Suppression of endogenous testosterone production attenuates the response to strength training: a randomized, placebo-controlled, and blinded intervention study

Thue Kvorning,1 Marianne Andersen,2 Kim Brixen,2 and Klavs Madsen1

1Institute of Sport Science and Clinical Biomechanics, University of Southern Denmark; and 2Department of Endocrinology, Odense University Hospital, Odense, Denmark

Submitted 24 March 2006; accepted in final form 18 July 2006

Kvorning, Thue, Marianne Andersen, Kim Brixen, and Klavs Madsen. Suppression of endogenous testosterone production attenuates the response to strength training: a randomized, placebo-controlled, and blinded intervention study. Am J Physiol Endocrinol Metab 291: E1325–E1332, 2006. First published July 25, 2006; doi:10.1152/ajpendo.00143.2006.—We hypothesized that suppression of endogenous testosterone would inhibit the adaptations to strength training in otherwise healthy men. Twenty-two young men with minor experience with strength training participated in this randomized, placebo-controlled, double-blinded intervention study. The subjects were randomized to treatment with the GnRH analog goserelin (3.6 mg) or placebo (saline) subcutaneously every 4 wk for 12 wk. The strength training period of 8 wk, starting at week 4, included exercises for all major muscles [3–4 sets per exercise × 6–10 repetitions with corresponding 6- to 10-repetition maximum (RM) loads, 3/wk]. A strength test, blood sampling, and whole body DEXA scan were performed at weeks 4 and 12. Endogenous testosterone decreased significantly (P < 0.01) in the goserelin group from 22.6 ± 5.5 (mean ± SD) nmol/l to 2.0 ± 0.5 (week 4) and 1.1 ± 0.6 nmol/l (week 12), whereas it remained constant in the placebo group. The goserelin group showed no changes in isometric knee extension strength after training, whereas the placebo group increased from 240.2 ± 41.3 to 264.1 ± 35.3 Nm (P < 0.05 within and P = 0.05 between groups). Lean mass of the legs increased 0.37 ± 0.13 and 0.57 ± 0.30 kg in the goserelin and placebo groups, respectively (P < 0.05 within and P = 0.05 between groups). Body fat mass increased 1.4 ± 1.0 kg and decreased 0.6 ± 1.2 kg in the goserelin and placebo groups, respectively (P < 0.05 within and between groups). We conclude that endogenous testosterone is of paramount importance to the adaptation to strength training.

It is well established that strength training elicits neural and morphological adaptations that may lead to increased muscle strength and muscle hypertrophy (19, 40, 46). Previous studies have shown that strength training induces acute changes in the circulating concentrations of testosterone, growth hormone (GH), and insulin-like growth factor-I (IGF-I) (26, 27, 29). It has been reported that endogenous testosterone together with, GH, IGF-I, insulin, and cortisol mediates the adaptive changes in muscles following strength training (e.g., muscle protein synthesis) (27, 31, 49). Accordingly, increased muscle protein synthesis has been observed after strength training (11, 36, 57). Furthermore, dramatic anabolic effects on skeletal muscles have been reported after exogenous testosterone suppletion in humans (6, 7, 47). The interaction, however, between strength training, levels of circulating endogenous testosterone, protein synthesis, muscle hypertrophy, and increased strength are not fully understood.

Treatement with gonadotropin-releasing hormone (GnRH) analogs (e.g., goserelin) inhibits pituitary secretion of LH and thus testicular production of testosterone (12). In previous studies, manipulation of the circulating testosterone concentrations by simultaneous treatment with GnRH analogs and exogenous testosterone, a positive relationship between testosterone concentrations and fat-free mass, muscle size and strength was reported (7, 47). Also, suppression of endogenous testosterone production by GnRH analogs in young men results in decreased protein synthesis, decreased strength, and decreased fat oxidation (37). However, the specific importance of endogenous testosterone is not fully clarified, although it is tempting to speculate that testosterone initiates recuperation after strength training. Thus the question is whether endogenous testosterone is critical for increasing muscle strength and muscle hypertrophy in response to strength training (11, 19, 21, 22, 24, 27, 33, 34, 54).

Therefore, the aim of the present study was to investigate the importance of testosterone for increasing muscle mass and muscle strength gain in a randomized, placebo-controlled, double-blinded intervention study. Endogenous production of testosterone was suppressed by the use of a GnRH analog, which enabled us to study the role of testosterone in the adaptations to strength training. We hypothesized that suppression of endogenous testosterone would inhibit the adaptation to strength training and attenuate the increase in lean body mass and muscle strength compared with a placebo group performing identical strength training.

MATERIAL AND METHODS

Subjects and study design. Twenty-six subjects volunteered to participate in the study. The subjects were participating in leisure sport once or twice a week but had only minor previous experience with strength training, no more than 1 h/wk. None of the subjects was participating in strength training activities on a regular basis before entering the study. The study conformed to the guidelines in the Declaration of Helsinki and was approved by the local Ethics Committee (VF 20040173). All subjects were informed of the risks and purposes of the study before their written consent was obtained. The subjects were carefully matched in pairs with regard to isometric knee strength.
extension strength, body mass index, and age. Within each pair, the subjects were randomized to placebo (saline) or 3.6 mg of goserelin (GnRH analog) injections once every fourth week, three times in total. Clinical examination of the subjects was performed before the experiment, and two subjects were disqualified due to exclusion criteria (metabolic disorders, low testosterone levels, angina pectoris, lower back disorders, prescription medication for heart or lung diseases, or any recent physical trauma). Two subjects did not complete the study, one due to an injury unrelated to the study and one due to side effects of the GnRH analog treatment (hot flushes). Therefore, twenty-two young men completed the study (Table 1). The subjects and investigators involved in training and testing were blinded regarding the allocation of the subjects whereas two investigators (M. Andersen and K. Brixen) administering the study drugs and monitoring safety parameters were aware of the allocation. The schedule of study procedures is shown in Fig. 1.

**Testing procedures.** The subjects underwent three test procedures; tests 1, 2, and 3, which included blood sampling, isometric strength testing, and whole body dual-energy X-ray absorptiometry (DEXA) scan (Fig. 1). The subjects were familiarized with the study procedures ~2 wk before entering the first test. This included measuring of anthropometrics of the subjects and a careful introduction to the testing procedures. Furthermore, each subject completed the entire strength testing protocol and was introduced to the strength training exercises. Thus the technique was carefully corrected until proper technique was achieved. Subsequently a 10-repetition maximum (RM) load was measured for all exercises in the training program to determine the initial training load.

**Treatment with goserelin.** A 3.6-mg depot of goserelin (Zoladex, AstraZeneca) was injected subcutaneously (abdomen sub cutis) once every 4 wk to reduce and maintain endogenous testosterone concentrations within castrate range. Goserelin prevents the reappearance of LHRH receptors and consequently inhibits the secretion of LH from the pituitary gland and thus testicular production of testosterone (12). All subjects received three injections in total starting right after the first test (Fig. 1). The relatively short treatment period was chosen due to ethical considerations; however, at the same time, the extent of the treatment period was intended to be long enough to induce measurable changes in strength, etc. (2, 6, 8, 17, 38, 41).

**Training.** A standardized warm-up was performed before training consisting of four sets of squats with 20 repetitions without load and with 1 min of rest between sets. The subjects from both groups trained with the same strength training program, including exercises for the entire body (Table 2). The training loads were increased due to RM tests at the start of each of the three periods, but also, whenever the subjects showed reserve of strength the load was increased. Subjects showed a reserve of strength when they eventually were able to perform more repetitions than expected with their respective previously measured RM load. Both groups showed the same progression in training loads, therefore culminating in the same training volumes after the strength training period (Table 3). All training sessions were supervised in order to control the training techniques, training loads, and training log books. Both groups carried through the same amount of training (except for one training session), therefore subjects in the goserelin group completed 23.7 training sessions on average, and the placebo group completed 23.6 training sessions on average. All subjects participated in a minimum of 22 training sessions. Previous studies with similar strength training programs have demonstrated significant acute increases in the level of testosterone (20, 28) and significant increases for muscle strength and muscle mass (2, 6, 8, 17, 38, 41). Optimal nutrition is important to benefit from strength training (15, 50), so each subject received 0.5 liter of skimmed chocolate milk (containing 17.5 g protein, 50 g carbohydrate and 2.5 g fat) directly after each strength training session, thereby ensuring sufficient supplement of proteins and carbohydrates for recovery.

**Blood sampling.** Subjects reported to the laboratory between 0700 and 0900 and had been fasting from 2400 the day before and refraining from strenuous physical activity for 48 h. Blood samples were drawn from an antecubital vein at the same time of day for each subject during tests 1, 2, and 3 after 30 min of supine rest for determination of resting level of serum endogenous total testosterone, free testosterone, GH, androstendione, dihydrotestosterone, estradiol, sex hormone-binding globulin (SHBG), and dehydroepiandrosterone sulfate. Thirty milliliters of blood were drawn for serum samples, immediately chilled on ice, and centrifuged at 3,000 rpm for 10 min at 20°C. All serum samples were then distributed to appropriate tubes and stored at −80°C until analyzed. Body temperature was measured during each blood sampling, showing identical temperatures in all three tests.

**Standardized breakfast.** After blood sampling, a standardized breakfast was served for the subjects followed by 1 h of rest before they proceeded to strength testing. The amount of food was adjusted in relation to body weight. Consequently, the subjects were divided in three groups (i.e., light, medium, and heavy body mass group) receiving three sizes of breakfast, containing 6.47 ± 0.46 kcal/kg,
0.22 ± 0.02 g protein/kg, 1.25 ± 0.10 g carbohydrate/kg, and 0.06 ± 0.00 g fat/kg.

Analysis of hormones. Serum total testosterone, androstenedione, and dihydrotestosterone were measured using an in-house assay based on extraction, chromatography, and a final, specific radioimmunoassay (RIA), as described in Lykkesfeldt et al. (35). Serum dehydroepiandrosterone sulfate was measured by RIA on diluted serum using tritiated dehydroepiandrosterone sulfate and antibodies raised against DHA-3-hemisuccinate-BSA. The method was calibrated with the method described in Lykkesfeldt et al. (35). Serum GH and SHBG were measured by a time-resolved fluorimmunoassay by AutoDelfia (Turku, Finland). Free testosterone (non-protein-bound) was calculated as described by Bartsch (5). Serum estradiol was measured using an in-house RIA after extraction chromatography, as described by Emment and Collins (14).

Isometric strength testing. After a 5-min standardized warm-up procedure on a bicycle ergometer, the dominant leg was tested in a KinCom dynamometer (KinCom 500H, software version 4.03; Chattec). Subjects were seated in an upright position, and the protocol included isometric knee extensions. The dynamometer was adjusted to fit the individual subject, and the specific positions were used in all tests afterward. The lateral femoral epicondylye was chosen to represent the axis of knee joint rotation and was therefore aligned to the axis of rotation on the dynamometer. The length of the lever arm was adjusted to ensure that the ankle pad was fixed 2 cm above the lateral and medial malleoli. Isometric contractions were performed at a locked position of 70° knee flexion (0° = full extension). Subjects were instructed to extend the knee as explosively and forcefully as possible, and three attempts were performed with maximal contraction held for 3 s. During testing, all subjects were instructed to hold arms crossed on the chest and not to move limbs that were not involved in the strength test. Visual feedback was given, with the highest value shown on the computer monitor. Forty-five seconds of recovery between trials were given, and the highest absolute value for isometric measurements was used for further analysis. The isometric measurements of torque were sampled on an external computer with a sampling rate of 1,000 Hz and corrected for the influence of gravity (1). All measurements were filtered by a 4th-order zero-lag Butterworth low-pass filter (10 Hz cut-off frequency) and analyzed for peak torque. The isometric strength measurements in test 1 were obtained to serve as control comparisons; however, for ease of illustration, only tests 2 and 3 are depicted.

Whole body DEXA scan. Subjects were DEXA scanned (Hologic 4500A, Waltham, MA) before and after the training period (tests 2 and 3). The DEXA scan was conducted between 0800 and 1600 and ≥24 h after training sessions (to avoid any impact of changes in hydration). Whole body and regional lean body mass and fat mass were measured. The coefficient of variation (CV) for lean body mass is 0.5–1%. The results presented for whole body measurement do not include the head.

Statistics. Differences in means within or between groups were tested using paired and unpaired t-tests for strength and DEXA measurements and for training load and volume. Hormone measurements were analyzed by two-way ANOVA with repeated measurements. Statistical analyses were performed using StatView (SAS Institute, 1998). All data are presented as means ± SD, and the level of statistical significance was set at \( P \leq 0.05 \).

RESULTS

Pre values. No significant differences between the placebo group and the goserelin group regarding baseline values before the strength training period were observed in any of the variables measured.

Endogenous testosterone, free testosterone, and GH. The change in endogenous testosterone levels differed significantly between the groups (\( P < 0.01 \)). Thus serum testosterone decreased in the goserelin group from 22.6 ± 5.5 nmol/l measured in test 1 to 2.0 ± 0.5 and 1.1 ± 0.6 nmol/l measured in tests 2 and 3, respectively (\( P < 0.01 \)), whereas it remained constant in the placebo group throughout the intervention period (22.2 ± 4.5, 24.7 ± 5.3, and 22.0 ± 4.8 nmol/l in tests 1, 2, and 3, respectively; Fig. 2). Also, the change in endogenous free testosterone levels differed significantly between the groups (\( P < 0.01 \)), with a decrease in the goserelin group from 0.62 ± 0.11 to 0.05 ± 0.01 and 0.02 ± 0.01 nmol/l measured in tests 1, 2, and 3, respectively (\( P < 0.01 \)), whereas it remained unchanged in the placebo group (0.60 ± 0.10, 0.69 ± 0.17, and 0.57 ± 0.11 nmol/l in tests 1, 2, and 3, respectively; Fig. 2). Serum GH levels remained unchanged throughout the entire intervention period in the placebo and goserelin groups (data not shown).
Isometric strength. The change in isometric strength differed significantly between the groups (P < 0.05). Thus isometric strength increased significantly after 8 wk of strength training in the placebo group, from 240.2 ± 41.3 to 264.1 ± 35.3 Nm (P < 0.05), whereas the goserelin group showed no significant change (224.8 ± 44.4 and 230.0 ± 39.8 Nm; Fig. 3).

Relative isometric strength. The changes in relative isometric strength in the two groups trended toward differing significantly (P < 0.05). However, it increased significantly after 8 wk of strength training in the placebo group, from 2.9 ± 0.5 to 3.1 ± 0.5 Nm/kg (P < 0.05), whereas the goserelin group showed no changes (2.8 ± 0.5 and 2.8 ± 0.5 Nm/kg).

Lean mass of the legs. The increase in lean mass of the legs in the placebo group was significantly different from the goserelin group (P < 0.05). Thus lean mass of the legs increased in the goserelin group from 10.02 ± 1.21 to 10.39 ± 1.21 kg and in the placebo group from 10.37 ± 1.20 to 10.94 ± 1.29 kg (P < 0.05; Fig. 4).

Lean body mass. The changes in lean body mass (head not included) in the two groups showed a trend toward significant difference (P = 0.07). Lean body mass increased significantly within both groups, from 56.8 ± 5.7 to 58.1 ± 5.1 kg in the goserelin group and in the placebo group from 57.5 ± 5.7 to 59.8 ± 6.3 kg (both P < 0.05).

Fat mass and fat percentage. The changes in fat mass and fat percentage (head not included) differed significantly between the groups (P < 0.05). Thus fat mass increased significantly in the goserelin group, from 15.6 ± 8.4 to 17.0 ± 8.3 kg (P < 0.05), whereas the values for the placebo group decreased from 16.8 ± 5.7 to 16.2 ± 5.4 kg (P < 0.05). Fat percentage increased significantly from 20.0 ± 8.2 to 21.1 ± 7.8% (P < 0.05) and decreased significantly from 21.4 ± 5.5 to 20.2 ± 5.1% (P < 0.05) in the goserelin and placebo groups, respectively (Fig. 5).

Androgen and estrogen status. The serum levels of dihydrotestosterone and estradiol decreased in the goserelin group and were significantly different from those in the placebo group (P < 0.01). The level of androstenedione decreased significantly over time in the goserelin group (P < 0.01), whereas dehydroepiandrosterone sulfate and SHBG remained unchanged (Fig. 6). The above-mentioned variables remained unchanged in the placebo group.

DISCUSSION

To our knowledge, this is the first study on the effect of strength training during suppression of endogenous testosterone production. In accord with earlier studies, treatment with goserelin successfully suppressed both endogenous total and...
free testosterone in the treated group (7, 37, 47). Our study demonstrates that suppression of serum testosterone below 10% of normal levels strongly attenuates the increase in lean mass and muscle strength and increases fat mass during strength training.

In the placebo group, the magnitude of increments in isometric and relative isometric strength (2, 8, 38, 41) and the magnitude of increases in lean body mass (6, 17) were in agreement with previous studies. We also found that resting levels of testosterone and free testosterone were unchanged in the placebo group. Similar findings have been reported previously (3, 9, 26).

The placebo group adapted to the strength training period by significantly larger increases in both lean leg mass and isometric strength. Although those in the goserelin group were able to have the same progression in training load as those in the placebo group, they did not gain muscle mass or increased isometric strength in the laboratory test. It could be explained by a neural adaptation and improved coordination of the prime mover muscles relevant for the respective exercises, which were not transferable to the laboratory test. Nevertheless, these

Fig. 5. Fat percentage difference (posttraining minus pretraining). Values are means ± SD. *Significant increase (P < 0.05); #significant difference between groups (P < 0.05).

Fig. 6. Androgen and estrogen status. Values are means ± SD. *Significant time effect (P < 0.01); #significant difference from placebo (time and treatment effect, P < 0.01).
results demonstrate a direct link between endogenous testosterone and the adaptability to strength training. This is in concurrence with our earlier study where the effect of strength training and endogenously elevated anabolic hormone levels was investigated (21). In that study, subjects were divided into an arm-training-alone group and a leg-plus-arm-training group to increase circulating levels of anabolic hormones. The significantly larger relative increase in isometric arm strength found in the leg-plus-arm-training group compared with the arm-training-alone group was related to the larger hormonal responses of testosterone and GH in the former compared with the latter group. The hormonal response to strength training has already been studied for years (3, 11, 20, 25, 27, 31, 33, 36, 46, 57). Our results strongly support the current theory of interaction between endogenous testosterone and androgen receptors in the subsequent phase of recovery, stimulating protein synthesis, muscle hypertrophy, and strength.

We argue that the absence of endogenous testosterone explains the attenuated response to the strength training period seen in the goserelin group. In support of this, chronic testosterone deficiency in young men decreases protein synthesis (37). In addition, by inducing chronic changes in circulating testosterone concentrations by GnRH analogs and testosterone administration in five graded doses, a positive relationship was found between testosterone concentrations and fat-free mass, muscle size, strength, and power, and fat mass (7, 47). This is furthermore supported by the fact that chronic supraphysiologic concentrations of testosterone (e.g., exogenous testosterone supplementation) induce increases in muscle mass and strength compared with a placebo group, and further increases are seen when it is combined with strength training (6).

Changes in the neural system are important when one is trying to explain the increase in muscle strength seen in the present study. However, several studies have shown changes in muscle mass after 8 wk of strength training (6, 10, 17, 39, 44, 48, 53) and even after 4 wk of training (55). Still, the relative importance of neural changes and increased lean mass, respectively, for the observed increase in muscle strength is difficult to quantify. Thus both neural factors and factors related to increase in lean mass and transitions in the quality of the contractile proteins could be involved in the early phase adaptation to strength training. No measurements of electromyography were included in our study; thus we are unable to further address the possible impact of neural factors on isometric strength. However, the increase in lean mass was greater in the placebo group and coincided with a significant increase in isometric strength seen only in the placebo group, strongly suggesting that endogenous testosterone is important for gain in lean mass and muscle strength.

Androstenedione, dihydrotestosterone, and estradiol showed unchanged levels in the placebo group, similar to earlier observations (9, 26). On the other hand, androstenedione, dihydrotestosterone, and estradiol were significantly lowered in the goserelin group in response to treatment. Changes in androstenedione may to some extent explain the decrease in circulating testosterone concentrations (32).

Endogenous testosterone levels may affect adaptation to training by other mechanisms than the direct anabolic effect on protein synthesis and degradation. Testosterone regulates and influences many physiological mechanisms. Thus testosterone acts on neural tissue through different mechanisms, ranging from neurotransmitter synthesis and release to development and remodeling of synaptic circuitry (4). Moreover, changes in circulating steroid hormone levels may impair the sensitivity of the neuroendocrine system (4). However, the goserelin group showed the same progression during the training period, indicating that some kind of neural adaptation in fact occurred. In addition, it has been reported that testosterone indirectly stimulates secretion of GH and IGF-I when testosterone is converted to estradiol. Since GH and IGF-I may be important for the integrated endocrine system mediating muscle hypertrophy (23, 52), the low levels of estradiol in the goserelin group indicate that this pathway was reduced and may thereby reduce the magnitude of muscle hypertrophy following the strength training period. In addition, a possible direct effect of testosterone on GH and IGF-I release exists. Thus Mauers et al. (37) reported decreases in IGF-I mRNA concentration by inducing androgen deficiency in young men, and they argue that androgens are necessary for local IGF-I production. Similarly, Ferrando et al. (16) reported increased IGF-I protein expression following testosterone supplementation to old men. Furthermore, testosterone may act as an antiglucocorticoid to suppress protein degradation (e.g., blocking the effect of cortisol) and may be involved in the exercise-induced glycogen supercompensation (42, 56). In support of this, hypogonadism is reported to be associated with poor glucose utilization (58).

In the present study, strength training did not neutralize the side effect of hypogonadism regarding storage of fat, as fat mass increased significantly in the goserelin group whereas fat mass decreased in the placebo group. The level of endogenous testosterone in the goserelin group is comparable to that in severe hypogonadism, which is associated with increased body fat content (58). Medical treatments using GnRH analogs have comparable side effects, such as decreased fat oxidation and increased adiposity. Tests at baseline and after 10 wk of treatment (7.5 mg luproin injected 3 wk apart) revealed that suppression of endogenous testosterone in young men was associated with decreased fat oxidation, decreased resting energy expenditure, and increased adiposity (37). In another study, using GnRH analogs for a period of 48 wk for the treatment of prostate cancer (22.5 mg luproin injected 12 wk apart), similar results were reported (45).

Despite low levels of serum testosterone, the goserelin group demonstrated a significant increase in lean body mass, and two subjects showed extreme increases in lean body mass compared with their fellow subjects. The respective subjects gained 3.9 and 2.8 kg lean body mass compared with the mean gain of 1.3 ± 1.2 kg for the goserelin group. Several mechanisms may be responsible for this observation. First, the adrenal glands secrete ~10% of the total testosterone production and are not suppressed by GnRH analogs (13). The very low level of endogenous testosterone in the goserelin group, however, may still have an effect on the adaptation to strength training. Second, recent studies have revealed that several other mediators may be involved in the adaptation to strength training: the androgen receptors, IGF-I, IGF-I, androgen, myogenin, myoD, myostatin, etc. (18, 33, 43, 51, 54). These mechanisms may not be influenced by suppression of endogenous testosterone.

Finally, since strength training was performed simultaneously with the treatment with GnRH analogs, minimizing loss of muscle mass and muscle strength, the subjects in the
goserelin group did not go through an obligatory retraining period. As anticipated, hot flushes, fatigue, and decreased libido were seen in some of the subjects in the goserelin group; however, only one subject stopped therapy prematurely. Moreover, serum testosterone had returned to pretreatment levels in all subjects at the final testing 6 mo after the study.

In summary, the present study demonstrates that suppression of endogenous testosterone production attenuates the increase in lean mass, increases storage of fat, and abolishes the increase in muscle strength during strength training in normal young men. We conclude that endogenous testosterone is of paramount importance for the muscular adaptation to strength training.

ACKNOWLEDGMENTS

First of all, we thank the subjects who participated in the study. Second, we thank the laboratory technicians Gitte Scheel Klemmensen, Bente Tøt, Donna Arbuckle-Lund, Kirsten Westermann, and Anette Riis Madsen. We also thank Cuno Rasmussen, Prof. Per Aagaard, Anders Holsgaard Larsen, Emil Pedersen, and Jacob Soendergaard for their helpful cooperation during the study.

GRANTS

We thank Anti Doping Denmark and the Team Denmark Foundation for their financial support and Arla Foods for sponsoring the chocolate milk.

REFERENCES
