD+E (30.6 ± 1.3 SEM) was ~9% lower than AD (33.5 ± 1.0 SEM) ($p<0.05$). Bodyweight displayed a group effect (AD > all other groups; $p < 0.0001$). Body weight gain was greater in AD vs all other groups ($p < 0.05$).

Chapter 4

Discussion

This study was centered on comparing a plant-based diet to an animal based diet and exercise regarding T and estradiol concentrations, as well as body composition in young and growing male rats. Our data show T, estradiol, and LBM measures did not differ between PD and AD diets. Furthermore, three months of exercise training appears to support T and estradiol concentrations in the presence of an AD.

Diet and Testosterone

Historically, T concentration has been shown to be supported in response to consuming an AD[4]. In contrast, a PD has been associated with a decrease in endogenous T[34,41]. However, results are mixed and often noted within human subjects living in a free environment—introducing confounds into the design. Based on such reports, the goal of this study was to test the hypothesis that a PD would sustain T concentrations in male rats over a three-month period compared to rats fed an AD. In support of our hypothesis, our main finding demonstrated that a

PD had no significant impact on endogenous T concentrations. Thus, a PD can support T concentrations comparable to an AD (1.89 ng/ml ± 0.27 SEM vs 1.78 ng/ml ± 0.27 SEM, respectively). In agreement with our results, Messina et al.[42] through a meta-analysis indicated diets formed of primarily soy do not negatively affect T concentrations in men. In a similar study, Kalman et al.[36] observed no significant differences for total/free T and LBM after supplementing human subjects with 50 g of protein per day derived from four different protein sources (soy concentrate, soy isolate, soy and whey mixture, and whey blend) for 12 weeks. Furthermore, animal data has revealed an increase in serum androgen levels in male rats exposed to soy isoflavones over a lifetime, which support the main finding of the present experiment[43,44]. These data show that a PD does not lead to decreased serum T concentrations.

Influential Factors and Mechanisms of Testosterone

Testosterone is a powerful anabolic steroid hormone that influences multiple functions, such as deceasing fat mass, increasing LBM, and influencing the reproductive system[21]. In the present study, there were no differences between groups for T concentrations. These data show that PD's do not have a negative impact on circulating T concentrations, although results from earlier experiments have reported decreased serum T concentrations associated with a PD. One possible explanation for these differences could be due to the compositional make-up of the diets used between groups. The types of fat included in the diet as monounsaturated, polyunsaturated, and saturated fats can affect T concentrations[4]. Furthermore, soy protein is believed to cause feminizing effects in men due to phytoestrogens content, which mimic estrogen[47]. However, this feminizing effect may not be true due to soy derivatives modulating the levels of sex steroids such as testosterone and estrogen[44,48]. In theory, this concept is logical, considering that both T

and estrogen are needed in a delicate balance for male and female reproduction and both hormones are under the influence of the hypothalamic-pituitary complex of the brain[49].

In agreement with present findings, male Long-Evans Rats' T concentrations remained nearly identical (3.2 vs 3.0 ng/ml) in groups exposed to a high isoflavone content compared to a low isoflavone content[43]. Furthermore, F1 generation rats with a lifetime exposure to soy isoflavones increased serum T concentrations by 155% (3.03 ng/ml vs to 1.19 ng/ml) ($p < 0.05$) post puberty to adulthood on diets comprised of 235.6 mg and 1046.6 mg of total soy isoflavones/kg pelleted diet compared to a casein based diet with 0 mg isoflavone content[44], respectively. However, Key et al.[8] showed vegans had 7% higher total T and 3% lower free T in comparison with omnivores.

An explanation for the differences across studies is not at once clear, however, such conflicting reports indicate that the effect of soy on T concentrations in animal- and human-models remains to be fully characterized. For example, equol is an isoflavonoid molecule that may be responsible for the positive estradiol influences seen in the earlier studies. Equol is a selective estrogen receptor beta agonist (ERβ) with many tissue-specific actions throughout the body[43]. Specifically, the mechanism of action for isoflavonoids include SERM-like binding to estrogen receptor subtypes and estrogen related receptor gamma (ERR-γ), interactions to decrease androgen hormone actions and in the case of equol specific binding to the potent androgen, 5 α-dihydrotestosterone (5α-DHT)[50]. Moreover, T concentration could vary among experiments due to altered enzyme activity caused by isoflavone exposure[21,33,51]. This activity could produce changes in steroid metabolism and clearance via the liver or altered free versus T bound to its binding proteins[21,51]. In addition, the period in which rats are considered mature is defined by the age they can begin to reproduce, which could affect the results. Commercially

available rats typically live to 25-40 months of age, reaching maturity by 70-90 days of age, middle age by 10 months, and old age by 18-24+ months[52,53]. Animals in the earlier stages of maturation will typically possess higher T levels as compared to animals that are in the latter stages of life which is typically associated with a reduction in serum T[52,53]. The decrease in serum T with age has been linked to reduced LH levels and gene encoding factors in addition to animals being exposed to soy isoflavones, which could affect feedback regulation of testicular androgen production[33,49,52]. Nevertheless, the results of the present study offer important information that factors such as diet, macro nutrient make-up of diet, and the source of the macro nutrients within the diet should be considered when investigating the influence diet has on T concentration.

Diet and Estradiol

Estradiol concentrations were examined to determine if a PD had any influence on circulating estradiol levels as shown in earlier studies. The results indicate that diet did not alter estradiol concentrations as there were no significant difference among groups PD (2.70 pg/ml ± 0.42 SEM; $p = 0.31$) and exercise (2.54 pg/ml ± 0.36 SEM; $p = 0.28$) groups compared to AD (1.88 pg/ml ± 0.42 SEM) and no exercise (1.87 pg/ml ± 0.47 SEM) groups, respectively. These estradiol concentrations are within the expected range for this strain and age of rat[54,55]. The PD+EX group (3.25 pg/ml ± 1.28 SD; $p = 0.22$) overall produced higher estradiol levels and had a 67% increase in estradiol concentration as compared to all other groups.

Consistent with past findings, estradiol levels have been shown to increase in human- and animal-models due to the consumption of a vegan/vegetarian type diet[34,41,45]. However, other studies have found the opposite to be true as well in both human- and animal-models[42,43]. Conflicting results may possibly be due to the varying isoflavone content in plant-based diets and

or soy supplements used throughout multiple studies. Another hypothesis is that the production of active metabolites from soy or isoflavones varies depending on complete/incomplete digestion in the gastrointestinal track. However, the role of estradiol within the body is often overlooked and misunderstood. Estrogen has a stimulatory and inhibitory influence on the hypothalamus-pituitary-gonadal axis, leydig cells, sertoli cells, and a host of T producing cells which has been recently documented[48]. In both human and animal models with either low estradiol and T, the administration of exogenous estradiol has been shown to increase libido and sexual function[24]. Conversely, estradiol in higher than normal concentrations can produce feminizing effects such as enlarge breast tissue, a reduction in LBM, and reduced serum T levels via inhibition of luteinizing hormone on leydig cells[56]. There appears to be delicate balance between T and estrogen that is needed to support physiological parameters of the body. This information suggests that altering the T/E ratio may be more of a contributing factor to adverse effects within the body than changes in the concentrations to either T or estradiol alone. There are multiple mechanisms by which T and estradiol secretion can be altered ranging from altered hypothalamic-pituitary activity to the clearance and enzymatic action of the liver. A better understanding of the effects by which PD's alter hormone levels may offer insight on possible solutions to hormone related cancers such as breast and prostate cancer. Further study employing larger sample sizes are needed to more fully elucidate the role of a PD on circulating estradiol.

Body composition

In observational studies, individuals who follow plant-based diets typically have lower body weights compared with individuals following other dietary patterns[57,58], suggesting that such diets may be useful for treating overweight and obese status. Despite *ad libitum* feeding in all groups, body mass was greater in the AD group compared to all others. Surprisingly, PD

groups despite being allowed to freely feed throughout the three-month period, were slightly lower in body mass as compared to the AD groups and remarkably lower in body fat. Furthermore, despite the dramatic difference in body fat between the AD and PD groups, only small differences were noted in LBM. These findings highlight the idea that animal protein is not necessary for development of LBM. This is a concern often expressed by nutritionists and athletes in reference to those individuals who prefer to adopt a diet that is devoid of animal protein. Our findings indicate clearly that normal growth, including lean body mass, can occur in the presence of plant protein exclusively.

Aerobic Exercise on Testosterone

Acute bouts of resistance[17,20] and endurance exercise[59,60] increase T levels in human participants. In response to prolonged endurance exercise (e.g., a marathon), T levels will typically decline[19]. The aerobic exercise groups (AD+EX and PD+EX) produced higher T levels by 43% (2.07 ng/ml ± 0.24 SEM; $p = 0.14$) compared to the no exercise groups (AD + PD) (1.45 ng/ml ± 0.31 SEM). In partial agreement with the present observations, maintenance of T concentration due to an acute bout of aerobic exercise has been previously reported[61]. In addition, 40 men were placed on a moderate-intensity (50% of VO_2 Max) and low-frequency (50 minutes of EX; 1x/wk) aerobic exercise program for 12-weeks. The results revealed aerobic training had no effect on serum T concentrations[61]. Differences in results are likely due to the duration and intensity of the aerobic exercise program, as well as the model used (humans versus animals), and other confounding factors often present with human studies. The intensity of an aerobic exercise program can stress the body to the point in which an increase in lactate production, decrease in lactate clearance, and the reduction in blood pH can negatively affect hormone concentrations. In contrast, the level of serum total T is believed to be elevated by the

hemoconcentration after initiating submaximal exercise[61]. However, continuing the submaximal exercise may cause additional blood flow to the exercised muscles and less flow to the testicles. The hormonal secretion then begins to decline in addition to declining hepatic blood flow decreasing hepatic clearance[61]. These discrepancies may shed some light on specific mechanisms of decreased T associated with high intensity and increased duration of aerobic exercise thus offering a potential guide when prescribing or creating an aerobic exercise prescription for athletes or recreationally trained individuals.

Limitations

There were several limitations in this study. Foremost, there were major differences between diets administered to the subjects. The sources of protein, carbohydrates, and fat varied between diets. It was not simply the protein type that varies (soy versus casein). Additionally, circulating T levels within the body are altered as a consequence of the type of fat included in the diet[4] a factor that could have played a role in the present study. The same could be true for the type and quantity of carbohydrate.

Moreover, free T was not calculated in this study due to lack of analytical techniques available for such measurements. The measurement of free T compared to total T may be more applicable and meaningful, considering that free T is often thought of as the bioavailable form of T. Of course, total T is most commonly measured and debate exists over whether free T is necessary for measurement.

Furthermore, the current study used a low-volume and moderate-intensity exercise training program. Higher intensity and longer duration programs have been used in other studies that reported an influence on T levels[18,59,61].

Finally, the sample sizes used to complete the study were relatively small, although common for such animal studies. Moreover, the duration of treatment was relatively short and involved a period during which normal growth should have been occurring. It is unknown whether similar findings would be observed over a longer time course and/or during later stages of life. Future studies may seek to answer such questions.

Conclusion

In summary, dietary intake had little impact on T concentrations in male rats. In addition, exercise appears to support T and estradiol in the presence of an AD. Lastly, LBM and T levels were supported in PD group as compared to an AD. These findings imply that a PD is capable of sustaining T levels and LBM, while resulting in a much lower fat mass in young and growing male rats. If these data can be extrapolated to humans and hold true during later stages of life, they offer evidence that plant-based diets can support T and lean mass gains.

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Appendix 1

Extended Literature Review

Overview: Testosterone, Dietary Intake, and Associated Variables

The influence of diet on circulating T concentration is an area of interest to both recreational and elite athletes. Understanding hormonal responses to feeding may benefit those individuals looking to improve muscle mass, strength and performance, as well as decrease fat mass. Physiological adaptations associated with elevated endogenous T are increased muscular hypertrophy, strength, LBM, and decreased fat mass[29]. Manipulating the macro- and micro-nutrient content of meals and how that variation influences endogenous T has produced mixed results[1,3,6].

Testosterone is a well-described sex hormone that plays important roles in the bodies of men and women. In men, it regulates sex drive (libido)[24], bone mass[23], fat distribution[23], muscle mass and strength[62]. Due to the influence of T on anabolic growth of skeletal muscle, for this purpose, elevating T is probably one of the most sought after goals of recreational and elite athletes. Testosterone is positively associated with muscle growth and has been investigated thoroughly in both humans and animals[20,23,29].

Sex hormone binding globulin (SHBG) is a sex steroid transporter secreted by the liver[63]. Approximately, 65% of serum T is primarily bound to SHBG, whereas a lesser amount of T is bound to albumin (~33%)[63]. Consequently, bioavailable T levels have been inversely related to the levels of SHBG[63]. To date, the literature remains inconclusive as to whether diet plays a direct role in the regulation of androgens or SHBG.

Macronutrient composition of a meal can have a major impact on circulating T concentrations. Replacing dietary carbohydrates with protein has been shown to decrease T[1]. However, Longcope et al.[6,7] has shown an increase in SHBG when ingesting a diet low in protein, possibly reducing bioavailable T by increasing the transport binding protein of T. Collectively, this suggest that the ratio of carbohydrate/protein plays an influential role in circulating T concentration indirectly by fluctuating SHBG. Volek and his colleagues[64] exposed 11 healthy men to a high fat diet (64% fat) formed primarily from monounsaturated fat for eight weeks. Results showed at the end of eight weeks there was a significant reduction in postprandial total T (-22%) and free T (-23%) one hour after consuming a fat-rich meal, which remained significantly below baseline for 8 hours compared with week 0 (baseline). Key et al.[8] compared levels of SHBG, free T and oestradiol in fifty-one male vegans and fifty-seven omnivores. The vegan group displayed 7% higher total T, 23% higher SHBG, 3% lower free T and 11% higher oestradiol compared to the omnivore group. It appears that the type of fat (saturated, monounsaturated, polyunsaturated) influences postprandial T concentrations, which is in opposition with earlier research[65]. The fat make-up of vegan (no animal products) diets typically consist of higher amounts of poly- and mono-unsaturated fats compared to saturated fat which is typically seen in a Western type diet (animal based). These dietary fats appear to produce an increase in SHBG thus affecting free T but, does not affect the homeostatic control of total T[8].

Testosterone overview

Testosterone, a steroid hormone from the androgen group is produced and secreted primarily from the leydig cells of the testes in men[21]. Approximately, 90-95% of plasma T is derived from the leydig cells[49]. A small percentage of plasma T is also derived from the adrenal cortex[49]. In men, T is the primary sex hormone responsible for the development of reproductive

tissues and promotes secondary sex characteristics such as muscle growth[29], muscle strength[14], and body hair[49]. While T production is higher in men than women, the production of this hormone is important in the health and well-being of both genders.

Testosterone is commonly measured as free (unbound) or total (unbound and chemically bound). Approximately 1-2% of T circulates free in the blood while the remaining 98-99% is bound to either SHBG (50-60%) or to albumin (40-50%)[66]. Testosterone has a high affinity for SHBG, therefore making only the free and albumin-bound T available for biological action [66]. Bioavailable T is best measured in the morning due to diurnal changes regulated by the circadian rhythm [66,67]. Total serum T in men lower than 300ng/dl (10.4 nmol/l) is considered below the lower limit of normal total T levels. This can be corrected by T treatments (exogenous testosterone) such as intramuscular injections, transdermal patches, buccal tablets and transdermal gels[26]. Insufficient levels of T (hypogonadism) due to increased uptake or lack of production can present serious health consequences due to the numerous roles T plays within the body. One such role is the maintenance of skeletal muscle tissue, which is supported by T.

The influence of T on muscle hypertrophy and strength has been investigated extensively in humans and animals[68,69]. Supra physiological doses of T enanthate in conjunction with a strength training program[28] is associated with a significant increase in muscle strength (Squat: 38 vs 19%; Bench press: 22 vs 10%), muscle hypertrophy (Triceps: 14 vs 11.9%; Quadriceps: 13 vs 6%), and increased LBM (6.1 vs 3.2 kg) compared to the enanthate + no exercise group. Similarly, Rogerson et al.[70] explored the short-term effects (< 6 weeks) of T enanthate on muscular strength and power in healthy young men. Results showed increases in one repetition maximum bench press (110 vs 100 kg; Effect size (ES): 0.5), one repetition leg press (350 vs 340 kg; ES: 0.2), and peak power during a 10-second cycle sprint (1400 vs 1300 W; ES: 0.3).

Finkelstein et al.[24] provided 198 men ages 20-50 with goserelin acetate (to suppress endogenous T and estradiol) and randomly assigned them to receive a placebo gel or 1.25, 2.5, 5, or 10 g of T gel (AndroGel®) daily for 16 weeks. In the same study, an additional 202 healthy men received goserlin acetate, placebo gel or T gel, and anastrozole (Arimidex; to suppress the conversion of T to estradiol). Results showed percentage body fat increased in groups receiving placebo or 1.25 or 2.5 g of T daily with anastrozole. Lean mass and thigh-muscle area decreased in men receiving placebo and in those receiving 1.25 g of T daily without anastrozole. Furthermore, leg press strength fell only with placebo administration. Lastly, sexual desire declined as the T dose was reduced. Similarly, sixty-one healthy men were given monthly injections of a Gonadotropin releasing hormone antagonist to suppress T production in conjunction with graded doses of T enanthate (25, 50, 125, 300, or 600 mg) for 20 weeks to determine the effects on muscle size and strength[71]. Results showed significant increases in muscle hypertrophy in T doses above 50 mg ($r = 0.66$, $P = 0.0001$). Strength was significantly increased in those receiving 50-, 300-, and 600-mg doses ($r = 0.48$, $P = 0.0005$) and changes in muscle volume ($r = 0.54$, $P = 0.003$). Wang et al.[62] randomized 227 men to either a 50 mg/day of Testosterone gel, 100 mg/day of Testosterone gel or a Testosterone patch for six months (180 days). The results showed increases in sexual function, mood, muscle strength (+11-13 kg: weight lifted), LBM (2.74 vs 1.28 kg) and a reduction in fat mass (-1.05 vs -0.90 kg) were positively correlated for the 100-mg group compared to the 50-mg group. Overall, these finding suggest that utilizing an exogenous source of T can potentially enhance athletic performance on tests requiring maximal/rapid force development and short maximal efforts. Collectively, these studies have shown that lean mass and strength are reduced and fat mass is increased in men with low T levels. Men with hypogonadism report less sexual activity, fewer sexual thoughts and fewer spontaneous erections

than men with normal T levels. Therefore, T supplementation can certainly affect physiological changes associated with hypogonadism by increasing lean mass, decreasing fat mass, and improving sexual function in men with hypogonadism.

Animal studies have shown the effect of T on stimulating the metabolic pathways for muscle protein synthesis which are involved in increasing LBM[68]. Mice were separated into three treatment groups: Sham (no treatment), castrated (Cas), and castrated receiving Nandrolone decanoate (Cas+ND). Results revealed castrated mice expressed a decrease in muscle myofibrillar protein synthesis (-29%) through Akt/mTOR signaling. However, the decrease in myofibrillar protein synthesis was reversed with administration of Nandrolone (anabolic steroid) by 4.5-fold (p = 0.004) when compared to castrated mice and 100% compared to the sham group (p=0.012). However, in a similar study, an orchiectomy had very little effect on downstream targets of the Akt/mTOR signaling pathway between three groups receiving Nandrolone (NAN), dihydrotestosterone (DHT), and an orchiectomy (ORX). Moreover, the castrated group showed a significant reduction in lean mass and body weight compared to NAN and DHT groups. Conversely, the NAN treatment group increased lean mass (+8.7%) and reduced absolute fat mass (-61%) compared to the DHT and ORX groups.

Data is consistent with regards to the positive effects T has on muscular growth and fat mass reduction. Furthermore, additional studies are needed on muscle wasting mechanisms due to reduced T. These findings may have clinical implications in the treatment of a variety of symptoms associated with T deficiency; symptoms such as muscle wasting, reduced bone mass/bone mineral density, sexual dysfunction, reduced energy and stamina, and impaired quality of living.

In addition, T influences overall effect on well-being and vitality. Overall well-being can suffer drastically when circulating T levels decline, often producing a range of symptoms. Symptoms include: low bone mineral density, sexual dysfunction, cognitive impairment, gynecomastia and depression[25]. Multiple studies have been conducted to show a decrease in these T associated symptoms with treatment from exogenous T. In addition to treatment, a health-related quality of life (HRQOL) questionnaire was administered, which subjectively measures patients' physical, social, and emotional well-being[25]. Recently, 274 elderly men randomized into T (n=138) and placebo groups (n=136) noted that those in the T group had improved muscle strength by 8.6%, increased LBM of 1.1 kg, a reduction in fat mass by 0.6 kg, and an improvement in the quality of living (self-administered questionnaire) compared to the placebo group[29]. Findings agree with earlier studies showing the potential clinical significance for improving the quality of life for elderly individuals using T.

Methods of Increasing Testosterone

Exercise

Several methods are believed to stimulate an increase of T *in vivo*. Resistance training has been shown to acutely elevate T (total & free), growth hormone (GH), and cortisol for up to 15-30 minutes' post exercise in men[16,17,72]. The magnitude of T, GH, and cortisol is greatest when large muscle mass exercises are performed with moderate to high intensities, increased volume, and short rest intervals[16,17]. When large muscle mass exercises are performed early in a workout, it has been speculated they have a positive effect on endocrine changes affecting smaller muscle groups. Small muscle groups which are typically used later in the workout may benefit in part to an enhanced T response which may lead to increased strength and hypertrophy[16]. There are several theories explaining acute increases of hormones post resistance

training. The major muscle involved, intensity and volume, nutritional intake, and training experience, may all influence these associated increase of endogenous T[16].

Exogenous Steroids

Exogenous steroid use is popular among recreational athletes and bodybuilders due to its effect on athletic performance and muscle size. Due to testosterones powerful anabolic effect on muscle tissue, this steroid becomes very appealing to athletes looking to gain a competitive edge. Aside from exercise, exogenous T can dramatically increase concentration levels of circulating T. Examples of common T treatments include injections (testosterone enanthate)[70], gels (Androgel®)[24], oral agents[73], and patches (Androderm®)[26]. However, as with all drugs, there may be some unwanted side effects[26]. This may be particularly true for oral and injectable forms of T. Symptoms such as elevated liver enzymes, decreased HDL-cholesterol, elevated estrogen levels, hematocrit and hemoglobin increases, and gynecomastia[26,42].

Botanical Supplements

Dietary supplements are consumed by more than half of adults in the US[74]. The most common reported reason for supplement use was to improve or support overall health. Currently, there are countless varieties of dietary supplements on the market claiming to increase T. Typically, these supplements contain either the raw ingredient or a proprietary blend of multiple ingredients.

Avena Sativa is a wild oat grass that has been used for centuries for a broad spectrum of health benefits. Of interest to bodybuilders, *avena sativa* claims to free up T from its bound state, making it more available to help promote muscle growth[75,76]. In addition, some research suggest

avena sativa may increase luteinizing hormone, which could possibly lead to increased levels of T[75].

Eurycoma longifolia, commonly known as *Tongkat Ali*, is a small flowering evergreen from Southeast Asia that has been traditionally used to enhance virility. Studies have confirmed its abilities to enhance sexual motivation, libido, and virility, perhaps through biosynthesis of T and other androgens[77-79]. Tambi et al.[80] reported a T increase in seventy-six men with late-onset hypogonadism who were given 200 mg of a standardized water-soluble extract of *Tongkat ali* for one month. Testosterone concentration increased from 5.66 ± 1.52 to 8.31 ± 2.47 nm (ES = 1.32), which represents an increase of 46.8%. Similarly, Ang and colleagues[77] showed that administering multiple doses of *eurycoma longifolia* orally to castrated mice promoted sexual performance when mounting, intromission and ejaculation. Mechanism behind its actions are thought to be elevated follicle stimulating and luteinizing hormone which the latter affects T production[77].

Mucuna Pruriens is an herbal compound widely used in Ayurveda (Indian herbal medicine) derived from the velvet bean and is a naturally occurring source of the neurotransmitter L-Dopa. L-Dopa is a precursor to the production of dopamine, norepinephrine, and epinephrine, and may amplify the body's natural release of luteinizing hormone (LH) and GH as well as other hormones particularly as you sleep. Specifically, LH handles signaling the testes to produce testosterone[21]. Yamanda et al.[81] administered oral L-Dopa (1000mg/kg) to male rats for fourteen days. Results showed oral L-Dopa supplementation increased serum LH levels four hours' post ingestion and LH levels returned to baseline levels within eight hours after administration. This agrees with Muthu and colleagues[82] who showed similar results in Wister male rats administered an extract of *Mucuna pruriens seeds* at similar doses of 1000mg/kg and

1500 mg/kg of body weight for thirty days. Results showed *Mucuna pruriens* seed treated rats expressed increased serum testosterone (3.0 vs 0.5 ng/ml) and testicular testosterone (9.1 vs 4.0 ng/dl) levels and testicular cholesterol (7.6 vs 3.2 mg/g) levels compared to the control group. These findings show the role *mucuna pruriens* play in altering testosterone production indirectly by targeting LH, serum and testicular testosterone and testicular cholesterol. All of which play a significant role in the overall production of endogenous testosterone. Moreover, *Mucuna pruriens* can aid with increasing LBM, recovery, immune support and feeling of vitality[82-84].

The potential benefits of supporting sufficient testosterone levels are numerous and highly sought out among men looking to improve overall health and well-being, body composition, and athletic performance. When looking to improve on these parameters of life, herbal supplementation may be an alternative method to pharmaceuticals. However, additional research and clinical trials are needed in human subjects to demonstrate the efficacy of herbal supplementation for purposes of elevating testosterone and improving health related outcomes.

Micronutrients

The ergogenic effect of many nutritional supplements has been investigated extensively throughout the years. The supplement industry in particular advertises countless supplements, claiming to increase testosterone[85], reduce body fat[86], and enhance libido[87]. Trace elements such as boron[88], vitamin D[89], and zinc[90] have been at the forefront of many of these claims. Boron may perhaps influence the function of hormones such as vitamin D, estrogen, thyroid hormone, insulin, and progesterone[88]. Vitamin D has been associated with influencing sperm count and motility, support of the male reproductive system and increasing testosterone in men[89,91,92]. Zinc, on the other hand, has been linked to hypogonadism, and growth retardation when deficient[90]. Many studies have focused on the role of these trace elements in male and female reproduction

in both human and animal models. Although some data appear promising, there still is much to learn around the role of micronutrients to increase circulating testosterone.

The altering of dietary micronutrient content has likewise been researched in relation to circulating testosterone. Zinc deficiency is prevalent throughout the world, including the USA, and has been associated with hypogonadism in men[93]. The effects of a zinc-containing supplement zinc monomethionine aspartate (ZMA) on serum testosterone was examined in 14 exercised trained men for an eight-week period[90]. The daily dose of zinc intake of all participants ranged from 11.9-23.2 mg/day. Total and free testosterone concentrations did not increase over the course of the study. In addition, the effect of vitamin D on circulating testosterone levels has been investigated in human models[89,91,92]. Findings from these studies support claims that there is an association between vitamin D testosterone levels[89,91,94,95]. Preliminary studies show that these micronutrients may possibly play a role in regulating testosterone levels. However, the specific mechanism regulating testosterone has not been shown. Research in this area is inadequate and additional studies are a necessity to further contribute to our current knowledge base.

Macronutrients

Varying the ratio of macronutrients such as carbohydrates, fats, and protein have been suggested to change circulating testosterone levels. There have been numerous studies that have analyzed the effects of varying dietary macronutrient content in human and animal models to influence testosterone levels. Alleman & Bloomer[2] examined hormonal response to carbohydrate and lipid meals during acute postprandial period. Results indicated that testosterone levels decreased at once following a meal regardless of meal size or macronutrient make-up. However, a significant time effect was noted ($p = 0.04$), with testosterone levels decreasing during the postprandial period and being statistically lower at the one-hour period compared to pre-meal. A

prior investigation predating Alleman and Bloomer displayed there may be a positive relationship with carbohydrate[1] and saturated fat[64] intake on circulating testosterone levels. The dissimilarity between studies, although both were isocaloric, could be due to the type or amount of carbohydrate, fat, and protein administered. Moreover, macronutrient ratios in the human diet can be an important regulatory factor for testosterone plasma levels. Finally, the source of macronutrients (i.e., coming from either whole food or supplement) can produce conflicting results. Gonzalez and his colleagues examined the effects of protein supplementation (20 g protein, 6 g carbohydrates, and 1 g fat) on circulating testosterone concentration and its role on anabolic signaling via mTOR signaling pathway in ten trained men[96]. No differences were observed between trials for elevated testosterone concentrations and anabolic signaling. Furthermore, milk-based protein, which is ~80% casein and ~20% whey was used to determine the effect protein ingestion had on anabolic signaling and endocrine response. Differences were noted on the source of the protein and amino acid availability between slower digesting proteins such as casein compared to whey, a rapidly digested protein. Due to the low carbohydrate and fat content, the macro nutrient content might not have been high enough to elicit an anabolic response. These differences may have influenced the results in which would call for future testing to determine the proper protein and carbohydrate ratio on Akt/mTOR activation via anabolic signaling.

In men, a low fat, high fiber diet was shown to decrease total and free testosterone concentrations[9]. In a similar study completed in women, testosterone levels decreased by 27% within two hours of both low fat, high fiber and a high fat, low fiber meal[10]. However, testosterone levels were reduced for 2 hours longer after the high fat, low fiber meal compared with the low fat, high fiber meal. Vegetarian men who consumed more fiber and polyunsaturated

fats were reported to have increased SHBG, total testosterone and decreased free testosterone levels compared to non-vegetarians[8]. In addition to fiber, it has been noted that a diet chronically high in fat appears to increase endogenous testosterone production[9] compared to acute ingestion of fat[3]. However, acute intake of dietary fat has resulted in an increase in serum testosterone[65]. Due to conflicting data, it is of importance to figure out the role carbohydrates (fiber) and fat have on circulating testosterone levels. These findings can potentially be applied to many health-related illnesses ranging from hypogonadism to impaired glucose tolerance.

Consumption of mixed meals and the role of combination feeding in supporting testosterone and supporting muscle mass continues to be studied. Specifically, the influence of macronutrient ratios related to testosterone has yielded differing findings [2,64,65]. Although ample evidence exists related to carbohydrate and protein intake related to insulin response[97-99], what is not completely understood is how carbohydrate or protein ingestion impacts testosterone. Mechanisms behind this are unclear but it has been proposed that the insulin may influence testosterone levels in blood[98]. As research has shown, insulin is an important driver of nutrients in to skeletal muscle, which aids in the maintenance of skeletal muscle mass.

The maintenance of skeletal muscle mass can be defined as the net result of protein synthesis and degradation[36]. Active individuals have adapted feeding strategies to favorably alter muscle protein synthesis in hopes of inducing physiological adaptations. One inquiry that is now being researched is the affect protein has on circulating testosterone levels. A recent study showed a protein supplement (20g protein, 6 g of carbohydrates and 1 g fat) had no effect on testosterone levels in men[96]. These findings are consistent with earlier studies looking at the influence of protein on testosterone levels[100]. Longcope et al.[6] revealed a diet low in protein decreased testosterone levels by increasing levels of SHBG. Despite reduced testosterone

concentrations, results from another study showed a reduced resting and post-exercise increase in testosterone concentrations in athletes consuming plant based proteins compared to protein derived from animal sources[58]. However, Kalman et al.[36] showed an increase in testosterone/estradiol ratio in men consuming a soy & whey mixture compared to soy concentrate, soy isolate and whey blend. Specifically, for twelve-weeks men were supplemented with fifty grams per day of one of four different protein sources (soy concentrate, soy isolate, soy isolate and whey blend, and whey blend only) in combination with a resistance-training program. Results indicated that at the end of twelve-weeks, subjects experienced no significant increase in LBM, in addition to no differences between groups for percentage body fat, body weight, SHBG and total and free testosterone. However, testosterone/estradiol ratio increased across all groups (+13.4, $p = 0.005$) and estradiol decreased ($p = 0.002$). Within group analysis revealed significant increases in the testosterone/estradiol ratio in soy isolate + whey blend group (+16.3, $p = 0.030$) and estradiol was significantly lower in the whey blend group (-9.1 ± 8.7 pg/ml, $p = 0.033$) compared to soy concentrate and soy isolate. Interestingly, data show that a plant-derived protein source is capable of sustaining LBM. Furthermore, total- and free testosterone concentrations in all groups were kept, which suggest that plant protein sources can support endogenous testosterone. Although testosterone levels did not change significantly, there was a significant decrease of testosterone/estradiol ratio following the twelve-week period. A protein blend made up of multiple sources of protein may prove to be beneficial in supporting testosterone and increasing LBM post physical activity. Total protein intake during this investigation was particularly low (50 g of protein per day) which should be considered in future studies which may be interested in comparing the influence of animal- and plant-based diets on testosterone and LBM.

Impact of Protein Type on Testosterone

Animal Protein

Skeletal muscle mass is regulated via changes in muscle protein synthesis (MPS) and muscle protein breakdown[12]. Of the two, the stimulation of MPS is believed to be the primary metabolic pathway responsible for regulating the maintenance or gain in skeletal muscle mass[101]. Directly influencing this process is physical activity, food intake, and dietary protein. Both animal- and plant-derived proteins provide amino acids which are used for synthesizing proteins in addition to acting as signaling molecules to induce MPS response[12]. However, there appears to be a difference in MPS response time between animal- and plant derived protein sources. This difference may be due to the digestibility and lack of specific essential amino acids in plant- as opposed to animal-based proteins. Animal protein is believed to be superior to plant-based proteins primarily due to its high essential amino acid profile and rapid digestibility and absorption compared to plant proteins[11]. On the other hand, most plant proteins have incomplete amino acid profiles and lack specific essential amino acids which are directly involved in stimulating MPS. The exception is soy protein, which is a complete protein source[11].

Protein is an important component of dietary intake and is needed for growth and a multitude of cellular processes[51]. Cellular processes such as biogenesis[51], protein synthesis and endocrine responses[36]. Studies have examined animal protein and its influence on endogenous testosterone[46]. Animal proteins such as whey[102], egg[103], beef[104] and casein[105] have been utilized in human and animal models only to produce conflicting results. Casein, a milk derived protein is known for its slow digestibility and absorption rate compared to whey isolate/hydrolysate and soy isolate. Boirie et al.[106] showed blood amino acid levels peaked in one hour after ingestion of whey or casein. However, the whey group peaked at higher levels and returned to baseline after

four hours. The casein group peaked at a lower level but didn't return to baseline until seven hours later. Moreover, casein has a complete essential amino acid content which has been considered superior to other proteins in regards to increasing muscle protein synthesis[107]. Gonzalez et al.[96] examined the effect of milk protein (~20% whey and ~80% casein) on mTOR signaling following a resistance exercise protocol designed to promote elevations in circulating hormone concentrations in trained men. It was shown that ingestion of the milk protein resulted in no response in signaling proteins Akt/mTOR or testosterone levels. It was suggested that leucine content may have been too low to elicit a response. Leucine is a key amino acid that triggers mTOR signaling and a rise in muscle protein synthesis[108]. The total leucine content of the protein supplement used was 1.86 g (0.02 g/kg of body weight), which may have not enough to maximally stimulate muscle protein synthesis. It has been suggested that 0.05 g of leucine per kilogram of bodyweight is needed to maximize the anabolic response[12]. Due to the slower digestion rate of casein, it may offer a lesser stimulus for anabolism thus contradicting its role as a superior protein based on earlier claims. It was speculated that the amount of protein and carbohydrates provided in this investigation may have been too low to elicit a response from the signaling proteins and endocrine response compared to similar studies[101,105].

Previously, it was shown that endocrine responses were lacking in regards to ingesting milk protein (~20% whey and ~80% casein) post exercise[96]. Further experiments conducted by Badgeret al.[46] investigated the developmental effects of soy protein isolate, casein, and whey protein in male (n=61) and female (n=192) rats. Results showed that soy protein isolate accelerated puberty in female rats (2 days earlier) and whey protein delayed puberty in male (1.3 days) and female rats, as compared with groups receiving casein. Furthermore, results showed male rats fed soy protein isolate had normal serum testosterone levels, however, female rats fed

soy protein isolate had reduced serum 17 β-estradiol concentrations by 30%. Although the mechanism by which whey and soy protein produced these effects is unknown, multiple mechanisms are now being investigated. Several proposed mechanisms are: increased synthesis and decreased degradation of SHBG synthesis; weak estrogenic agonist/antagonist activity through the estrogen receptor α; and estrogen receptor β-mediated actions and altered hormone production[46]. These findings further point toward the endocrine disruption of endogenous hormones in humans and animals.

Hormones and hormone-like substances which are present in food of animal origin are believed to be the cause of several endocrine disruptions[31,32]. Progesterone and estradiol are steroid compounds naturally present in animal tissues and fluids (meat, milk, etc.). For this reason, these hormones cannot be completely avoided in food of animal origin, since they are part of the animal's metabolism[31,32]. Cow's milk has considerable amounts of hormones and is of concern[31]. Regal et al[31] used a liquid chromatography-tandem mass spectrometry method for detection and quantification of four naturally occurring steroid hormones in commercial bovine milk (n = 10). These hormones consisted of pregnenolone (P5), progesterone (P4), 17-hydroxypregnenolone (17-OHP_5), 17-hydroxyprogesterone (17-OHP_4). Specifically, 17-OH-progesterone and 17-OH-pregnenolone are precursors of estrogens and androgens respectively[109]. Samples were taken over the course of three consecutive days and for all steroids, the analyzed recovery was between 70% and 110% with an average R value $\geq$ 0.96.

The relationship between steroid hormones and several human health problems has been previously reported, such as prostate cancer[110] and breast cancer[111]. It has been hypothesized that a Western compared to vegan type diet may influence hormonal levels, which can be potentially dangerous, even in small amounts which may lead to major changes in endocrine responses/

functions in humans and animals. However, a modified *ad libitum* diet low in animal fat and refined carbohydrates but rich in low-glycemic-index foods, monounsaturated and polyunsaturated fatty acids and plant based proteins has been shown to favorably change hormonal profiles of men and women[111,112]. Therefore, such a plant-based diet may be favorable for overall health.

Plant Protein

Most plant proteins are considered inferior compared to animal derived proteins due to their incomplete amino acid profile and lower essential amino acid (EAA) content[11]. Research today deals with protein quality in regards to its influence on muscle protein synthesis in the human and animal trials[11]. Plant proteins such as pea, oat, wheat, hemp, rice and soy have been reviewed, specifically in regards to their amino acid profile and their influence on muscle anabolic response. Interestingly, none of the plant-based proteins have been investigated in regards to their effect on the anabolic hormone testosterone, except for soy.

Although a plant protein, soy beans have a complete amino acid profile and are ranked with beef protein in regards to their protein digestibility corrected amino acid score (PDCAAS). For instance, soy beans have a high PDCAA score of 0.91 compared to beef with a score of 0.92[11]. Due to soy's PDCAA score, one could expect soy beans to be as effective in stimulating MPS as beef. This reasoning has led to many investigations on soy and its impact on MPS and testosterone production in human and animal models[43,113]. Currently, the unanswered question is whether plant-based proteins as a dietary supplement are efficacious at altering testosterone and MPS which in turn would increase LBM or support it. If so, what are the mechanisms behind this action? At the forefront of this discussion are the highly debated endocrine-disrupting chemicals.

There is a growing interest in the potential health related problems posed by endocrine-disrupting chemicals (EDC's) to the reproductive system[47]. EDC's include synthetic organic compounds such as pesticides, fungicides, pharmaceutical agents, as well as natural plant-derived EDC's referred phytoestrogens[31]. Soy and soy products contain isoflavones, a class of phytoestrogens that interacts with endogenous estrogen signaling pathways[33]. Isoflavones have a chemical structure similar to the hormone estrogen and have been classified by some as selective estrogen receptor modulators (SERM)[33]. SERM's possess mixed estrogen agonists/antagonists in part due to their preferential binding to estrogen receptor beta compared to estrogen receptor alpha[33]. However, isoflavones are the reason that soy has become controversial. Concerns that the estrogen-like properties of these compounds might lead to adverse effects in men and women have been raised.

Due to sensationalized media stories on these topics, men may have been influenced to stay away from soy and their derivatives, due to its potential feminizing effects. Feminizing effects include gynecomastia, as well as increased estrogen and decreased testosterone concentrations in blood[42,113]. Hamilton-Reeves et al.[114] observed changes in hormone concentrations of fifty-eight men after providing them soy protein isolate, alcohol washed soy protein isolate, or milk protein for six months. The androgen receptor expression in the prostate was significantly decreased in both soy protein isolate groups (1.37 vs 1.26) compared to the milk protein isolate group. However, androgen receptor expression was significantly elevated from baseline in the milk protein isolate group (1.23 vs 1.42) compared to the two soy protein groups. In the same study, serum estradiol (66 vs 79 pmol/L), estrone (141 vs 171 pmol/L) and androstenedione concentrations (2.9 vs 3.4 nmol/L) of alcohol washed soy protein isolate was significantly elevated at the six-month mark compared to the milk and soy protein isolate group.

Testosterone and free testosterone concentrations did not change between soy protein isolate (12 vs 13 nmol/L; 33 vs 32 pmol/L), alcohol washed soy protein isolate (13 vs 13 nmol/L; 34 vs 32 pmol/L), and milk protein isolate (12 vs 12 nmol/L; 29 vs 31 pmol/L) groups respectively, compared to baseline. In agreement, Dillingham et al.[115] demonstrated that the ingestion of soy protein isolate (high/low isoflavones) as opposed to milk protein isolate for fifty-seven days resulted in elevated estradiol (77.9 vs 85.1 pmol/L) and estrone concentrations (155.7 vs 172.4 pmol/L) in thirty-five men. Further results showed total testosterone significantly decreased (22.1 vs 19.8 nmol/L*)* in the soy groups compared to the milk protein isolate group with no changes in androgen concentrations between all groups. Similarly, Thrope et al.[34] showed that consumption of scones for six weeks made with wheat or soya flour (containing 120 mg/day isoflavones) in nineteen volunteers resulted in a decrease in total serum testosterone (19.3 vs 18.2 nmol/L*;* 95% CI 1.01, 1.12; $p = 0.03$). Overall, these findings imply that the origin of meals (animal or plant) may upregulate or down regulate the androgen receptor mRNA expression[34]. Furthermore, data appears conflicting when trying to figure out how soy protein impacts testosterone. Differences may be due to how the protein is administered. For example, administration could be a whole food source, extracts, or a powdered supplement. It should be noted that the studies using whole food sources and extracts reported significant reductions in testosterone concentrations in addition to elevated estrogen levels. These findings could prove beneficial in showing how dietary habits influence endocrine function. This in part may directly influence the production and the circulating levels of testosterone, thus potentially altering LBM.

Indeed, it has been suggested that the ingestion of plant-based meals of soy origin could produce feminizing effects in men and health related issues in women with certain cancers[116] due to its role as a potential estrogen agonist/antagonist. However, Messina et al.[42] reviewed nine

clinical (avg. n = 19.4 men) studies evaluating the effects of isoflavone exposure on feminizing effects in men. Results showed neither isoflavone supplements nor isoflavone rich soy exerted feminizing effects on men by decreasing total and free testosterone, in addition to increasing estrogen concentrations. In agreement, Hamilton-Reeves et al.[113] conducted a meta-analysis on thirty-six studies showing soy isoflavones produced no effects on testosterone, free testosterone, & SHBG in men with an overall effect size of 0.02 (CI – 0.05, 0.09). Deibert et al.[117] showed weight loss over a six-month period without losing muscle mass in pre-obese and obese subjects who were administered a high-soy-protein diet. In comparison to the above findings, animal studies have produced mixed results, ranging from increased testosterone levels to delayed/early onset of developmental effects [43,44,46].

Limitations

Varied study designs and methodological approaches may be the limiting factor for consistency when viewing results from the studies focused on soy ingestion and circulating hormones. Study designs ranged from 3-12 weeks in duration and investigated the effects of consuming whole soy foods[34], soy protein isolate[115], or extracted isoflavones[118] on circulating reproductive hormone levels. Investigating the above in the context of mild caloric restriction or *ad libitum* feeding has been done, which may confound the overall findings. Furthermore, circadian rhythm, sample size, age, gender and current health status may play a role in the interpretation of the results. Finally, findings from animal studies suggesting that the increase/decrease in feminizing effects observed with isoflavones may not apply to men, because of the differences in isoflavone metabolism between rodents and human. Some rodents can use high-density lipoproteins and low-density lipoproteins in conjunction with cholesterol to make testosterone[119] compared to humans who typically use low-density lipoproteins[51]. Equol is

bacteria that metabolizes soy isoflavones and these bacteria are more common in the animal population than in humans. As noted earlier, the concern raised by some is that soy protein—based on its essential amino acid profile and PDCAAS—might not be able to support LBM in the same manner as animal protein. However, results have shown weight loss without losing muscle mass in subjects ingesting a high-soy-protein diet[117] or a soy-based meal replacement[120].

Conclusion and Future Research

This study will further examine the role of diet on testosterone and LBM in male rats. The primary question to be answered is how diet influences total and free testosterone. Secondary aims will be to determine the influence of nutrition on estradiol concentrations, total body mass, LBM and fat mass. Investigating the influence of nutrition on circulating testosterone levels in an animal model may prove beneficial for the generalization towards human models. Results from this study may indicate that animal protein is not the only protein form capable of yielding increased LBM and a maintenance or increase in circulating testosterone.

The potential benefits of supporting adequate testosterone levels are abundant and highly sought out among individuals wishing to improve their overall health and outlook on life, their physical and mental performance, and their overall physique. When considering methods to increase or support testosterone levels, protein supplements or nutrient partitioning may prove an effective alternative to pharmaceuticals.

Appendix 2

Table 1. Dietary composition of the Animal Diet and Plant-Based Diet

Nutrient	**Animal Diet**		**Plant Diet**	
	gm%	kcal%	gm%	kcal%
Protein	20	17	15	15
Carbohydrate	50	43	58	59
Fat	21	40	11	25
Fiber	5	0	13	1
Total		100		100
kcal/gm	4.7		3.9	
Casein	195	780	0	0
Soy Protein	0	0	170	680
DL-Methionine	3	12	3	12
Corn Starch	50	200	0	0
Corn Starch-Hi Maize 260 (70 % Amylose and 30% Amylopectin)	0	0	533.5	2134
Maltodextrin 10	100	400	150	600
Sucrose	341	1364	0	0
Cellulose, BW200	50	0	100	0
Inulin	0	0	50	50
Milk Fat, Anhydrous	200	1800	0	0
Corn Oil	10	90	0	0
Flaxseed Oil	0	0	130	1170
Ethoxyquin	0.04	0	0.04	0
Mineral Mix S1001	35	0	35	0
Calcium Carbonate	4	0	4	0
Vitamin Mix V1001	10	40	10	40
Choline Carbonate	2	0	2	0
Ascorbic Acid Phosphate, 33% active	0	0	.41	0
Cholesterol	1.5	0	0	0
Total	**1001.54**	**4686**	**1187.95**	**4686**
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	

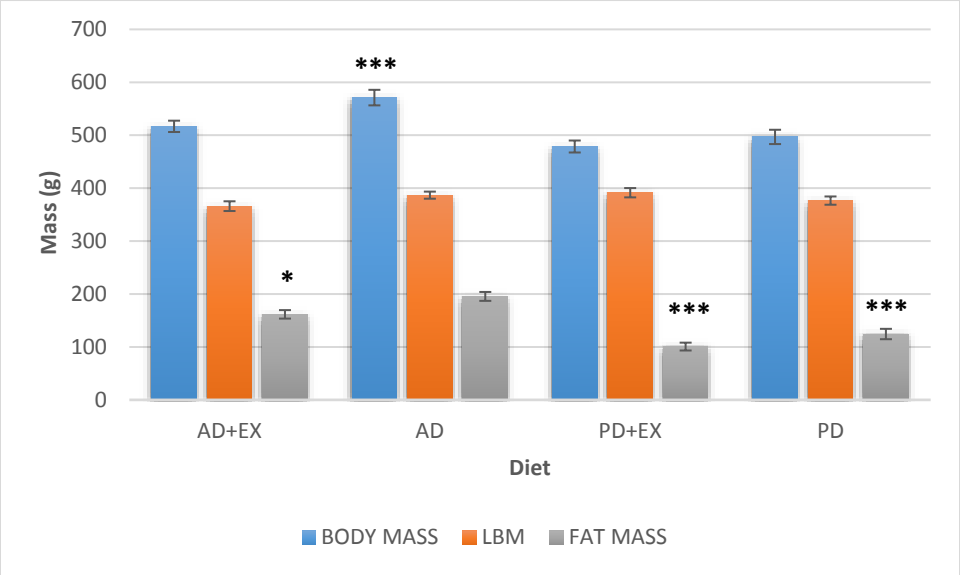

Figure 1. Body composition data of male rats assigned to two different diets with and without exercise. PD supports LBM ($p = 0.14$); *, *** differs significantly from control ($P < 0.05$, $P < 0.0001$, respectively). Group effect was noted for mean fat mass ($p < 0.0001$), with PD groups lower than AD groups; AD+EX was lower than AD ($p < 0.05$). AD body mass was significantly greater than all other groups ($P < 0.0001$)

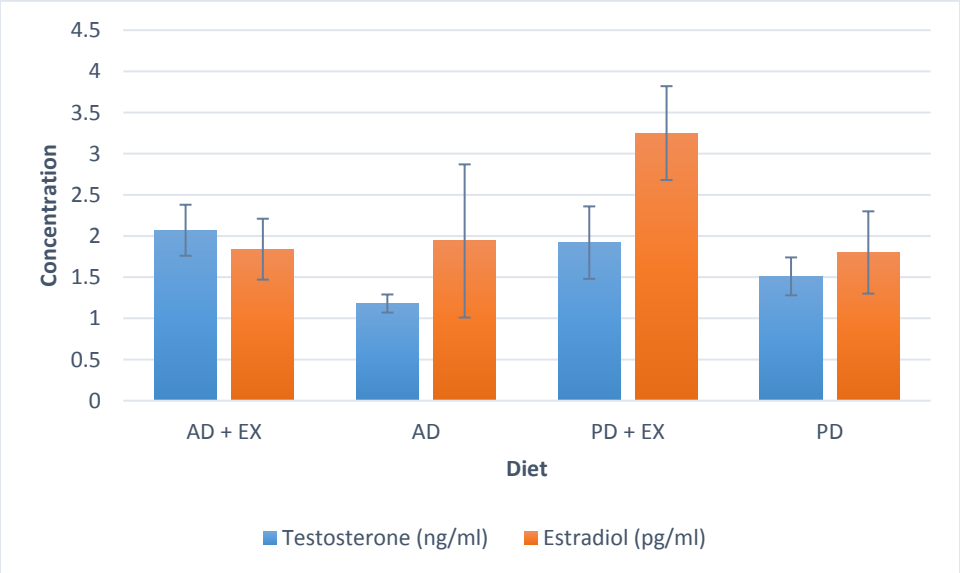

Figure 2. Testosterone and Estradiol concentrations of male rats assigned to two different diets with and without exercise. AD appears to decrease testosterone; exercise appears to be associated with higher testosterone. Elevated estradiol in PD+EX group may be associated with very small sample size.