

Structural Alterations of Skeletal Muscle Induced by Chronic Administration of D-Amphetamine and Food Restriction

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Abstract

Amphetamines are frequently used as anorexigenic drugs to facilitate fasting and weight loss, although some side effects are known. The objective of the study was to study the effects of fasting induced by amphetamine administration as compared to food restriction alone on skeletal muscle. Twelve male Wistar rats were assigned to three groups: One group (AMPH) received d-amphetamine sulphate during 14 days (20 mg/Kg/day, s.c.); group FRES was the pair-fed, food restricted control receiving each day the same quantity of food consumed by the AMPH group the day before; a control group (CONT) was fed *ad libitum*. Food intake and body weights were controlled daily. After 14 days the animals were sacrificed and the soleus muscles were removed for light and electron microscopical evaluation and muscle fiber morphometry. Towards the end of the experimental period, food intake returned to normal in the AMPH group due to amphetamine tolerance. The AMPH and FRES animals experienced a comparable weight loss of about 15%. The muscles fibers of the AMPH and FRES group showed a slightly (n.s.) higher incidence of central nuclei than the CONT group. Their muscle fibers atrophied by about 25%. Signs of degeneration and regeneration were observed at the ultrastructural level in both experimental groups. It is concluded that the deleterious effects on skeletal muscle are rather the common result of the alimentary restriction than the particular effect of amphetamine.

Key words: amphetamine, food-restriction, skeletal muscle damage, muscle atrophy, rats.

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Anorexigenic drugs, like amphetamines, are often used as aids to reduce voluntary food intake [14, 27, 28], despite of their side effects [10, 25]. Amphetamine administration induces disturbances in the hormonal balance [2], in the metabolism, and in the function of many organs and tissues [6]. In humans, several toxic side effects were reported in the literature, namely hyperthermia, disseminated intravascular coagulation, and acute renal failure [3, 25]. These effects may be related to extensive muscle damage being manifested as rhabdomyolysis and clinically diagnosed by indirect signs [8]. In fact, the presence of numerous markers for muscle damage in blood and in urine [12, 24] was frequently described in laboratory animals and in humans after the administration of amphetamine.

Although the origin of amphetamine related muscle damage is not completely understood, the restriction of caloric intake induced by this drug may contribute to tissue catabolism [9, 18, 22], since drastic hormonal and metabolic alterations can be induced by acute food restriction [7, 17, 19]. For instance, an increased rate of nitrogen excretion [11] and high rate of net release of amino acids from skeletal muscle [20] were described after short-term fasting. A reduced growth rate of muscle and a smaller cross sectional area of fibers were also described [16, 22].

Since the concept of muscle damage induced by amphetamine is so far only supported by indirect evidences, the aim of this investigation was to study the structural alterations in skeletal muscle after repeated administration of amphetamine as compared to food restriction.

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Materials and Methods

Twelve male Wistar rats (160-180 g) were housed in individual cages and acclimatized during 7 days under standard laboratory conditions ($22\pm1^{\circ}\text{C}$; 12 h light / 12 dark) following the institutional guidelines for the care and use of laboratory animals. The animals were assigned to three groups: one group (AMPH, $n = 4$) received d-amphetamine sulphate during 14 days (20 mg/Kg/day, s.c.) and was allowed to ingest food *ad libitum*. Another group was the pair-fed control, in reality food restricted, group (FRES, $n = 4$), receiving each day the same quantity of food like consumed by the AMPH group the previous day. The third group was the control that was allowed to ingest food *ad libitum* (CONT, $n = 4$). All groups had free access to water. The FRES and CONT group received daily s.c. injections of 0.9% saline. All groups received the same injection quantities (1 ml/Kg b.w.) once every morning. Body weights and food intake were monitored in all groups each day. After 14 days, the animals were sacrificed by cervical dislocation and their soleus muscles were dissected for further analysis.

Ten to twelve 1 mm cubic pieces of muscle were immediately transferred to 2.5% glutaraldehyde in cacodylate buffer to ensure rapid fixation. Using routine methods, the samples were further fixated with 2% osmiumtetroxide, dehydrated in graded alcohol, and embedded in Epon 812. Semithin sections were stained with methylene blue and were examined histologically under a light microscope, especially for the incidence of central nuclei. Moreover, the cross sectional areas of at least 400 fibers per muscle were morphometrically evaluated at a magnification of $\times 250$ using a MOP-Videoplan (Zeiss, Germany). The absolute data were converted into percentages, thereby setting the values of CONT muscles as 100%. Ultrathin sections stained with uranyl acetate and lead citrate were examined qualitatively in a Jeol 100 CXII electron microscope.

The data were expressed as means with standard deviations. The significance of differences between the groups at each time interval was tested using the one-way ANOVA for repeated measures and post hoc analyzed using the Scheffe's test, with an $\alpha = 0.05$ as level of significance.

Results

Food intake decreased markedly in the AMPH group starting with the first day of drug administration (Fig. 1). The FRES group ingested the same quantity of food like the AMPH group, with one day of delay due to the feeding protocol. Some tendency of recovery in food ingestion was observed around the fifth day, but food intake was still significantly lower in the AMPH and FRES groups after 10 days as compared to controls. Afterwards, food intake increased to control quantities. The loss of body weight (Fig. 2) was similar in the AMPH and FRES groups and reflected the food intake. After a progressive weight loss up to roughly 15% over 8 days, body weight remained stable. The differences in body weight were significant in both experimental

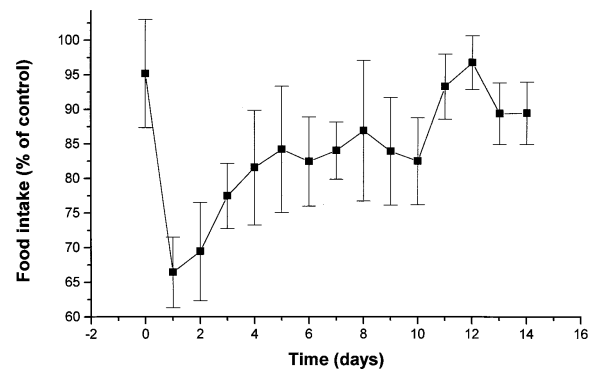


Figure 1. Food intake of the AMPH group as related to controls (= 100%).

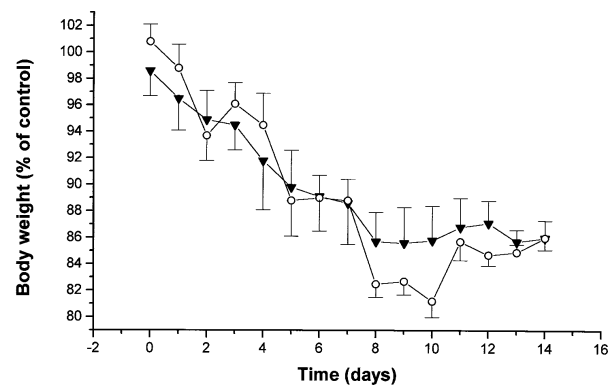


Figure 2. Relative body weight of the AMPH (circles) and FRES (triangles) groups as related to controls (= 100%).

Table 1. Mean values and standard deviation of fiber area (expressed as % of the area of CONT group) and fibers with central nuclei.

Groups	Fiber area (% of CONT)	Central nuclei (% of fibers)
CONT	100.0 $\pm$ 31.2	1.9 $\pm$ 1.8
AMPH	72.1 $\pm$ 33.0*	4.1 $\pm$ 2.1
FRES	75.3 $\pm$ 24.4*	3.5 $\pm$ 2.4

(*p < 0.05 vs. CONT)

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groups compared to CONT from the second day throughout the experimental period; body weight did not differ between the AMPH and FRES groups.

The main results of muscle morphometry are shown in table 1. Fibers with central nuclei were found with an incidence of $4.1 \pm 2.1\%$ in the AMPH group and of $3.5 \pm 2.4\%$ in the FRES group, respectively, without statistical significance from the $1.9 \pm 1.8\%$ observed in the CONT group. Muscle fiber areas decreased significantly by 28 and 25% in AMPH and FRES, respectively, hence both experimental groups revealed a similar degree of atrophy as compared to the CONT group.

As to muscle histology at the light microscopic level, no severely damaged fibers were detected in the three groups. A general enlargement of the interstitial space with some local leukocyte infiltration was observed in the AMPH and FRES groups. The ultrastructural appearance of the CONT muscles was normal and no signs of pathological alterations were observed. In contrast, the examination of the muscles of both, the AMPH and FRES groups revealed signs of degeneration and regeneration to a similar extent (Fig 3). The interstitial space was enlarged, filled with collagen fibers and containing activated fibroblasts. A conspicuous number of polymorphonuclear cells were also found in the interstitium. The muscle fiber sarcolemma often appeared scalloped with pseudopodia-like processes protruding into the interstitium. In many fibers, residual bodies were found but not many lysosomes. The satellite cells, located adjacent to the muscle fibers and contained in a common basement membrane, appeared prominent and sometimes activated as judged by signs of protein synthesis. Single and fusing myoblasts were also observed. Apart from some slight vacuolization, no conspicuous signs of intrafiber edema were encountered, and the mitochondrial structure appeared normal. The capillary endothelium showed a normal structure.

Discussion

As a result of amphetamine administration, food intake was immediately reduced in the AMPH group. This had been expected due to the anorexigenic effect of the drug [14]. This effect, however, ceased with prolonged administration, which has to be interpreted as a tolerance to amphetamine [13, 26]. So the food intake had returned to CONT ingestion at 11 days. Therefore the fourteenth day had been chosen to terminate the experiments, when food intake appeared stable over three days. Since the FRES group had been fed the same amounts like group AMPH (with a one-day delay), both groups virtually experienced the same food restriction. Accordingly, their relative body weight (as compared to CONT) decreased in the same magnitude, which rather has to be attributed to food restriction / less food intake than to a direct effect of amphetamine administration.

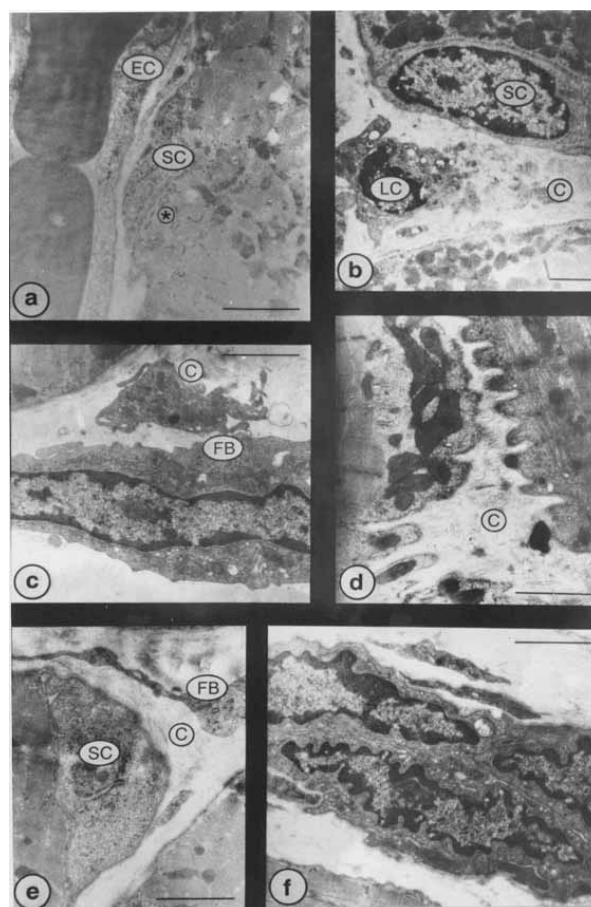


Figure 3. Electron micrographs of soleus muscle of AMPH (a, b, f) and FRES (c, d, e) groups, bar represents 5 μ m. **a:** Activated satellite cell (SC) with abundant rough endoplasmic reticulum also in the adjacent muscle fibre (asterisk), EC = endothelial cell; **b:** A leukocyte (LC) in the interstitium filled with collagen (C), SC = satellite cell; **c:** Activated fibroblast (FB) embedded in collagen (C); **d:** Two muscle fibers with scalloped sarcolemma, C = collagen; **e:** Activated satellite cell (SC) and fibroblast (FB) embedded in collagen (C); **f:** Three fusing myoblasts.

The stabilization in body weight during the last experimental days reflects the normalized food intake.

Amphetamine is known to increase body temperature as well as cortisol and ACTH concentrations [2, 6] which per se would lead to a catabolic situation. Comparing, however, the quantitative data (body weight, muscle atrophy) of both experimental groups, these effects are assumed to stand back behind the direct effects of less food intake (AMPH) and food restriction (FRES), respectively. Food restriction, on the other hand, leads to an increase of cortisol concentration [15] and to some decrease of insulin-like growth factor I and of the density of its muscle receptors [4].

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The quantitative data on muscle fibers speak in favor of muscle wasting, as substantiated by marked muscle atrophy in both experimental groups. A muscle fiber atrophy of about 25% is reflected by a loss of body weight of about 15%. The total muscle mass, however, should be reduced less, taking into account the observed augmentation of interstitial connective tissue. Hence, food restriction does not only lead to a loss in muscle mass, but also to some substitution of functional muscle tissue by connective tissue. It therefore has to be emphasized that food restriction severely affects skeletal muscle and not exclusively adipose tissue as desired by individuals fasting excessively.

Central nuclei as found more frequently in the muscle fibers of both experimental groups (although without significant difference to controls) are a typical sign of necrosis and regeneration [21]. Qualitative electron microscopy supported this concept of degeneration and regeneration, and such was observed to a comparable extent in both experimental groups. Some of the structural characteristics can be attributed to degeneration, like a scalloped sarcolemma as a typical sign of breakdown of the cytoskeletal filaments in fibers undergoing atrophy [1] and the presence of polymorphonuclear cells acting as scavengers of cell debris [5]. The presence of residual bodies as remnants of lysosomes indicates an autophagic process [23]. The fact, however, that only residual bodies have been encountered and no „active“ lysosomes were found should suggest that the muscle fibers were already recovering and most of the degeneration had been taken place earlier.

Several signs of an increased protein synthesis and of regeneration support this suggestion, like the presence of activated satellite cells and fusing myoblasts that eventually will form new muscle fibers. Such regeneration was also described in other types of atrophy [1] and in regenerating denervated muscles [21]. The apparent dominance of regenerative processes, although taking place in an atrophied and structurally affected muscle, speaks in favor of immediate recovery from food restriction that in fact has not been present anymore during the last days of the experimental period.

Although the quantitative data and structural findings showed virtually the same result in both, the AMPH and FRES groups at the end of the experimental period suggesting the same etiology based on a reduced food intake, it can be assumed that the time course of the pathophysiological processes may be different.

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