REGULATORY TOXICOLOGY

Ecdysteroids as non-conventional anabolic agent: performance enhancement by ecdysterone supplementation in humans

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Abstract

Recent studies suggest that the anabolic effect of ecdysterone, a naturally occurring steroid hormone claimed to enhance physical performance, is mediated by estrogen receptor (ER) binding. In comparison with the prohibited anabolic agents (e.g., metandienone and others), ecdysterone revealed to be even more effective in a recent study performed in rats. However, scientific studies in humans are very rarely accessible. Thus, our project aimed at investigating the effects of ecdysterone-containing products on human sport exercise. A 10-week intervention study of strength training of young men ($n = 46$) was carried out. Different doses of ecdysterone-containing supplements have been administered during the study to evaluate the performance-enhancing effect. Analysis of blood and urine samples for ecdysterone and potential biomarkers of performance enhancement has been conducted. To ensure the specificity of the effects measured, a comprehensive screening for prohibited performance-enhancing substances was also carried out. Furthermore, the administered supplement has been tested for the absence of anabolic steroid contaminations prior to administration. Significantly higher increases in muscle mass were observed in those participants that were dosed with ecdysterone. The same hypertrophic effects were also detected in vitro in C2C12 myotubes. Even more relevant with respect to sports performance, significantly more pronounced increases in one-repetition bench press performance were observed. No increase in biomarkers for liver or kidney toxicity was noticed. These data underline the effectivity of an ecdysterone supplementation with respect to sports performance. Our results strongly suggest the inclusion of ecdysterone in the list of prohibited substances and methods in sports in class S1.2 "other anabolic agents".

Keywords Sports performance · Doping · Ecdysterone · Spinach extract · Humans · Resistance training

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00204-019-02490-x) contains supplementary material, which is available to authorized users.

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Introduction

Ecdysteroids are widely marketed to athletes as dietary supplements advertising to increase strength and muscle mass during resistance training, to reduce fatigue and to ease recovery. Several studies have reported a wide range of pharmacological effects of ecdysteroids in mammals, most of them beneficial to the organism. The most active phytoecdysteroid, ecdysterone (a "Russian secret", chemical structure in Fig. 1), was already suspected to be used by Olympic athletes since the 1980s. At present, increasing numbers of dietary supplements containing ecdysteroids are marketed as "natural anabolic agents". Their advertisings promise to increase strength and muscle mass during resistance training, to reduce fatigue, and to ease recovery. Extensive investigations on the possible growth-promoting effects of ecdysterone in various animal species (rats, mice, Japanese quail and cattle) and a few in humans were reported (Bathori et al. 2008; Courtheyn et al. 2002; Dinan 2001, 2009; Dinan and Lafont 2006; Gorelick-Feldman et al. 2008; Haupt et al. 2012; Kumpun et al. 2011; Lafont and Dinan 2003; M McBride 2013; Parr et al. 2014; Slama and Kodkoua 1975; Slama et al. 1996; Tchoukouegno Ngueu 2013; Toth et al. 2008; Wilborn et al. 2006). Stimulation of protein synthesis was already reported in the 1960 (Arking and Shaaya 1969; Burdette and Coda 1963; Okui et al. 1968) and Bathori et al. (Bathori et al. 2008) reported its anabolic effect in humans.

Conversely to anabolic–androgenic steroids (AAS) that increase muscle mass mainly through their binding to androgen receptor (AR), no nuclear receptor that is homologous to the ecdysone nuclear receptor (EcR) found in insects has yet been described in mammals (Gorelick-Feldman et al. 2008). Ecdysterone has been characterized as devoid of binding ability to either AR, estrogen receptor (ER, where ERalpha was targeted), or glucocorticoid receptor (Bathori et al. 2008; Seidlova-Wuttke et al. 2010). However, only rec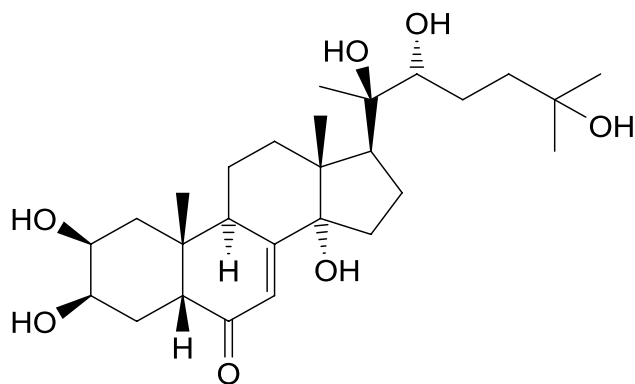ently, binding of ecdysterone to the ERbeta could be shown in vitro and in silico (Parr et al. 2013, 2014, 2015b). An effect even exceeding that of the AAS metandienone was found in vitro (Parr et al. 2015a) and Chermnykh et al. reported that ecdysterone showed an anabolic effect stronger than that of metandienone already without combination with training while metandienone, in contrast, showed no effect if not combined with training (Chermnykh et al. 1988). Doses higher than 5 μg/kg body weight (BW) were reported as active while lower doses did not result in anabolic activities (Bathori et al. 2008; Chermnykh et al. 1988; Wilborn et al. 2006). Even if there are lots of rumors on ecdysterone misuse by athletes, only few scientific studies are available to demonstrate its performance-enhancing effect. After 20 days of supplementation, Azizov reported a significant increase in running capacity of mice. In forced swimming tests, they reported that rats supplemented with ecdysterone were able to swim significantly longer than the control animals (Azizov and Seifulla 1998). An increased grip strength in rats was also reported and a phosphatidylinositol-3-phosphate kinase (PI3K)-mediated mechanism is discussed (Gorelick-Feldman et al. 2008).

In contrast to cell culture and animal studies, ecdysterone supplementation to improve performance has not yet been extensively investigated in humans. Apart from the working group around Wilborn (Wilborn et al. 2006), no detailed investigation in humans has yet been carried out. Therefore, in our study, we report on the evaluation of the effect of a long-term administration of an ecdysterone-containing dietary supplement with a special focus on the increase in performance during resistance training in humans.

Serum and urine samples were analyzed for endogenous hormones and liver enzymes. Furthermore, a complete anti-doping screening was carried out to exclude underlying effects from potential cross contaminations in the supplement.

Methods

Training investigation (in vivo)

Subjects

Forty-six voluntary healthy male subjects ($n = 46$) took part in the investigation successfully. Six subjects were noticed as drop out due to missing at more than ten percent of all training sessions in the study period or personal reasons.

At the beginning, they were 25.6 years (SD 3.7 years), 181.9 cm (SD 6.3 cm) tall and weighed 80.0 kg (SD 9.1 kg). All subjects provided a 1-year barbell training experience and ability to perform basic strength exercises as back squat, deadlift, and bench press. For inclusion

Fig. 1 Chemical structure of ecdysterone

in the study, all subjects were non-smokers, did not take any medication or other dietary supplements, and were injury-free for at least half a year.

This study was approved by the Ethics Committee of the German Sport University Cologne and carried out on the basis of the Helsinki agreement in double-blind design. All participants provided written informed consent prior to their participation. They were assigned to four different groups (matched according to performance and body composition): placebo group (PL, $n = 12$), ecdysterone1 group (Ec1, $n = 12$), ecdysterone2 group (Ec2, $n = 10$), and control group (CO, $n = 12$).

Supplements and dosage

As source of ecdysterone, the dietary supplement "Peak Ecdysone" (PeakPerformance Products SA, Roodt-sur-Syre, Luxemburg) was used. The product is labelled to contain 100 mg of ecdysterone from spinach extract plus 100 mg of leucine.

The volunteers out of the Ec1 group took two capsules of "Peak Ecdysone" per day as recommended on the label of the product. The Ec2 group took a high dosage of ecdysterone (eight capsules of "Peak Ecdysone" each day) over the entire intervention period. The PL group took two placebo capsules each day over the same period. The CO group took only two capsules of "Peak Ecdysone" without training. Each group took half of their nutritional supplementation dose in the morning after breakfast and the other half on training days immediately after training or on non-training days in the evening.

Training system

The PL, Ec1, and Ec2 participated in a 10-week resistance-training program with three training sessions a week and a two split training plan (details as supplemental material). Each training plan consists of six barbell exercises for the whole body. Every training day was followed by a resting day. In weeks 1–6, the subjects performed three sets of 12 repetitions for each exercise. After week 6, they performed only three sets with eight repetitions. Participants increased their training weight (2.5–5 kg) under the supervision of the supervisor every week, except in weeks four and seven (recovery weeks), starting with an intensity of 70% of their one-repeat ability (1-RM). The technically correct execution of the respective exercises was always in focus. If this could not be guaranteed, the weight was reduced or not increased according to plan. Before (t1) and after (t2), the training period medical examination as well as performance evaluations were carried out. Between the tests and the training intervention, there was a 72–96 h break. Endocrinological and compliance parameters (anti-doping screening) were also checked after 5 weeks (t_{half}). The complete examination procedure is shown in Fig. 2.

Outcome measures

Anthropometric parameters

In the pre- (t1) and post-test (t2) medical examination, anthropometric parameters as weight and height of the participants were determined. All subjects were requested to be sober to the decreases (12 h of no food intake). Body composition (fat-free mass, muscle mass, fat mass, and total body water) was measured by bio-electrical impedance analysis using Akern BIA 101 (Akern GmbH, Mainz, Germany).
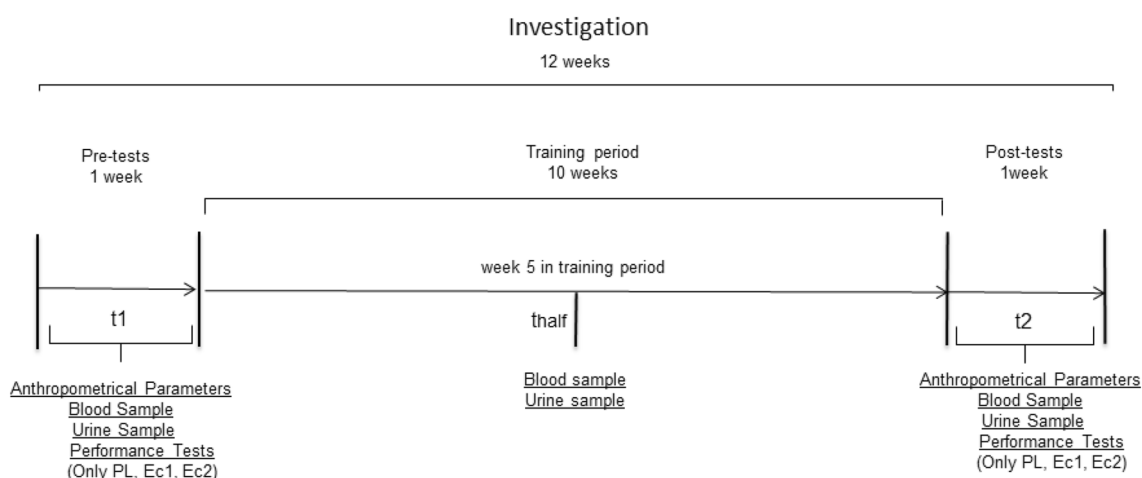
Fig. 2 Investigation design and procedure

Test parameters for performance

After the medical examination, a standardized breakfast [60 g of cornflakes, two bananas, and 300 mL of milk (1.5% fat)] was provided. Afterwards, performance tests were performed as described below. Tests for training effects were performed as counter movement jump (CMJ, power), one-repetition (1-RM) back squat (BS, lower body strength), and 1-RM bench press (BP, upper body strength). As measure for CMJ, the flight time was determined with an optojump photo cells (Microgate, Bozen, Italy). All subjects had three attempts with best result being recorded. For the strength measurements, four warm-up sets were performed (started with 50% of 1-RM and 10 repetitions followed by weight increase and number of repetitions reduction), followed by four maximum force tests. The weight was steadily increased after a successful test. If a load was not successfully mastered twice in a row, the test was terminated.

Blood and urine samples

Serum and urine samples were collected for further analyses. Blood serum concentrations of estradiol (E2), testosterone (Testo), luteinizing hormone (LH), insulin-like growth factor 1 (IGF1), and thyroxin (T4) were determined using specific immunoassays (ELISAs) at t1 and t2. In addition, serum concentrations of Testo, LH, and T4 were also determined at t_{half}.

To identify potential side effects, clinical parameters for liver and kidney toxicity were determined in the blood serum at t1 and t2. Analyzed parameters were creatinine, glutamate–oxaloacetate transaminase (GOT), glutamate–pyruvate transaminase (GPT), and gamma-glutamyl transferase (GGT). Analysis was performed in a laboratory specialized in clinical routine diagnostics (Labor Dr. Wisplinghoff, Cologne, Germany).

Urine samples were used for anti-doping screening to exclude intentional or unintentional co-administration of prohibited substances in sports. Furthermore, the urinary profile of endogenous steroids may also provide evidence on biological influences potentially induced by the administration of ecdysterone. The analyses were performed in accordance with the procedures used in the WADA-accredited Anti-Doping Laboratory of Rome, Italy (Laboratorio Anti-doping FMSI). According to the technical document, the "steroid profile" is composed of the following analytes (as free steroids content obtained from the unconjugated steroid fraction plus those released from the conjugated fraction after hydrolysis with β-glucuronidase from E. coli): androsterone (A), etiocholanolone (Etio), 5α-androstane-3α,17β-diol (5αAdiol), 5β-androstane-3α,17β-diol (5βAdiol), testosterone (Testo), and epitestosterone (EpiT) as well as ratios of specific steroid pairs, i.e., Testo/EpiT; A/Testo; A/Etio;

5αAdiol/5βAdiol; and 5αAdiol/EpiT. A detailed description of the analytical methods is given in Supplementary Materials.

Statistical analyses

The current version of Gpower (3.1.9.2, Universitaet Dusseldorf, Germany (Faul et al. 2009)) was used for expressiveness and power analysis to determine the sample size. The data collected were used to test for normal distribution using the Kolmogorov–Smirnov test. Subsequently, with a 2×4 Ankova and Bonferroni test time $\times$ group effects and with paired T test, the individual time and group effects were analyzed. The current version of SPSS (25.0, IBM Statistics, Armonk, NY, USA) was used. Significant differences are set at $p < 0.05$.

Cell culture investigation of supplement activity (in vitro)

For in vitro investigation of the supplement activity, a C2C12 cell line-based assay was used. The standard protocol for C2C12 cells was adhered to as in Zheng et al. (Zheng et al. 2018). This myoblast cell line derived from murine satellite cells has shown its potential as an in vitro model to study muscle hypertrophy. To obtain the test solution, 4.8 mg of the capsule content (according to the labelling 2.4 mg of ecdysterone and 2.4 mg of L-leucine) were dissolved in 10 mL of DMSO. This solution is diluted 1:1000 (v:v) prior to the assay.

After 48 h treatment of the C2C12-derived myotubes with solutions of the supplement, the diameters of the myotubes were determined. Reference solutions of dihydrotestosterone (DHT), estradiol (E2), and ecdysterone were used as controls. The test procedure was performed as three independent replicates.

Results

Anthropometrical parameters

After 10 weeks of treatment (training and/or supplement administration) anthropometrical, medical and performance parameters were evaluated.

The body weight of pre- and post-tests did not show significant differences within all groups (details provided as in Fig. 3, left and in Supplementary Materials). The participants from Ec1 and Ec2 increased their body weight significantly over 10 weeks (Ec1 = 2.58 kg (SD 1.90 kg); Ec2 = 3.11 kg (SD 1.51 kg), $p < 0.05$; *). There is also a significant difference in body weight change between Ec2 and CO (time $\times$ group effect, $p < 0.05$; #). In muscle mass (MM),
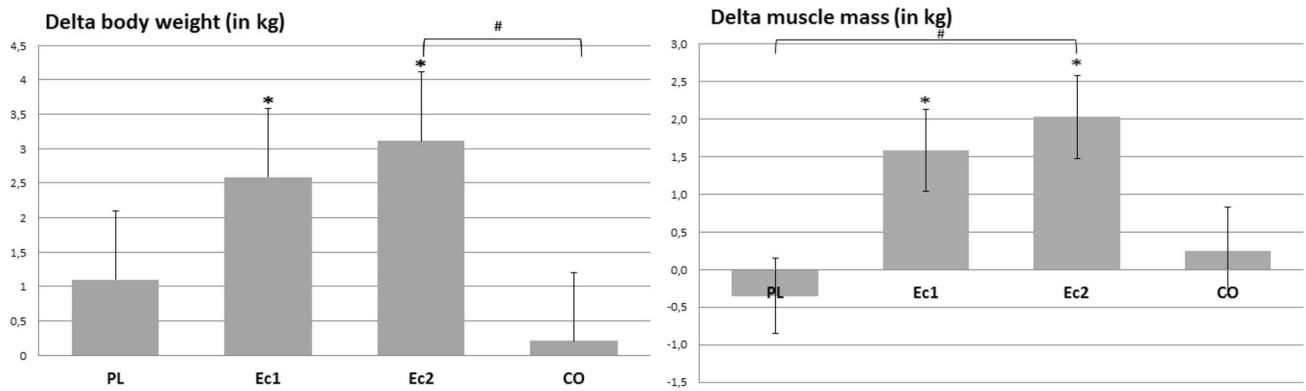
Fig. 3 Individual changes in body weight (in kg, left) and of muscle mass (in kg, right) at pre- and post-intervention test, * indicates time effect; # represents group × time effect (both $p < 0.05$)

a time effect could also be observed in Ec1 and Ec2 (Fig. 3, right, $p < 0.05$; *). In change of MM, there was a significant difference between the PL and Ec2 (time × group effect, $p < 0.05$; #). The Ec2 group increased MM more than 2 kg [2.03 kg (SD 1.76 kg)], while the PL reduced the MM in average 0.35 kg (SD 1.73 kg). The Ec1 group also increased their MM (in average 1.58 kg (SD 1.88 kg)), but without significant difference ($p = 0.115$) to PL. The CO has only a slight change in the MM of 0.25 kg (SD 2.02 kg), with no significant difference to a training group (Fig. 3 right).

In fat mass (FM) and total body water (TBW), there was no significant difference in pre- and post-tests within the groups and no time × group effect could be observed.

Power and strength performance

Power performance: counter movement jump (CMJ)

After 10 weeks of nonspecific bounce training, all three training groups increase their jump height [PL: 1.94 cm (SD 1.74 cm); Ec1: 2.01 cm (SD 1.99 cm); Ec2: 2.39 cm (SD

1.62 cm), details are provided as Supplementary Material]. There are time effects in all three training groups. However, there is no significant difference in time × group effects in CMJ.

Strength performance: 1-RM back squat and bench press

All three training groups increased their 1-RM back squat. The PL group improved their squat from 107.5 kg (SD 15.34 kg) to 124.17 kg (SD 13.59 kg), improvement: 16.67 kg (SD 4.49 kg), 15.5%. Ec1 had an increase of 18.50 kg (SD 6.16 kg) from 104.17 kg (SD 18.58 kg) to 122.71 kg (SD 17.90 kg) (improvement 17.75%) and Ec2 from 100.50 kg (SD 11.06 kg) to 120.00 kg (SD 13.23 kg) (improvement 19.50 kg, 19.4%). However, no significant difference between the groups and no time × group effect was observed (Fig. 4 left). In the second strength performance test parameter, the 1-RM BP, it was observed that all three training groups increased their performance significantly. The PL group had an increase of 3.33 kg (SD
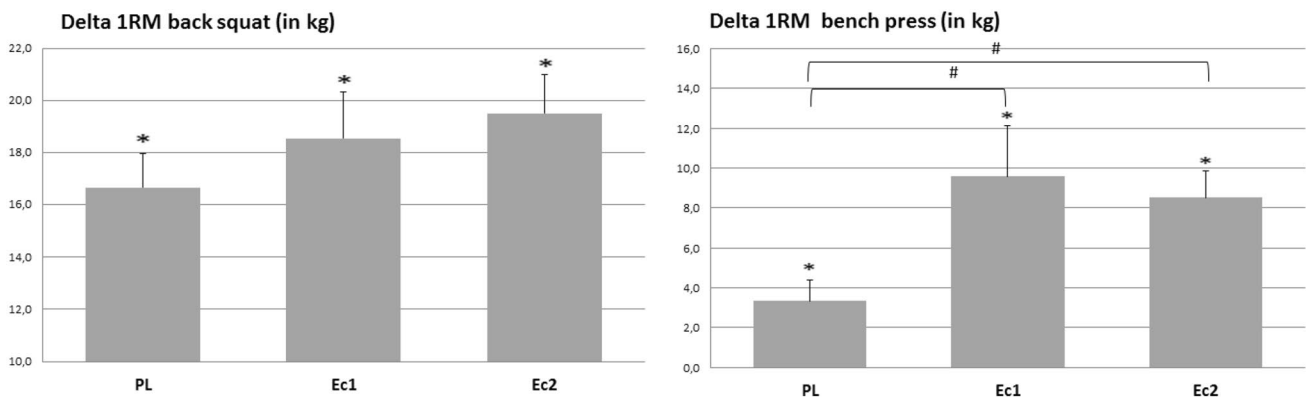
Fig. 4 Results of strength tests performed pre- and post-intervention, 1-RM back squat (left), 1-RM bench press (right), * indicates time effect; # represents group × time effect (both $p < 0.05$)

3.74 kg) (relative 3.59%) from 92.71 kg (SD 13.46 kg) to 96.04 kg (SD 11.15 kg). In contrast, both supplement groups increased their 1-RM bench press more than 8 kg. In Ec1 an increase from 82.92 kg (SD 15.73 kg) to 92.50 kg (SD 13.73 kg), improvement 9.58 kg (SD 2.79 kg), 11.5%) and in Ec2 from 88.75 kg (SD 13.08 kg) to 97.25 kg (SD 10.30 kg), improvement: 8.50 kg (SD 4.44 kg), 9.5%) was observed (Fig. 4 right).

Supplement analyses

The extraction procedure for ecdysterone resulted in an amount of 6 mg ecdysterone per capsule for this product. In addition, the supplement was checked for the absence of contamination with other performance-enhancing drugs. No contamination of these products with substances that are prohibited in sports was found (Supplementary Material).

Serum sample analyses

Serum concentration of ecdysterone in blood sample

Serum concentrations of ecdysterone were determined in the groups and results are displayed in detail in Fig. 5. As expected, concentrations increased with time. Furthermore, dose-dependent values were detected, i.e., the highest ecdysterone concentration was obtained in Ec2 group, where volunteers took the highest dose (8 capsules) of supplement. Baseline values of ecdysterone (concentrations close or even below LOQ) were seen prior to administration (t1) and in the PL group.

Endocrine hormone analysis

To investigate potential effects of ecdysterone, training and combinations thereof on the endocrine system blood serum concentrations of E2, Testo, LH, IGF1, and T4 were determined at t1 and at t2. In addition, Testo, LH, and T4 serum concentrations were also determined at t_{half}.

The average change of the respective serum hormone concentrations between t1 and t2, normalized for individual serum concentrations of the participants at t1, is shown in Fig. 6.

No changes in serum Testo and LH were seen. However, in IGF1 serum concentrations, a significantly different pattern of change compared to the placebo group (time effect, $p < 0.05$; * and time $\times$ group effect $p < 0.05$; #) was observed. While training resulted in a decrease of IGF1 for the placebo group at t_{half}, treatment with ecdysterone could antagonize this. In E2 each group decreased its concentration, but only in Ec1, a time effect was observed ($p < 0.05$; *).

The decrease of T4 concentrations induced by training was the same in the ecdysterone groups compared to the placebo group (time effect, $p < 0.05$; *). The comparison with the concentrations determined at t_{half} confirmed this. Five-week uptake of ecdysterone resulted in a significant change in the T4 serum concentrations compared to the control group ($p < 0.05$; #) (Supplementary Material). After 10 weeks, there was no significant difference between any group any more.

Testo and LH serum concentrations were also determined at t_{half} (Supplementary Material). In Testo, each group decreased their concentrations. In PL, Ec2, and CO, a time effect is also observed ($p < 0.05$; *) Despite significant differences in various endocrinological parameters at different times, no change can be attributed exclusively to ecdysterone supplementation.

Biomarker analysis for side effects

Serum concentrations of the biomarkers of liver and kidney toxicity [creatinine, glutamate–oxaloacetate transaminase (GOT), glutamate–pyruvate transaminase (GPT), and

Fig. 5 Serum concentrations of ecdysterone ([ng/mL of serum], grouped boxpots) in the different treatment groups at t1, thalf, and t2, one outlier (Co group) eliminated
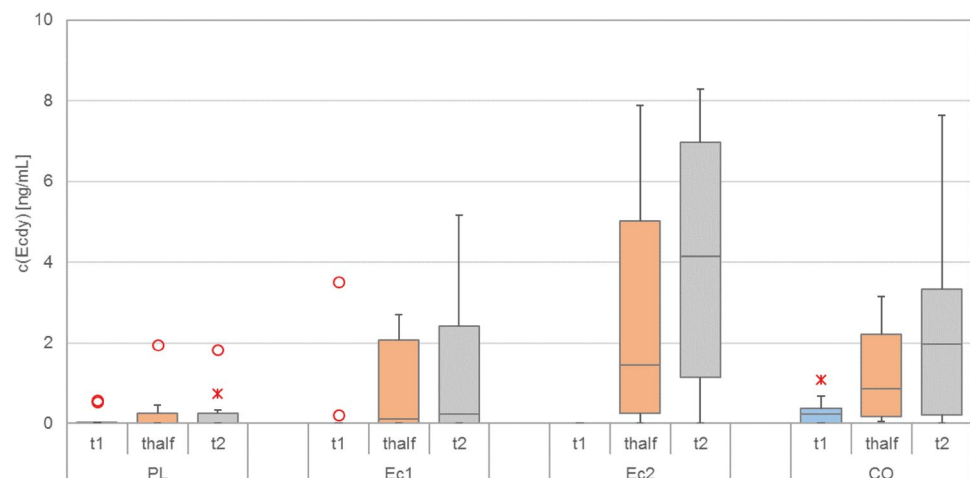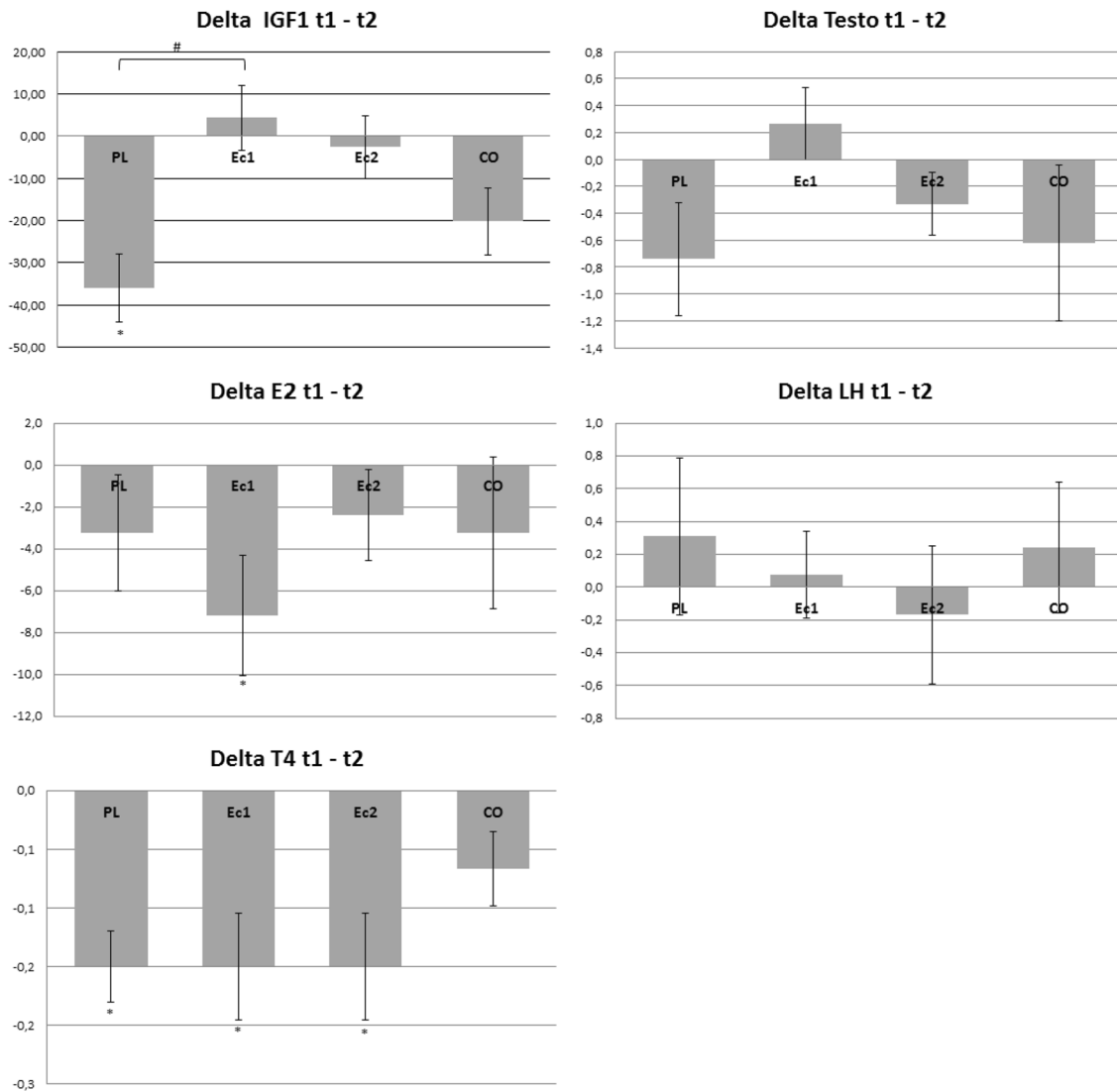
Fig. 6 Average change of the respective hormone serum concentrations between T1 and T3, setting the individual serum concentrations of the participants, normalized for individual serum concentrations of the participants at T1. * Indicates time effect; # represents group × time effect (both $p < 0.05$)

gamma-glutamyl transferase (GGT)] did not change significantly over the 10-week intervention period for all groups.

Urine sample analyses

No hint on significant alteration of the steroid profile after administration was detected. No other performance-enhancing drugs or significant alterations in the urinary steroid profile were detected in anti-doping screening.

In vitro investigation of the supplement

C2C12 cells, a myoblast cell line derived from murine satellite cells, have been used as an in vitro model to study muscle hypertrophy through ecdysterone. After 48 h of treatment, the diameters of myotubes were determined. The results are compared with different controls (Supplementary Material). The results show an anabolic activity of the supplement extract (Ecdy cap) in vitro. Increased diameters of C2C12 derived myotubes were detected, which were significantly different from the control (time*group effect, $p < 0.05$; #). Hypertrophy was found similar to that obtained by dihydrotestosterone (DHT), estrogen (E2), or pure ecdysterone reference (Ecdy lab). There are no differences within the treatment with the different hormones.

Discussion

Our data showed positive effects of ecdysterone on anthropometric (BW and MM) and performance parameters (1-RM BP). These results could be confirmed by the in vitro study. In addition, a dose-dependent effect could also be observed in various parameters (BW, MM, and serum concentration of ecdysterone). Furthermore, side effects that are explicitly attributable to ecdysterone supplementation could not be demonstrated. Negative effects on creatinine, GOT, GPT, or GGT could not be observed.

Similarly, no significant alteration of urinary steroid hormones was detected. This suggests that the anabolic effect of ecdysterone is based on a mechanism that is different than that of testosterone, DHT, and synthetic AAS. Furthermore, this is also in line with no pseudo-endogenous steroid administration, either as non-compliance to the study protocol or due to a cross contamination of the supplement.

Anthropometric and performance parameter

The positive effect of ecdysterone administration and training on body weight (BW) and muscle mass (MM) could be clearly shown (Fig. 3). In both parameters, a positive time effect was generated in Ec1 and Ec2. In addition, a dose-dependent effect could be observed. Ec2 (high dose) additionally showed significant time × group differences to CO and PL. Similar positive effects on body weight and muscle hypertrophy have been demonstrated in 600 mg testosterone administration (Bhasin et al. 1996). Although the effects are not as strong as with testosterone supplementation (in human), significant differences between PL, Ec1, Ec2, and CO can be detected. These results are corroborated by the in vitro study (hypertrophy in C2C12 myotubes). The anabolic effects of the Ecdy cap are similar to the hypertrophic effects of DHT, E2, and Ecdy lab (Supplementary Material). Similar anabolic effects in vitro have been reported earlier by different groups (Parr et al. 2014, 2015a; Zheng et al. 2018). In addition, the positive effect of ecdysterone in various animal experiments was also demonstrated earlier as well (Bathori et al. 2008; Courtheyn et al. 2002; Dinan 2001, 2009; Dinan and Lafont 2006; Gorelick-Feldman et al. 2008; Parr et al. Parr et al. 2014, 2015a). As a result, it can be concluded that ecdysterone has a positive anabolic effect on muscle growth in humans, similar to cell culture and animal studies.

In performance, all three training groups increased their power (CMJ) and strength (1-RM BS, 1-RM BP) performance significantly. In CMJ, the improvement of the training groups was most likely due to motoric learning. Thus, the increased leg strength may have caused the increase in performance (Wisløff et al. 2004). However, on average, the jump performance is not fully developed, so that neural adaptations are more likely to improve the jump performance. For the training intervention, a linear periodization model was used, which systematically increases the weight to maximize strength. In 1-RM BS, the improvement in each group is similar to the investigation of Schoenfeld et al. (Schoenfeld et al. 2015) and Joao et al. (Joao et al. 2014), who also used a linear periodization model. This means that the conceptual training design achieves similar results as the previous studies. Compared to the strength development during testosterone supplementation and strength training (Bhasin et al. 1996), there was no significant difference between the placebo and the two ecdysterone groups. However, there is a tendency for an increase in performance by the supplementation of ecdysterone as well as a dose-dependent effect (Fig. 4 left). This can be confirmed by the enhancement of the average training weights in the squat of the individual training groups (Supplementary Material). In upper body strength development, significant differences between the groups were observed. Both ecdysterone groups increased their performance significantly compared to the placebo group (Fig. 4 right). These results are in contrast with Wilborn (Wilborn et al. 2006), who found no differences between ecdysterone and the placebo group. The different observations can be attributed to different training systems as well as ecdysterone concentrations. Wilborn et al. did not use a systematic linear training model, but a wave-shaped training model with individual increase and a different ecdysterone concentration. Both factors play a decisive role, which can lead to different observations. In this study, it can be clearly recognized that ecdysterone has a positive effect on upper body performance.

Supplement analyses

The quantification of ecdysterone in the supplements revealed an amount of 6 mg per capsule, which is considerably lower than the amount labelled on the bottle (i.e. 100 mg per capsule). Thus, a daily dose of 12 mg of ecdysterone, i.e. 0.15 mg/kg BW in an 80-kg volunteer, was administered in Ec1 and CO, while the high-dose group (Ec2) received a daily dose of 48 mg of ecdysterone or 0.6 mg/kg BW in an 80-kg volunteer. In agreement to this, a dose-dependent increase of ecdysterone serum concentrations after ingestion (Fig. 5) and bioactivity of the supplement extract in the in vitro assay were detected (Supplementary Material). The label of the supplement also indicated leucine as ingredient (100 mg/capsule). Effects of leucine administration on skeletal muscle performance have been reported. However, in these studies, the daily uptake

of leucine was in the range of 80 mg/kg bodyweight, which would mean 6.4 g in a 80 kg person (Borack and Volpi 2016; Gnanou et al. 2006). Therefore, we can exclude that the small daily dose of leucine provided via the capsules may have any relevance for the observed physiological effects in this study.

Serum parameters

Serum concentration of ecdysterone

The determination of ecdysterone in blood serum resulted in increasing concentrations in all supplementation groups (Ec1, Ec2, and CO) during the study. A clear dose-dependent increase in ecdysterone is detected; thus, concentrations in Ec2 group (administration of 8 capsules) were considerably higher than in Ec1 and CO group (both administration of 2 capsules). Variances in concentrations in all groups most likely result from different individual pharmacokinetic parameters and slightly different time intervals, since last supplement administration, even if all volunteers administered the last capsule in the morning when also sample collection took place. Detectable baseline concentrations of ecdysterone (most of them close to the lower limit of quantification) at t1 as well as in t_{half} and t2 of the placebo group (PL) most likely resulted from regular diet.

Endocrine hormone parameters

During the 10-week intervention study, various hormonal changes were observed. However, no change can be exclusively related to the supplementation of ecdysterone (Fig. 6 and Supplementary Material). Possible tendencies and positive effects on IGF1 could possibly be explained by intensive training. Intensive training may negatively affect IGF1, which could be counteracted by supplementation with ecdysterone (Fig. 6). However, further investigations will be needed to confirm this assumption.

Furthermore, an influence of the intensive training on T4 could be determined. There are no differences between the three training groups (PL, Ec1, and Ec2).

For Testo, E2, and LH, no explicit change by supplementation and/or training was observed. This corroborates the assumption that ecdysterone has no direct influence on expression of Testo, E2, or LH. In summary, further investigations are, therefore, necessary to obtain more detailed information on the influence of ecdysterone on hormone expression.

Conclusion

This project demonstrates the performance-enhancing effect of ecdysterone in humans. Thus, our results strongly suggest including ecdysterone in class S1 "Anabolic Agents". As it is

reported in the literature, the mechanism of action of ecdysterone appears as independent from the androgen receptor activation, but it is rather exhibited by the activation of the estrogen receptor beta. However, further investigations on the activity of ecdysterone are recommended. They should also include a controlled administration trial of ecdysterone in humans to elucidate the metabolism of ecdysterone and to evaluate possibilities for its improved detection in doping control analyses.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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