Changes in Body Composition of Old Rats at Different Time Points after Dexamethasone Administration

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Abstract: Background: Aging leads to changes in skeletal muscle quantity and quality and is accompanied with increase in body mass and fat mass, whereas fat-free mass either decreases or remains unchanged. The body composition of rodents has been an important factor for clinical trials in the laboratory. Glucocorticoids such as dexamethasone are widely used in clinical medicine, but may induce myopathy, characterized by muscle weakness, atrophy, and fatigue. In animals treated with glucocorticoids, a dose-dependent reduction of body weight has been observed. This weight loss is usually followed by muscle atrophy and a reduction of several muscle proteins, contributing to impaired muscle function. This study was designed to describe changes in body composition and BMC of 22-month-old rats during 10- and 20-day recovery period after 10-day dexamethasone administration.

Method: Data on body mass, lean body mass, fat mass and bone mineral content of the rats were obtained with dual energy X-ray absorptiometry scan.

Result: Significant reduction in body mass, lean body mass, fat mass and fast-twitch muscle mass was observed after dexamethasone treatment. Body mass, fat mass and fast-twitch muscle mass stayed decreased during 20 days after terminating the hormone administration; lean body mass reached the preadministration level after 20-day recovery period. There were no significant changes in bone mineral density during the recovery period. Dexamethasone treatment gradually reduced hindlimb grip strength that also stayed decreased during the 20-day recovery period.

Conclusion: This study demonstrated that a 10-day period of overexposure to glucocorticoids induced longlasting changes in old rats’ body composition and these values did not attain the baseline level even after 20-day recovery period.

Keywords: Old rats, dexamethasone administration, body composition, skeletal muscle, recovery, glucocorticoids.

1. INTRODUCTION

Aging leads to changes in skeletal muscle quantity and quality and is accompanied with increase in body mass and fat mass, whereas fat-free mass either decreases or stays unchanged [1, 2]. Aging rats have many similarities with humans, like selective loss of Fast-Twitch (FT) muscle fibers and the decline in the number of motoneurons [3, 4]. The body composition of rodents has been an important factor for clinical trials in the laboratory. Among aging population increased lipid consumption and sedentary lifestyle are linked with metabolic pathologies [5].

Lipids are stored mainly in abdomen, liver and hips. Abdominal obesity is linked with diabetes mellitus and cardiovascular diseases [6, 7].

Glucocorticoids are cost-effective drugs that exert strong anti-inflammatory and immunosuppressive effect.

The number of elderly patients treated with glucocorticoids is higher and the range of clinical applications is more extensive than for many other treatments. Glucocorticoids have adverse side effects on long-term use and high doses. Among these side effects, glucocorticoids-induced myopathy is a serious complication for patients, and 60% of patients with glucocorticoids-induced myopathy develop muscle weakness of proximal skeletal muscles that are severe enough to interfere with the activities of daily living [8].

Muscle weakness develops during 4-5 days after glucocorticoids administration as a result of atrophy of FT muscle fibers [9, 10]. Rats with glucocorticoid-caused myopathy have been observed to lose both muscle strength [11] and fatigable force, and fatigued at a higher rate because of the reduced force of glycolytic muscle fibers [12]. Glucocorticoids also decrease bone mass and the effect is indirect or systemic on bone tissue remodelling [13, 14]. In bone tissue, glucocorticoids reduce the number and function of osteoblasts, resulting in a decreased osteoblastogenesis [15] and increase osteoclastic bone resorption [16]. It has
been shown that short-term treatment of postmenopausal (ovariectomized) rats with dexamethasone results in increased bone catabolism and decreased bone anabolism associated with misallocation of bone matrix proteins [17]. Dexamethasone treatment increased the aging muscle wasting more than in adults because of faster loss of myofibrillar protein [18, 11].

Recovery of skeletal muscle in aged rodents took twice as long as in young adults since glucocorticoids decrease the stimulatory effect of insulin and Insulin-like Growth Factor-1 (IGF-1) in the skeletal muscle of old rats [19]. The Mechano Growth Factor’s (MGF) participation in aging-associated sarcopenia has also been shown [20]. In FT muscle fibers glucocorticoids cause breakdown of thick and thin filaments and disintegration of myofibrils more intensively than in Slow-Twitch (ST) fibers [21, 22]. Skeletal muscle regeneration is faster in fibers with higher oxidative capacity [2]. Unfortunately, it is not known how old skeletal muscle is recovering after terminating the glucocorticoids administration. Dual-energy X-ray Absorptiometry (DXA) has been shown to be a non-invasive, non-traumatic method, expedient for measuring body composition in humans [23] and small laboratory animals [24]. DXA allows measures of body compositional longitudinal changes, relevant in human biology.

The present study was undertaken to assess the catabolic effect of dexamethasone on the body composition and the disappearance of the catabolic effect after 10 days from interruption of dexamethasone treatment.

We hypothesised that recovery from the catabolic state in most of the tissues takes twice more time than the hormone administration period.

2. METHODS

2.1. Animals

18 female rats, aged 22 months were used. The animals were bred at the local animal house, using Wistar rats parents (Harlan Laboratories, the Netherlands). Rats were housed in groups of four in standard transparent polypropylene cages under controlled 12 h:12 h light-dark cycle and temperature (21°C), with free access to tap water and food pellets (diet R70, Lactamin, Sweden). The experiments were conducted in accordance with EU legislation (directive 2010/63/EU) and the experimental protocol was approved by the Animal Experimentation Committee at the Estonian Ministry of Agriculture.

2.2. Experimental Groups

The animals were randomly distributed into four groups: control group (C, n = 5); dexamethasone group (DEX, n = 5) - daily injection of dexamethasone for ten consecutive days; recovery group (REC 10, n = 4) - ten days of dexamethasone treatment, followed by a period of 10 days without dexamethasone treatment; recovery group (REC 20, n = 4) - ten days of dexamethasone treatment, followed by a period of 20 days without dexamethasone treatment. The body mass of rats was measured daily during the experimental period.

2.3. Dexamethasone Treatment

Dexamethasone (Dexafort 3mg/ml; International B.V. Boxmeer, the Netherlands) was diluted to 200µg/ml with 0.15 M NaCl and administered intraperitoneally daily for 10 days, 50µg/100g body mass (bm). In REC 10 and REC 20 groups dexamethasone was administered for 10 days, and maintained without administration of the drug for a further 10 and 20 consecutive days before being sacrificed.

2.4. Total Body Composition by DXA

Total body composition was measured before (baseline) and after 10-day dexamethasone treatment, after 10- and 20-day recovery using a Dual energy X-ray Absorptiometry (DXA) (Hologic Discovery W, Bedford, USA) equipped with small animal software. Rats were anaesthetized before measurements. Anesthesia consisted in intra-peritoneal injection of the solution of ketamine (60 mg/1 kg bm) (Bioketan Vetoquinol Biowert Sp. Z o.o.) and xylazine (9 mg/1 kg bm) (Xylapan Vetoquinol Biowert Sp. Z o.o.). A specially designed small animal step phantom was scanned daily to calibrate the body composition results. Rats were placed in a prone position on the DXA platform and scanned. The whole body scan field was adjustable to a maximum of 32.9 cm (L) * 28.1 cm (W). The whole body measurement of rat required 4 minutes and 11 seconds and provided global and regional body composition results. High-resolution measurement of rat required 4 minutes and 5 seconds and provided lumbar spine bone mineral content (BMC, g) and bone mineral density (BMD, g/cm²). Scans were analyzed with Hologic APEX Version 3.3.0.1 analysis software, and values for fat mass (g), lean body mass (g), bone mineral content (BMC, g), bone mineral density (BMD, g/cm²), and body mass (g) were recorded.

2.5. Hindlimb Grip Strength

Grip strength of hind limb was tested using a grip strength meter for rodents (Grip Strength Meter 0167-004L, Columbus Instruments, US). Hind limb grip strength was measured before and after 10 days of dexamethasone treatment, 10 days and 20 days of recovery. The maximum grip force (strength in N) was included in the statistical analysis.

2.6. Muscle Mass

After the experimental procedures, all animals were euthanized by excess of anesthesia. Entire ST (soleus), FT (plantaris and gastrocnemius) muscles were surgically removed, trimmed clean of visible fat and connective tissue, weighed and immediately frozen.

2.7. Statistical Analysis

Data were expressed as mean ± Standard Error (SE). Differences between the means were tested for statistical significance with one-way Analysis of Variance (ANOVA) and Student’s t-test. Comparison of data before and after the study period was done using the paired Student’s t-test. Differences were considered significant at P < 0.05.
3. RESULTS

3.1. Body Composition

At the beginning of the experimental protocol, all groups exhibited similar baseline whole body composition parameters as demonstrated in Table 1.

Table 1. Body composition parameters (mean ± SE) before dexamethasone treatment of rats in control group (C), dexamethasone-treated group (DEX) and recovery (REC 10 and REC 20) groups. Data are mean ± SE mean.

| Group      | Body Mass g | Fat Mass g | Lean Body Mass g | BMC g |
|------------|-------------|------------|------------------|-------|
| C (n=5)    | 363.50 ± 13.46 | 138.38 ± 13.02 | 225.12 ± 4.49 | 14.66 ± 0.64 |
| DEX (n=5)  | 348.28 ± 10.51 | 136.82 ± 6.71  | 197.00 ± 7.82 | 14.46 ± 0.24 |
| REC 10 (n=4)| 365.20 ± 19.28 | 140.40 ± 15.91 | 209.47 ± 4.36 | 15.33 ± 0.50 |
| REC 20 (n=4)| 354.23 ± 18.20 | 138.40 ± 17.64 | 201.47 ± 4.90 | 14.33 ± 0.37 |

Fig. (1). Representative images of old rats obtained from a Dual energy X-ray Absorptiometry (DXA). BASELINE - before dexamethasone treatment; DEX - 10 days of dexamethasone treatment; REC 20 - 20 days of dexamethasone treatment, followed by a period of 20 days without dexamethasone treatment.

Fig. (2a) illustrates bm changes in animals during the 10-day period of dexamethasone (50 ug/100 g bm) administration and 10 and 20 days after discontinuation of the hormone administration. All rats started the experimental protocol with similar bm (348.28 ± 10.51 g, 365.20 ± 19.28 g, 354.23 ± 18.20 g for DEX, REC 10 and REC 20, respectively). After 10 days of dexamethasone administration, the animals showed 24% lower bm (P < 0.001) compared with the baseline values. After 20 days since the removal of dexamethasone treatment, the rats exhibited a partial recovery of bm, obtaining 11.5% increment in bm (P < 0.05) (Fig. 2a).

The lean bm decreased from 197.00 ± 7.82 g to 177.6 ± 5.4 g (P < 0.01) after 10 days of hormone administration, stayed decreased also during 10-day recovery period (179.18 ± 4.08 g, P < 0.001) after terminating hormone administration and reached preadministration level after 20 days of recovery (Fig. 2b).

Both the absolute (g) and relative (%) amounts of fat were significantly lower after dexamethasone treatment. Fat mass decreased 45% (from 136.82 ± 6.71 g to 74.64 ± 6.02 g, P < 0.001), the relative mounts of fat from 39.28 ± 1.41% to 28.04 ± 1.51%, P < 0.001) after 10 days dexamethasone administration, no alteration was noted in fat mass after 10 and 20 days since the removal of dexamethasone treatment, compared with baseline values (Fig. 2c).

BMD and BMC are the common parameters of bone quantity and quality. Significantly lower BMC (P < 0.01) was observed in DEX group compared to the baseline value. Across time points of recovery BMC stayed decreased (Fig. 3). No changes emerged in the BMD after dexamethasone administration and during the recovery period.

3.2. Muscle Mass

ST soleus muscle mass remained unchanged throughout 10 days of dexamethasone treatment (112.7 ± 3.88 mg vs 110.50 ± 5.09 mg), showed the tendency to decrease after 10-day recovery period (105.5 ± 4.84) and muscle mass reached to the control group level after 20 days of recovery (113.00 ± 3.27 mg).
Changes in body mass (REC 20) during the experimental period. Data are presented as mean ± SE. ** P < 0.01 in comparison with the control group.

Gastrocnemius and plantaris muscle mass were significantly smaller after hormone administration in comparison with the control group and did not recover during 20 days after discontinuation of hormone injections.

3.3 Hindlimb Grip Strength

To assess the changes of muscle strength after dexamethasone treatment and recovery period, hindlimb grip strength was measured using a grip strength meter. Measurement of grip strength of each group is shown in Fig. (4).

The grip strength of the control group was the same throughout the experimental period. Dexamethasone treatment gradually reduced grip strength 25% (from 7.43 ± 0.22 N to 5.60 ± 0.21 N, P < 0.001). Grip strength stayed
decreased also during 10- and 20-day recovery period (5.30 ± 0.27 N, P < 0.001 and 6.57 ± 0.61 N, respectively) after terminating hormone administration.

4. DISCUSSION
This study demonstrated that a 10-day period of overexposure to glucocorticoids induced longlasting changes in old rats’ body composition and these values did not attain the baseline level even after 20-day recovery period. The lean bm decreased after hormone treatment, however, after 20-day recovery period, it reached the pretreatment level. It seems to be misleading to use lean bm to describe changes in skeletal muscle mass. Body fat mass decreased after dexamethasone administration and stayed on the same level during 20-day recovery period. This may be the result of an imbalance in lipogenesis and lipolysis, with enhanced lipolysis supported by neuroendocrine activation and tumor-related lipolytic factors [25].

Aging is accompanied with general weakness, impaired locomotion and decrease of strength [18]. Glucocorticoid treatment has been found to reduce muscle strength in young and old rats [26] as a result of atrophy of FT muscles [10]. The present study shows that in old rats ST muscle mass has a tendency to decrease after hormone treatment. The catabolic action of both aging and glucocorticoids depend on the activity of muscles [27]. Soleus muscle remained unchanged throughout 10 days of dexamethasone treatment, which is in agreement with previous publications showing that glucocorticoids have different catabolic effects among slow and fast fiber type muscles [28-30]. It is known that ST muscle fibers are involved in static and slow long lasting movements - e.g. concerning everyday motor activity - and this may explain why there is no significant catabolic action of glucocorticoids and atrophy of ST muscles in aged rats [27]. In our study, FT muscle mass decreased after dexamethasone administration and did not attain the pretreatment level after 20- day recovery (after 20 days since the interruption of dexamethasone treatment). Atrophy of FT muscles is the result of inhibition of Insulin-like Growth Factor-I (IGF-1) [31] and upregulation of two genes myostatin and glutamate synthase [32]. Increase of muscle activity increases the synthesis rate of myofibrillar proteins [33] and via mammalian target of rapamycin activating proteins within the nitrogen-activated proteinkinase signaling [34].

BMD and BMC are the common parameters of bone quantity and quality. The BMC decreased after 10 days of dexamethasone administration and stayed on the same level after 20-day recovery.

It has been shown earlier that glucocorticoids decrease bone mass by indirect or systemic way through bone tissue remodelling [13, 14]. Excess glucocorticoids reduce osteoblast function, resulting in decreased osteoblastogenesis [15] and increased osteoclastic bone resorption [16]. In comparison with young rats, the effect of glucocorticoids seems to be stronger in old rats [26].

CONCLUSION
The bm, lean bm, muscle grip strength, fat mass and the bone mineral content decreased significantly after 10 days of glucocorticoids administration. After 20 days since the discontinuation of dexamethasone treatment, the rats exhibited a partial recovery of bm, lean bm, and fat mass. There were no changes in bone mineral density after dexamethasone administration and during the recovery period. FT muscle mass decreased significantly after hormone administration and did not reach the pretreatment level during 20 days after terminating hormone injections. ST skeletal muscle has a tendency to decrease in elderly rats and mammals, this shows that old skeletal muscle is more sensitive to the catabolic effect of glucocorticoids. These data support the view that almost all the alterations in body composition induced by dexamethasone in the elderly are reverted after discontinuation of the treatment. This information is important, considering the frequent use of glucocorticoids in humans and animals.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
The experimental protocol was approved by the Animal Experimentation Committee at the Estonian Ministry of Agriculture, Netherlands.

HUMAN AND ANIMAL RIGHTS
No humans were used in this study. All the research procedures conducted on animals in this study were in accordance with the guidelines of the European Communities Council Directive (2010/63/EU), Netherlands.

CONSENT FOR PUBLICATION
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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