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Anabolic steroid and gender-dependent modulation of cytosolic HSP70s in fast- and slow-twitch skeletal muscle

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Abstract

Besides their clinical uses, anabolic steroids (AASs) are self-administered by athletes to improve muscle mass and sports performance. The biological basis for their presumed effectiveness at suprapharmacological doses, however, remains uncertain. Since the expression of high levels of some stress proteins (HSPs) has been associated with an increased tolerance to stress and chronic exercise up-regulates HSP72 in skeletal muscle, this investigation was aimed at testing whether the administration of suprapharmacological doses of AASs, either alone or in conjunction with chronic exercise, induced changes in HSP72. Nandrolone decanoate (ND), an estrene derivative, but not stanozolol (ST), a derivative of the androstane series, up-regulated the levels of HSP72 and changed the proportions of various charge variants of the cytosolic HSP70s in sedentary and exercise-trained rats, exclusively in fast-twitch fibres. Since the expression of HSP73-levels in skeletal muscle was dependent on gender but not on muscle type, and that of HSP72-levels was muscle type specific but gender-independent, ND effects on cytosolic HSP70s could not be explained solely by a functional relationship with sex steroids. The reported results indicate that, by up-regulating the expression levels of HSP72 in fast-twitch fibres, nandrolone decanoate could contribute to improving the tolerance of skeletal muscle to high-intensity training.

Keywords: Stress proteins; Skeletal muscle; Anabolic steroids; Nandrolone decanoate; Stanozolol; Exercise; Sexual dimorphism; Wistar rats

1. Introduction

The cellular heat shock or stress response is a universal mechanism that helps to preserve cells from stress and to protect them from repeated environmental challenges (for a review see [1,2]). It is characterized by the rapid, transcriptionally regulated induction of the synthesis of a group of proteins known as heat shock or stress proteins (HSPs). The most highly induced proteins of the cellular stress response in mammalian cells are those of the70 kDa family, a group of closely related proteins distributed in different cell compartments. HSP72 and HSP73 have been located within the cytoplasm and, following stress, in the nucleus. HSP73 is constitutively expressed and is considered to be only slightly inducible by stress, while HSP72 is usually synthesized in response to stress [3]. GRP78, located in the lumen of the endoplasmic reticulum, binds various proteins crossing through this compartment [4]. GRP75 is found within the mitochondria, where it is involved in the import of mitochondrial precursor proteins and

Abbreviations: AAS(s), anabolic or anabolic-androgenic steroid(s); HSPs, heat shock or stress proteins; HSP70s, various or all components of the 70 kDa family of stress proteins, as indicated; HSP72 and HSP73, cytosolic heat shock proteins of 70 kDa, stress-inducible and constitutively expressed, also known as HSP70 and HSC70, respectively; ND, nandrolone decanoate; PTPTR, progressive training programme of treadmill running; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; ST, stanozolol; 1D- and 2D-electrophoresis, one and two-dimensional electrophoresis, respectively.

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functionally interacts with the mitochondrial chaperonin HSP60 and other HSPs to facilitate protein folding and oligomerization of protein complexes [5]. It has been reported that the levels of both mitochondrial stress proteins are expressed constitutively in proportion to mitochondrial content [6].

Exercise activates the stress response in skeletal muscle fibres and other tissues (for a recent review see [7]). In skeletal muscle the rates of synthesis of HSP72, GRP78, GRP75 and HSP60 increased [8], indicating that exercise stress acted on heat shock inducible proteins and on other stress proteins less sensitive to increased temperatures but affected by glucose deprivation and other treatments that perturb N-linked glycosylation of nascent polypeptides [4,9]. Conditions such as hyperthermia [10], oxidative stress [11], hypoxia [12,13], glucose deprivation [9] and other types of metabolic stress [13,14], known to induce the stress response in different types of cells, may also occur in skeletal muscle fibres and other tissues during physical exertion.

Exercise also triggers acute and chronic adaptations of the endocrine system and modulates the output and serum levels of various hormones, including sex steroids [15–17]. Testosterone and several synthetic derivatives of sexual steroids, collectively called anabolic or anabolic-androgenic steroids (AASs) [18], are known to stimulate muscle protein synthesis and enlargement (for a comprehensive review see [19]). In addition to their therapeutic uses, AASs have been used for decades in the sport context aimed at increasing muscle size and strength and sports performance [20–22]. More recently, AAS abuse has extended to endurance-based sports under the assumption that they may also help to increase aerobic capacity [21] and to improve tolerance to high-intensity training. Currently, high amounts of AASs are still consumed in the sport context despite the fact that conclusive evidence supporting AAS effects on the skeletal muscle of eugonadal males is lacking and that the biological basis for their presumed effectiveness remains uncertain.

Since the expression of high levels of some HSPs has been associated with an increased protection of cells to withstand otherwise lethal injuries, and chronic exercise up-regulates the levels of HSP72 in skeletal muscle [23,24], this investigation was aimed at testing whether the administration of AASs, either alone or in conjunction with chronic exercise, involved changes in the expression levels of the cytosolic HSP70s. Two commonly used AASs were used, nandrolone decanoate (ND, an estrene derivative) and stanozolol (ST, an androstane derivative), both at a suprapharmacological dose of 10 mg/kg per week. Because of the structural relationship of AASs to sex steroids, the gender dependence of the expression levels of the cytosolic HSP70s in skeletal muscle was also investigated.

2. Materials and methods

2.1. Experimental animals

Male (effect of AAS administration on HSP expression levels) or male and female (gender-dependent expression of HSPs) Wistar rats served as experimental animals. The animals were bred and housed in the animal facilities of the Centre of Molecular Biology, at the Autonomous University of Madrid. They stayed in a temperature (22–24°C) and humidity (50–60%) controlled environment, with a 12 h photoperiod, and were provided with a standard laboratory diet and water ad libitum. Three groups of exercise-trained animals (C, non-treated; ND, nandrolone decanoate; ST, stanozolol) and the corresponding sedentary groups, made up the experimental subjects for the study of the effects of AASs in skeletal muscle HSP levels ($n = 4$ or 5 per group). Five male and five female rats served to study the gender dependence of the expression levels of stress proteins in skeletal muscle. All interventions were made following the recommendations included in the 'Guide for Care and Use of Laboratory Animals' (US Department of Health and Human Services, NIH) and European laws and regulations on the protection of animals.

2.2. Exercise-training programme and treatments

Before starting the experiments all rats were pretrained at a lower speed (10–20 m/min) 10–20 min/day for 5 days to make them familiar with treadmill running. They were then randomly distributed into the different experimental groups. Rats were exercise-trained on a motor-driven treadmill (Li 8706, Letica Scientific Instruments, Barcelona, Spain). We used a progressive training programme of treadmill running of 3-month duration (PTPTR) consisting of sessions in which the speed (from 20 to 30 m/min) and duration (from 30 to 85 min) gradually increased throughout, with a moderate increase in the slope (4 or 8%) during the last seven training weeks [24]. Although the animals adhering to the exercise-training regime usually completed their exercise-training sessions, exercise was occasionally interrupted on an individual basis when an animal was fatigued (i.e. upon refusal to run despite the mild electric shock). Both anabolic steroids, nandrolone decanoate (Deca-Durabolin, Organon Española, Barcelona, Spain) and stanozolol (Winstrol Depot, Zambon SA, Barcelona, Spain) were injected weekly into the m. gluteus medius at 10 mg/kg, alternating right and left hind limbs every week. Treatments started in the second training month and were prolonged until finishing the programme, the last injection taking place 4 days before sacrificing the animals. Untreated controls received injections of the same amount of vehicle

in the same location and with the same periodicity. The animals were weighed every training day. Sedentary animals were also weighed and handled at the same time as exercise-trained animals performed their training sessions. Following the appropriate interventions, three days after the last training session to discard immediate effects of acute exercise, the animals were weighed and anaesthetized and the soleus and extensor digitorum longus (EDL) muscles quickly dissected in vivo. The muscles were frozen in liquid N_2 and stored a −70°C until used.

2.3. Quantification of stress proteins by immunoblotting after one- or two-dimensional electrophoresis

Whole muscle protein homogenates were prepared in a buffer solution containing 50% glycerol as previously reported [8]. Stress proteins were separated from whole muscle homogenates by one (1D) or two-dimensional (2D) electrophoresis. Slab-gels (1D) were loaded either with 80 or 30 μg of protein per slot (depending on the abundance of the stress protein to be tested) and the electrophoretic separation performed by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE), using 12% acrylamide gels polymerized in the presence of 15% glycerol [8]. 2D-electrophoresis was performed using a pH-range of 4–6.5 in the first, isoelectric focusing dimension [8]. Protein sample preparation (using acetone precipitation) and running conditions were as previously reported [8]. Proteins separated in 1D- or 2D-gels were transferred electrophoretically (Bio-Rad Trans-Blot Cell) to nitrocellulose membranes (0.2 μm, Schleicher and Schuell), stained with Ponceau S to visually control protein transfer and to mark the position of reference proteins, and blocked with 5% non-fat dried milk in Tris buffered saline, washed and processed for specific recognition of heat shock proteins using monoclonal antibodies. The peroxidase-conjugated secondary antibody was detected by enhanced chemiluminescence (ECL, Amersham). Quantification was performed in the linear absorption range using a Laser densitometer (Molecular Dynamics, Image Quant Software v.3.0).

2.4. Monoclonal antibodies

The following antibodies were used to specifically recognise the indicated antigens: Anti-HSP70 monoclonal (C92F3A-5, StressGen SPA-810, at a 1:250 dilution), anti-HSC70 rat monoclonal (1B5, StressGen SPA-815; dilution 1:1000), anti-(HSC70 and HSP70) monoclonal (N27F3-4, StressGen SPA-820, dilution 1:1500) or anti-Heat shock protein 70 (HSP70) monoclonal (clone BRM-22, Sigma, dilution 1:3000), to recognise the cytosolic HSP70s either individually or together. All antibodies were tested for immunoreactivity and specificity in 1D- and 2D-immunoblots as reported previously [8]. Peroxidase-conjugated polyclonal goat anti-mouse IgG antibody (Transduction Labs.) or rabbit anti-rat immunoglobulins (Dako) were used as the secondary antibodies, always at a 1:1500 dilution.

2.5. Statistical analysis

Data were expressed as means ± SD of four or five animals per group. The statistical analysis was performed by multiple regression (C1, exercised; C2, ND-treated; C3, ST-treated; C4, exercised and treated with ND; C5, exercised and treated with ST) using the computer programme MicroTSP (version 6). Gender-dependent differences in the expression levels of HSPs were analysed statistically using Student's t-test. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Changes induced by anabolic steroids and chronic exercise in various physiological parameters indicative of anabolic steroid and exercise-training effects

Table 1 presents the effects of administering a suprapharmacological dose of the anabolic steroids ND or ST to sedentary or chronically exercised rats on body weight gain (referred to initial body weight), and on the wet weight and muscle-somatic indices (wet weight referred to body weight) of the soleus and EDL muscles of sedentary and exercise-trained rats. Adherence to the progressive programme of treadmill running, reduced body weight gain in both, non-treated and AAS-treated animals ($P < 0.01$), as compared with the corresponding sedentary controls. ND-treatment reduced body weight gain of sedentary ($P < 0.05$) and exercise-trained ($P < 0.01$) rats, indicating that nandrolone decanoate exerts additional effects that add to those of exercise. Exercise training did not modify the wet weights of either muscle, but increased the muscle-somatic indices as a consequence of the reduced body weights. ND, but not ST, reduced soleus wet weight in sedentary animals ($P < 0.05$) without changing the muscle-somatic index. In the EDL muscle, neither wet weight nor muscle-somatic index changed as a consequence of AAS administration. AAS-treated animals showed more consistent increases in muscle-somatic indices after exercise-training than untreated controls. ND, but not ST, induced a marked increase of the reno-somatic index (kidney-to-body weight ratio, $P < 0.01$) in both, sedentary and exercise-trained animals and of the cardio-somatic index ($P < 0.05$) (not shown). After completing the training programme the levels of the β-F1-ATPase of the inner mitochondrial membrane

increased in the soleus muscle, evidence of a metabolic adaptation to training (not shown).

3.2. Effects of AAS administration on the expression levels and isoform distribution of the cytosolic HSP70s in skeletal muscle of sedentary and exercise-trained rats

Fig. 1 summarises the data corresponding to the effects of administering a suprapharmacological dose (10 mg/kg/week) of ND or ST to sedentary or exercise-trained animals on the expression levels of the highly inducible stress protein HSP72 in soleus and EDL muscles. In sedentary animals, ND administration up-regulated the expression levels of HSP72 in the fast-twitch EDL muscle, but not in the soleus. In exercise-trained rats, ND showed opposite statistically significant effects on HSP72 levels in both muscle types.

Table 1
Effect of separate treatments with a suprapharmacological dose (10 mg/kg/week) of the anabolic-androgenic steroids nandrolone decanoate (ND) or stanozolol (ST), either alone or in conjunction with a training programme of treadmill running on body weight gain, muscle mass and muscle-somatic index of two skeletal muscles of the hind limb differing in fiber type composition

	Sedentary			Exercised[a]		
	Control	ND	ST	Control	ND	ST
Relative body weight gain[b]	1.87 ± 0.36	1.55 ± 0.17*	1.88 ± 0.14	$1.55 \pm 0.08^{\cdot\cdot}$	$1.26 \pm 0.12^{**,\cdot\cdot}$	$1.57 \pm 0.12^{\cdot\cdot}$
M. Soleus[c]						
Mass (mg)	249 ± 30	212 ± 17*	262 ± 24	245 ± 25	223 ± 32	243 ± 13
m-s index	0.51 ± 0.05	0.48 ± 0.03	0.53 ± 0.07	$0.56 \pm 0.06^{\cdot}$	$0.56 \pm 0.06^{\cdot\cdot}$	0.55 ± 0.03
M. EDL[c]						
Mass (mg)	221 ± 13	208 ± 12	214 ± 19	220 ± 19	209 ± 6	226 ± 23
m-s index	0.45 ± 0.04	0.47 ± 0.03	0.43 ± 0.04	$0.5 \pm 0.04^{\cdot}$	$0.52 \pm 0.03^{\cdot\cdot}$	$0.51 \pm 0.04^{\cdot\cdot}$

[a] Rats were exercised by adhering to an PTPTR of 3-month duration, as specified in Section 2.
[b] Data (means $\pm$ SD, $n = 4$ or 5 rats) represent the differences between body weights immediately before sacrifice and at the start of the experiment, referred to initial body weights.
[c] Data are means $\pm$ SD ($n = 8$ or 10 individual muscles) of the absolute wet weights (mg) or referred to body weight (expressed in g, muscle-somatic index). $^{*}P < 0.05$, $^{**}P < 0.01$ treated versus untreated; $^{\cdot}P < 0{,}05$, $^{\cdot\cdot}P < 0.01$, exercised versus sedentary.

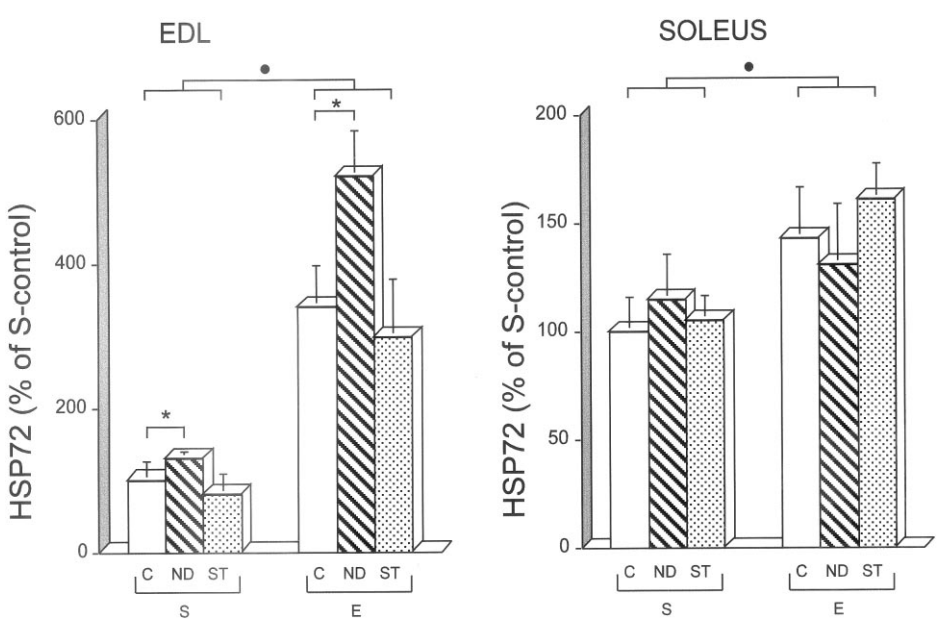

Fig. 1. Effect of the anabolic steroids nandrolone decanoate (ND) or stanozolol (ST) on the expression levels of the highly inducible cytosolic stress protein HSP72 in soleus and EDL muscles of sedentary and exercise-trained rats. Sedentary (S) or exercise-trained (E) Wistar rats, were treated with a suprapharmacological dose (i.m. injection of 10 mg per kg per week, for the last 2 months) of the anabolic steroids nandrolone decanoate (ND) or stanozolol (ST) or only with the oil vehicle (C). Soleus and EDL muscles were obtained in vivo from anaesthetized animals three days after the last training session, immediately frozen in liquid N_2 and maintained at -70°C until used. The relative amounts of HSP72 were obtained from immunoblots of total muscle proteins separated by SDS-PAGE using a monoclonal antibody that specifically recognized the inducible protein without crossreacting with the constitutive cytosolic member of HSP70s. The data represent means $\pm$ SD of four or five animals per group. $^{\cdot}P < 0.05$, exercise-trained versus non-trained animals, $^{*}P < 0.05$, anabolic steroid-treated versus non-treated animals.

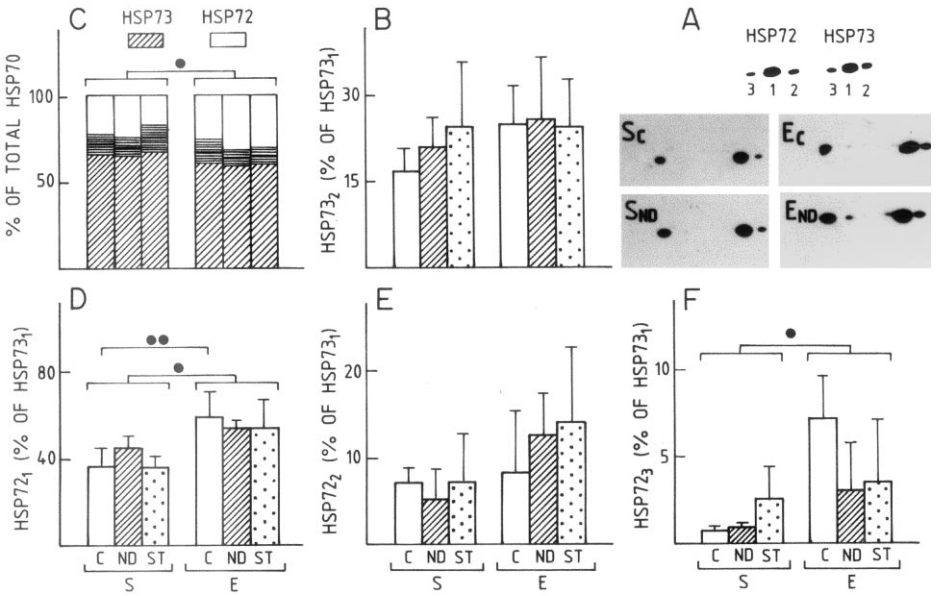

Fig. 2. Effect of the anabolic steroids nandrolone decanoate (ND) or stanozolol (ST) on the relative proportion of cytosolic HSP70s and on the abundance of their charge variants in the slow-twitch soleus muscle of sedentary and exercise-trained rats. The experimental groups of animals were the same as those already indicated in Fig. 1. Whole soleus muscle protein extracts were separated by two-dimensional electrophoresis and the zones corresponding to the location of the stress proteins HSP72 and HSP73 were transferred to nitrocellulose membranes and analysed by immunoblotting with an antibody that recognized both proteins, using enhanced chemiluminescence to detect the antigen–antibody complexes. The abundance of the different charge variants was normalized by reference to the major variant (variant 1) of HSP73. For further details see Section 2. (A) Schematic representation of the charge variants detected and the nomenclature used with examples of representative immunoblot patterns of soleus muscle cytosolic stress proteins of the family of 70 kDa from non-trained (S) or exercise-trained (E) Wistar rats, without treatment (C) or after administering nandrolone decanoate (ND); (B–F) graphic representation of the relative proportions of both cytosolic HSP70s and of their charge variants, under the different experimental conditions. Data represent means ± SD of four or five animals per group. $^{\cdot}P < 0.05$, $^{\cdot\cdot}P < 0.01$, exercise-trained versus sedentary rats.

It amplified ($P < 0.05$) the up-regulatory effect of chronic exercise on the expression levels of HSP72 in the EDL muscle, and partially counteracted the effects of exercise training on HSP72 in the soleus.

As previously reported [8], both cytosolic HSP70s were made up of at least three isoforms (charge variants) of the same molecular mass and very close isoelectric points, in both muscle types [8]. Figs. 2 and 3 summarize the effects of AAS administration on the relative abundance of cytosolic HSP70s and of their charge variants in the soleus and EDL muscles, respectively. In the soleus muscle, exercise training increased the proportion of the inducible component of cytosolic HSP70s in a statistically significant manner ($P < 0.05$, Fig. 2C), mainly because of increased amounts of its major charge variant, the variant 1 (Fig. 2D). No AAS-effects were evident in this muscle. In the EDL muscle, exercise-training also increased the proportion of HSP72 in total cytosolic HSP70s in a statistically significant manner ($P < 0.01$, Fig. 3C), and all of its charge variants increased as a consequence of training (Fig. 3D–F). Consistent with the data obtained in one-dimensional blots, the proportion of HSP72 in total cytosolic HSP70s increased in the EDL muscle of sedentary animals through ND-treatment ($P < 0.05$, Fig. 3C). ND also increased the relative amount of the acidic variant of HSP73 (variant 2, Fig. 3B) and of the major variant of HSP72 (variant 1, Fig. 3D). In exercise-trained rats, ND-treatment increased the proportion of isoform 2 of HSP72, a more acidic variant the proportion of which also was found to increase in skeletal muscle following exercise [8].

3.3. Gender-dependence of the expression levels of cytosolic HSP70s in skeletal muscle

Fig. 4 presents the effects of gender on the expression levels of the cytosolic HSP70s. The expression levels of the inducible protein HSP72, were not dependent on gender. However, the constitutive stress protein HSP73 was expressed in a sexually dimorphic fashion (Fig. 4), being about 40% higher in males than in females in both muscle types. HSP73 trended also to be expressed at higher levels in the soleus muscle than in the EDL, but differences between muscles were not statistically significant. HSP72 was expressed in a muscle type specific fashion. Its levels in the soleus were about 7-fold those in the EDL in males, and even higher (8- to 9-fold) in females.

Analysis of the relative proportions of the different charge variants of both cytosolic HSP70s in male and

female rats, obtained from 2D-immunoblots, indicated only small differences between sexes. In the soleus muscle of female rats the proportion of the most acidic variant (variant 2) in total HSP73 was higher ($P < 0.05$) than in males, which was the only significant difference between genders. This difference was not observed in the EDL muscle.

4. Discussion

This investigation on the effects of anabolic steroids on the expression levels of cytosolic HSP70s in the skeletal muscle of sedentary and chronically exercised individuals is an attempt to answer two different questions. One purely scientific question, relates to the mechanisms involved in controlling the expression levels of stress proteins in the skeletal muscle and to the possible implication of steroids in defining HSP levels in different fibres types and in contributing to the up-regulatory effects of exercise-training on some HSPs [23,24]. The other question, which pertains to sport sciences but is also the expression of a social concern, refers to the reasons why anabolic steroids continue to be self-administered by athletes in spite of the lack of conclusive evidence supporting their presumed effectiveness on skeletal muscle of eugonadal males and the accumulation of data indicating that their administration at suprapharmacological doses may have adverse effects on health [25–28].

In the absence of reliable evidence indicating that suprapharmacological doses of AASs are effective on extragenital skeletal muscle (excluding the highly androgen-sensitive muscles of the perineal complex of the rat and the guinea pig) of eugonadal males [19], an AAS-dependent modulation of the expression levels of stress proteins would provide support to the notion that AASs might act on skeletal muscle by improving tolerance to exercise-training. In this context, improving the tolerance of skeletal muscle to exercise-training, should be understood as increasing its resistance to exercise-induced damage and/or allowing it to recover from an acute exercise bout faster than would otherwise occur in the absence of treatment. Presently, little information is available concerning a possible correlation between increased HSP levels and increased tolerance to exercise-training in skeletal muscle. However, data from different laboratories analysing the expression levels of HSP72 in highly trained rowers [23] or in chronically exercised rats [24], indicating that the skeletal muscle of exercise-trained individuals expresses higher levels of HSP72, together with a report showing reduced injury in skeletal muscle following stress conditioning through heat shock [25], suggests that such a correlation could effectively exist. Furthermore, muscle fibres are apparently endowed with a mechanism to adjust HSP levels continuously by regulating the rate of synthesis of HSP72 up and down during active and resting periods, respectively [24]. Thus, the observations made in the heart of entire animals showing an increased tolerance to stress in cells expressing increased levels of some HSPs [29–32] could also apply to the skeletal muscle fibre and its tolerance to exercise-induced damage.

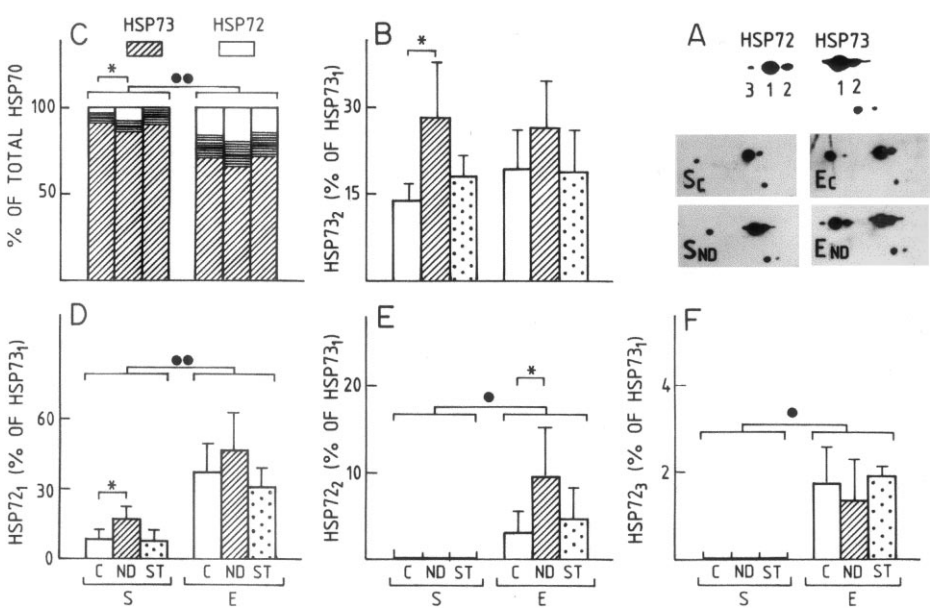

Fig. 3. Effect of the administration of the anabolic steroids ND or ST at a suprapharmacological dose on the relative proportion of the cytosolic HSP70s and on the abundance of their charge variants in the fast-twitch extensor digitorum longus (EDL) muscle of sedentary and exercise-trained rats. Except for the muscle type, all the experimental details of this figure are the same as given in the legend for Fig. 2. $^{\bullet}P < 0.05$, $^{\bullet\bullet}P < 0.01$, exercise-trained versus non-trained; $^{*}P < 0.05$, $^{**}P < 0.01$ anabolic steroid-treated versus untreated animals.

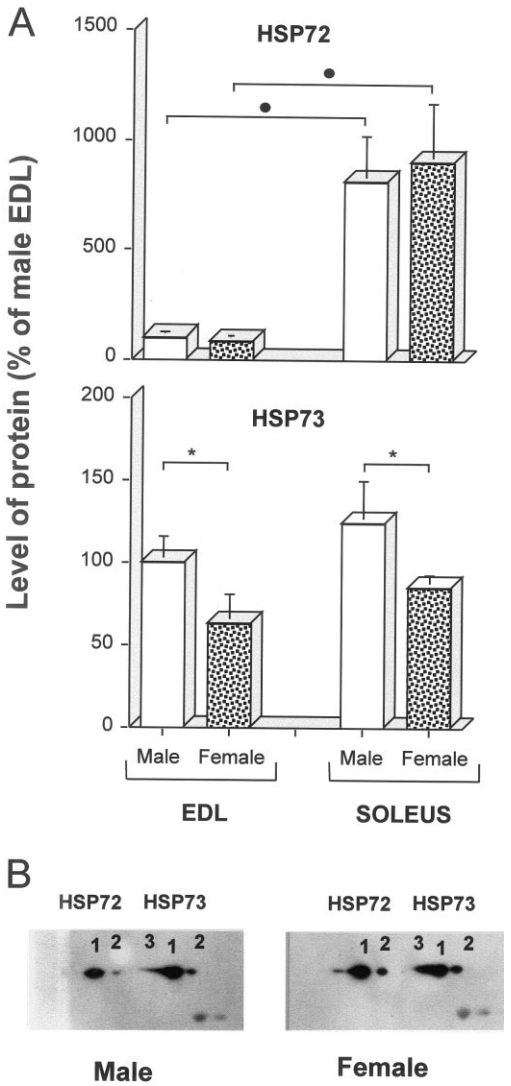

Fig. 4. Gender-dependence in the expression levels of cytosolic stress proteins in soleus and EDL muscles of Wistar rats. Soleus and EDL muscles were obtained in vivo from anaesthetized male (M) or female (F) Wistar rats, immediately frozen in liquid N_2 and stored at -70°C until used. Proteins were separated from whole muscle homogenates by one (panel A) or two-dimensional electrophoresis (panel B). The relative amounts were determined by immunoblotting, using monoclonal antibodies that specifically recognized each protein (panel A) or an antibody that recognized both cytosolic HSP70s (panel B). The peroxidase conjugated to the secondary antibody was developed using enhanced chemiluminescence. For further details see the text and Section 2. (A) Graphic representation of the data obtained by densitometric analysis of one-dimensional immunoblots. (B) Detail of representative immunoblots of two-dimensional patterns of cytosolic HSP70s (HSP73 located in the acidic side of the gel) illustrating the increased proportion of the most acidic charge variant of HSP73 in the soleus muscle of female as compared to male rats. Data in panel A are means $\pm$ SD of five animals per group; $^{\bullet}P < 0.01$, soleus versus EDL; $^{*}P < 0.01$, female versus male rats.

One of the conclusions derived from the results reported in this paper is that the anabolic steroids used in this study were heterogeneous with respect to their biological actions on cytosolic HSP70s in skeletal muscle. Apparently muscle fibres of male rats were more sensitive to suprapharmacological doses of ND than of ST, in spite of the fact that ST was apparently a better agonist of testosterone than ND, as deduced from the degree of feedback inhibition of the hypothalamus-pituitary-gonadal axis (unpublished observations). A second conclusion derived from these results is that the effects of ND on HSP72 in skeletal muscle, apparently mimicked the effects induced by exercise-training. However, since in the EDL muscle ND up-regulated HSP72 to a higher level than did exercise alone, ND may be acting on skeletal muscle by mechanisms other than those activated by chronic exercise.

Independently of the possible mechanism(s) underlying the up-regulatory effects of ND on HSP72-levels in fast-twitch fibres, we interpret this result as an indication of the ability of ND to confer an increased exercise tolerance onto fast-type muscle fibres. Since no information is yet available on the possible role of the different charge variants of HSP70 for the organization or function of this protein, an interpretation of the biological meaning of changes in their proportions is premature. However, interestingly, the only charge variant of HSP72 that increased in the EDL muscle of exercise-trained rats by ND administration was variant 2, the same variant the relative proportion of which was found to increase in the soleus muscle after a single bout of exercise [8].

The observation that HSP73, but not HSP72, was expressed in a sexually dimorphic manner in both muscle types suggests a role for sex steroids in controlling the expression levels of some HSPs. Gender-dependence adds a new degree of complexity to the in vivo regulation of HSP levels in skeletal muscle, known to be fibre-type specific [8,33]. Contrary to what was expected, however, the observed effects of ND cannot be interpreted in terms of its structural and functional relationship to androgens. Firstly, ST, that was apparently a more potent agonist of testosterone than ND as evidenced by a higher degree of inhibition of the hypothalamus-pituitary-gonadal axis (unpublished observations), was almost ineffective in regulating HSP70 levels. Secondly, the up-regulatory effects of ND mainly affected HSP72, the levels of which were not expressed in a sexually dimorphic fashion.

ND (19-nortestosterone) and ST (17α-methyl-2′H-androst-2-eno-(3,2-C)-pyrazol-17β-ol), an androstane derivative related to androstanolone (4,5-dihydrotestosterone) are closely related to the main natural androgen, testosterone. Most of the cellular responses of both AASs are probably initiated by interacting with the androgen receptor [34]. However, some of their biological effects could depend on an interaction with other steroid hormone receptors, including the estrogen receptor and the glucocorticoid receptor [35,36], or even by interacting with structures other than steroid recep-

tors [18,37]. Thus, the possibility that at least part of the effects of ND were due to interactions with steroid hormone receptors other than the androgen receptor, or with structures other than canonical steroid receptors, should be taken into consideration.

Although the results of this investigation in an animal model indicate that ND administration increased HSP72 levels in fast-twitch fibres and may thus increase their exercise tolerance enabling a better response to more intense training regimes, this conclusion should be considered with caution when applied to AASs self-administered by athletes. Because of the illegitimate use of anabolic steroids, the information concerning patterns and doses of administration is scarce. Frequently AAS users practice polypharmacy, with various AASs administered at a time, resulting doses 10–100 times larger than those recommended for replacement therapy, and following a pyramidal administration schedule [38]. Because of this, it was not possible to select a laboratory protocol that could be considered representative of the administration of AASs by athletes. Our study was limited to a comparative analysis of two commonly abused substances, pertaining to different structural series, administered separately at supraphar-macological doses, in an animal model. The obliged use of the animal model, on the other hand, allowed us to study homogeneous groups of subjects under controlled conditions that are very difficult, if not impossible, to meet in human studies involving AAS abuse.

In conclusion, the present report provides experimental evidence that nandrolone decanoate, an anabolic steroid of the estrene series, but not stanozolol, a derivative of the androstane series, up-regulates the levels of the highly inducible stress protein HSP72 and induces changes in the proportion of a minor charge variant of HSP73 and of the major charge variant of HSP 72, exclusively in the fast-twitch EDL muscle. A minor charge variant of HSP72 was also up-regulated in exercise-trained rats. Since a similar effect in the slow-twitch soleus muscle was lacking, these results suggest that some anabolic steroids might increase the exercise tolerance of skeletal muscle by up-regulating HSP72 levels in fast-twitch fibres. In an attempt to establish a possible relationship between this effect and the biological actions of sexual steroids, we have characterized a sexual dimorphism in the expression of the constitutively expressed stress protein HSP73. Since HSP72 levels were expressed in a gender-independent manner, the observed effects of nandrolone decanoate in skeletal muscle could not be interpreted as a consequence of an androgen-like activity of the anabolic steroid.

While these findings could contribute to explaining the way in which skeletal muscle fibres could benefit from the administration of some anabolic steroids, it seems unlikely that solely a selective effect on fast-twitch skeletal muscle fibres is the basis for a general mechanism explaining the presumed advantages of AAS administration on sports performance. The soleus and EDL muscles of the rat hind limb were selected for this study because of their relatively homogeneous composition of mainly slow-twitch or fast-twitch fibres, respectively, and because they represent two extremes in fibre-type composition, contractile properties and basal expression levels of HSP72. However, most human skeletal muscles are mixed-fibre, formed of different proportions of slow-twitch and fast-twitch fibres. As predicted from the orderly recruitment of fibre units according to size, the proportion of active fast-twitch fibres will increase as a function of exercise intensity. This could be one of the reason why increased levels of HSP72 in fast-twitch fibres could be of particular help to high-intensity training.

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