Dose-Dependent Hepatic Response to Subchronic Administration of Nandrolone Decanoate

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ABSTRACT

VIEIRA, R. P., R. F. FRANÇA, N. R. DAMACENO-RODRIGUES, M. DOLHNIKOFF, E. G. CALDINI, C. R. CARVALHO, and W. RIBEIRO. Dose-Dependent Hepatic Response to Subchronic Administration of Nandrolone Decanoate. *Med. Sci. Sports Exerc.*, Vol. 40, No. 5, pp. 842–847, 2008. **Background:** Androgenic anabolic steroids (AAS) are synthetic hormone derivatives of testosterone and are mainly used to enhance athletic performance and muscle mass, but medical applications also have been described. Short- and long-term side effects have been demonstrated in many organs, but the liver adverse effects are the most common and serious ones associated with AAS use. However, these effects have been supported by few clinical and experimental studies. **Objective:** To evaluate the hepatic function and structure after 5 wk of nandrolone decanoate administration at three different doses. **Methods:** Twenty-seven adult male Wistar rats were randomly assigned to the following groups: control, clinical, intermediate, and suprapharmacological doses of nandrolone decanoate during 5 wk. **Results:** The biochemical studies showed that nandrolone decanoate administration leads to a dose-dependent increase in serum levels of the aspartate aminotransferase (AST) ($P < 0.05$), alanine aminotransferase (ALT) ($P < 0.01$), and alkaline phosphatase (ALP) ($P < 0.001$), as well as a significant decrease in total proteins ($P < 0.01$), bilirubin ($P < 0.05$), total cholesterol and fractions ($P < 0.05$), and triglycerides ($P < 0.05$). Although a significant statistical difference was found for AST, ALT, and ALP when compared with the control group, their values remained within the normal range. The number of Kupffer cells was increased in the liver parenchyma ($P < 0.05$), and the content of collagen was increased in the central lobular vein wall, in the hepatic parenchyma, and in the portal space ($P < 0.05$). **Conclusions:** These results suggest that subchronic treatment with nandrolone decanoate, mainly administered at higher-than-clinical doses, are potentially deleterious to the liver, leading to incipient fibrosis.
Key Words: ANDROGENIC ANABOLIC STEROIDS, HEPATOTOXICITY, KUPFFER CELLS, LIVER FIBROSIS, COLLAGEN

Testosterone is a male sex hormone responsible for the androgenic and anabolic effects during male adolescence and adulthood. The androgenic anabolic steroids (AAS) are synthetic compounds structurally related to testosterone (20). AAS exert their actions by activating gene expression after binding to the androgen receptor, which is located in the cell nucleus, but the rapid non-genomic action of steroids, either by nonreceptor mediated actions at the cell membrane or by membrane-bound receptor sites, have also been proposed (4).

The AAS have licit uses, as in medical practice, and illicit ones, as by individuals who want to increase muscle mass and athletic performance (2,10). The use of AAS is illegal for nontherapeutic purposes in sports medicine, even if prescribed by a physician, and they are prohibited in sports by the World Anti-Doping Agency (WADA). In addition, since their first illicit and systematic use in the 1950s by weightlifters from the Soviet Union and the United States, there is evidence of the increasing use of AAS by athletes and nonathletes (31). In many countries, the prevalence of AAS use among high school–aged students has shown amazing and growing rates that range between 3 and 12%, among males as well as female students (30).

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TABLE 1. Food intake, body mass, and liver mass index.

	CON	CLI	INT	SUP
Food intake (g)	26.08 ± 1.57	25.92 ± 2.18	24.80 ± 1.67	20.34 ± 4.56*
Body mass (g)	55.42 ± 15.17	100.85 ± 15.13#	132.28 ± 12.57#	44.50 ± 14.38
Liver mass index	0.026 ± 0.001	0.030 ± 0.002	0.041 ± 0.004†	0.024 ± 0.009

Values are means ± SD. CON, control group; CLI, clinical group; INT, intermediate group; SUP, suprapharmacological group. The results for food intake show the daily average of the group. * $P < 0.05$ when compared with the other groups. The results for body mass show the average increase (final mass less initial mass). #$P < 0.001$ when the CLI and INT groups were compared with the CON and SUP groups. The index of relative weight of the liver was calculated as the final body mass divided by liver weight. †$P < 0.01$ when the INT group was compared with the other groups.

The AAS present several short- and long-term side effects, mainly when used without control (17). Among these side effects, hepatotoxicity is the most common and prominent of them (24). The hepatic adverse effects of AAS are slight alterations in the transaminase levels, moderate centrolobular inflammatory or degenerative lesions, and some cases of hepatocellular adenomas (3,13,25,28). Some studies have reported that female and male sex steroid hormone use can lead to an increase in the extracellular matrix deposition, including skin and tendon, by inhibition of matrix metalloproteinases activity (12,19).

Nandrolone decanoate (ND) is one of the most common AAS used by athletes and in medical practice (10). ND is used to increase body weight and muscle mass in HIV-infected patients, and also in patients undergoing renal hemodialysis (11,21). The ND treatment has presented important antianemic effects in patients with chronic kidney diseases (7). There is also evidence that the ND treatment decreases the number of bone fractures in elderly osteoporotic women (9) and reverts the altered nutritional status after esophageal resection for carcinoma (6). However, a literature review has revealed that most studies did not investigate the dose-dependent side effects of ND administration on hepatic structure and function, particularly on the synthesis of hepatic collagen. With such an aim, this study evaluated the effects of subchronic treatment (lasting 5 wk), with three different doses of ND, on rat liver structure and function, with a special emphasis on the collagen distribution.

METHODS

In all experiments, all American College of Sports Medicine (ACSM) guidelines for animal care were followed, and all protocols were authorized by the committee for ethics in animal research of the University of Vale do Paraíba (protocol no. L051/2004/CEP), in agreement with the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation.

Animals and experimental protocol. Adult male Wistar rats (180–250 g) were randomly assigned to four groups: control (CO, $N = 7$), clinical dose (CLI, 0.7 $mg \cdot kg^{-1} \cdot wk^{-1}$, $N = 7$), intermediate dose (INT, 5.3 $mg \cdot kg^{-1} \cdot wk^{-1}$, $N = 7$), and suprapharmacological dose (SUP, 10.3 $mg \cdot kg^{-1} \cdot wk^{-1}$, $N = 6$). The animals were caged in groups of seven in a room maintained at 25° ± 3°C with controlled lighting (lights on from 06:00 to 20:00 h) and pellet laboratory chow and water available *ad libitum*. All animals were weighted weekly, and food consumption was evaluated daily. The study lasted 5 wk, and the ND

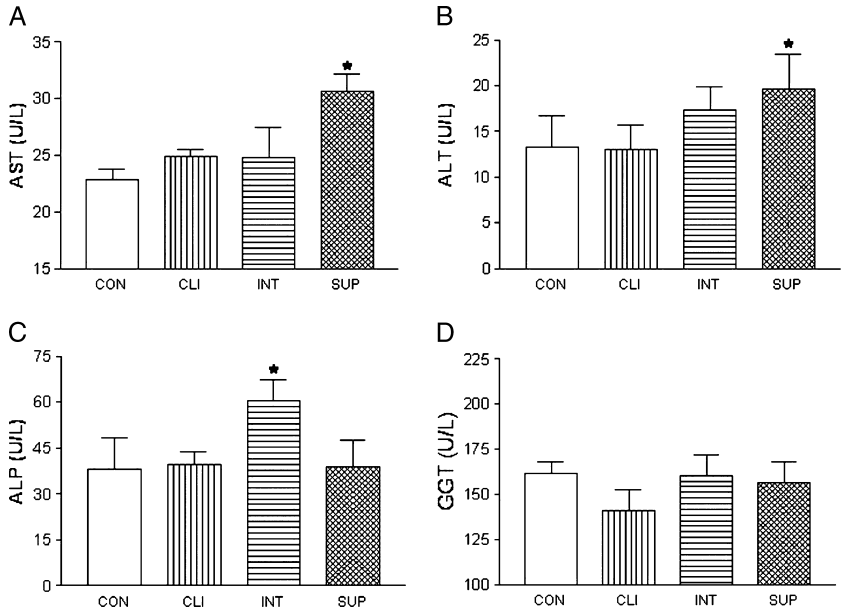

FIGURE 1—Liver enzymes. In *panels A and D*, the *bars* represent the serum levels of AST and GGT, respectively. In *panels B and C*, the *bars* represent the means and standard deviations of serum levels of ALT and ALP, respectively. *A*, * $P < 0.05$ when the SUP group was compared with the CON group; *B*, * $P < 0.01$ when the SUP group was compared with the CON and CLI groups. *C*, * $P < 0.001$ when the SUP group was compared with all the groups. CON, control group; CLI, clinical group; INT, intermediate group; SUP, suprapharmacological group.

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TABLE 2. Plasma levels of bilirubin, proteins, and lipoproteins.

	CON	CLI	INT	SUP
Free bilirubin ($mg \cdot dL^{-1}$)	0.28 ± 0.03	0.22 ± 0.01*	0.21 ± 0.04*	0.23 ± 0.40*
Total proteins ($g \cdot dL^{-1}$)	5.52 ± 0.29	5.47 ± 0.35	5.67 ± 0.51	4.7 ± 0.34#
Albumin ($g \cdot dL^{-1}$)	3.42 ± 0.40	3.43 ± 0.58	3.46 ± 0.58	3.10 ± 0.37
Cholesterol total ($mg \cdot dL^{-1}$)	55.42 ± 12.3	56.41 ± 8.7	56.85 ± 7.7	24.83 ± 7.70†
LDL cholesterol ($mg \cdot dL^{-1}$)	77.42 ± 6.23	76.14 ± 7.84	74.14 ± 4.18	64.66 ± 6.60†
HDL cholesterol ($mg \cdot dL^{-1}$)	52.28 ± 6.38	47.31 ± 7.34	46.71 ± 4.33	41.16 ± 3.30†
VLDL cholesterol ($mg \cdot dL^{-1}$)	25.14 ± 1.45	28.42 ± 1.49	28.85 ± 2.84	23.50 ± 4.00†
Triglycerides ($mg \cdot dL^{-1}$)	124.57 ± 7.0	142.57 ± 7.6	143.57 ± 13.7	118.0 ± 20.80†

Values are means ± SD. CON, control group; CLI, clinical group; INT, intermediate group; SUP, supratherapeutical group. For plasma levels of bilirubin: * $P < 0.05$ when the CLI, INT, and SUP groups were compared with the CON group. For plasma levels of total proteins: #$P < 0.01$ when the SUP group was compared with the other groups. For plasma levels of total cholesterol and fractions and for triglycerides: †$P < 0.05$ when all groups were compared.

(steroids treated groups) or vehicle (control group) was administrated by intramuscular injection, five times a week.

Blood samples and biochemical assays. Blood samples (5 mL) were collected into heparinized tubes from the cava vein after ketamine (50 $mg \cdot kg^{-1}$) and xylazine (40 $mg \cdot kg^{-1}$) anesthesia and centrifuged at 1000 rpm at 4°C for 10 min. Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltransferase. (GGT), total bilirubin, total proteins, albumin, total cholesterol, and fractions and triglycerides were determined by commercial spectrophotometric kits supplied by Analisa (Minas Gerais, Brazil).

Morphometric study. Liver fragments were fixed in 10% phosphate-buffered formalin and then processed for paraffin embedding and sectioning according to standard procedures. Histological sections (5 μm) were stained with H&E and Picrosirius solution. The latter has proved to be a useful method for the identification of the fibrillar collagen in tissue sections as it takes on an intensely red color (33). The density of uni- and binucleated hepatocytes and Kupffer cells was evaluated in H&E slides, as follows: using a 100-point grid with a known area (10,000 μm^2 at 1000× magnification) attached to the microscope ocular, we calculated the area of the hepatic parenchyma present in each microscopic field by counting all points hitting the area of interest. Subsequently, we counted the number of the studied cells within that area. The cellular density was determined as the number of each cellular type divided by the hepatic parenchyma area. Measurements were performed in 20 fields per animal. Results are expressed as cells per millimeter squared (33).

The morphometric evaluation of collagen content was performed in three different regions of interest: interstitium of the hepatic parenchyma (20 fields per slide), portal space (five portal space areas per slide), and the perivascular area of the centrolobular vein (five veins per slide).

The tissue fraction occupied by fibrillar collagen in these regions was estimated with the aid of a digital analysis system, using the Image Pro Plus 6.0 software (Media Cybernetics). The images were generated by a Nikon Optiphot microscope connected to a camera (Nikon DS-2Mv). The threshold for the collagenous fibers was established after enhancing the contrast up to a point at which the fibers were easily identified. The area occupied by fibrillar collagen was determined by digital densitometric recognition. The value of the fraction of fibrillar collagen in each region of interest was then obtained, dividing the collagen area by the total area of the region of interest. The results are expressed as percentages.

Statistical analysis. A one-way analysis of variance (ANOVA) and Student–Newman–Keuls *post hoc* test (parametric data) and one-way analysis of variance on ranks (ANOVA on ranks) and Dunn's *post hoc* test (nonparametric data) were used for the comparison of the different parameters among groups. Data are presented as means ± SD. The level of significance was set at $P < 0.05$.

RESULTS

Food intake, body mass, and index of relative mass of the liver. Results are summarized in Table 1. After the third week of treatment, food consumption

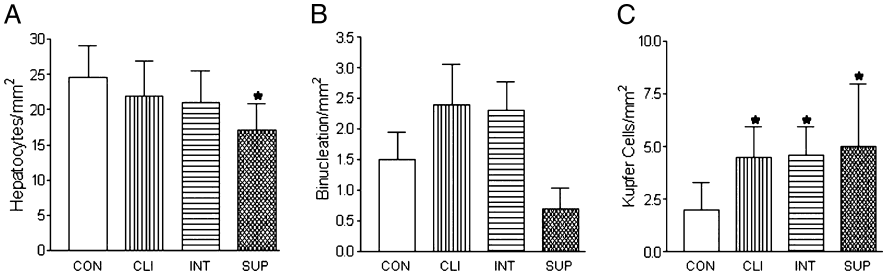

FIGURE 2—Liver morphometry. *A*, Number of hepatocytes per millimeter squared. * $P < 0.05$ when the SUP group was compared with the CON and CLI groups. *B*, Number of binucleations per millimeter squared. *C*, Number of Kupffer cells per millimeter squared. * $P < 0.05$ when the CLI, INT, and SUP groups were compared with the CON group. CON, control group; CLI, clinical group; INT, intermediate group; SUP, supratherapeutical group.

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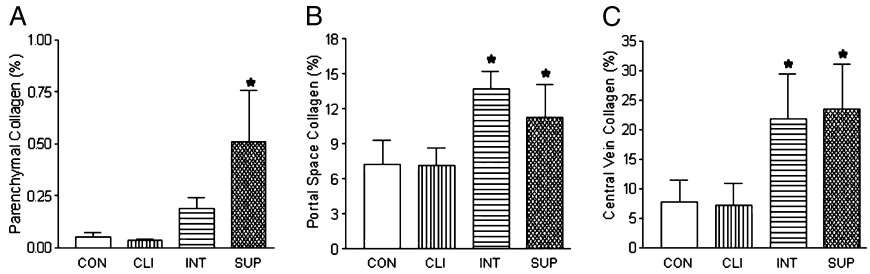

FIGURE 3—Hepatic collagen content. *A*, Collagen content in the liver parenchyma. * $P < 0.05$ when the SUP group is compared with the CON and CLI groups. *B*, Collagen content in the liver portal space. *C* Collagen content in the liver centrolobular vein. For *panels B and C*, * $P < 0.05$ when the SUP group is compared with the CON and CLI groups, respectively. CON, control group; CLI, clinical group; INT, intermediate group; SUP, suprapharmacological group.

significantly decreased in the SUP group, when compared with the other groups ($P < 0.05$). The body mass was significantly higher in the CLI and INT groups, when compared with the CON and SUP groups ($P < 0.001$). The relative mass of the liver was significantly increased in the INT group, when compared with the other groups ($P < 0.01$).

Liver enzymes. Figure 1A shows that the plasma levels of AST were significantly increased in the SUP group, when compared with the CON group ($P < 0.05$). The levels of ALT in the SUP group were increased when compared with the CON and CLI groups (Fig. 1B; $P < 0.01$). Interestingly, the plasma levels of ALP were significantly increased only in animals treated with INT doses, but not with SUP doses (Fig. 1C; $P < 0.001$). Figure 1D shows the plasma levels of GGT; no significant differences were observed among the groups.

Liver proteins and lipoproteins. Results are summarized in Table 2. The plasma levels of bilirubin were significantly decreased in the CLI, INT, and SUP groups, when compared with the CON group ($P < 0.05$). The plasma levels of total proteins was significantly decreased in the SUP group when compared with the other groups ($P < 0.01$). The plasma levels of albumin were not significantly altered in any group. The plasma levels of total cholesterol and fractions and triglycerides were significantly decreased in the SUP group, when compared with the other groups ($P < 0.05$).

Liver morphometry. In Figure 2A, the SUP group shows a significant decrease in the number of uninucleated hepatocytes, when compared with the CON and CLI groups ($P < 0.05$). In Figure 2B, no difference was observed regarding binucleations, when all the groups were compared. In Figure 2C, the CLI, INT, and SUP groups showed a significant increase in the number of Kupffer cells, when compared with the CON group ($P < 0.05$).

Hepatic collagen content. Figure 3A shows that the collagen content in the liver parenchyma was significantly increased in the SUP group when compared with the CON and CLI groups ($P < 0.05$). In Figure 3B and C, the graphs demonstrate that the collagen content in the liver portal space and centrolobular vein, respectively, was significantly increased in the INT and SUP groups, when compared with the CON and CLI groups, respectively ($P < 0.05$). In Figure 4A–D, the photomicrographs illustrate the collagen content in the liver portal space in the CON, CLI, INT, and SUP groups, respectively.

DISCUSSION

In the present study we evaluated, for the first time, the hepatic response to 5-wk administration of three different dosages of nandrolone decanoate (ND) in rats. Our results showed that ND leads to a dose-dependent increase in plasma levels of enzymatic markers of liver toxicity and, more importantly, to an increase in collagen deposition in the liver parenchyma, portal space, and centrolobular vein.

The use of ND by professional and recreational athletes as well as in clinical and sports medical practice, is high and well established (2,10,20). However, despite the considerable number of clinical and experimental studies on ND (2,8,10), few studies have evaluated its effects on the liver (13,24,25,28), and few studies have evaluated the effects of different dosages of ND administration on organs

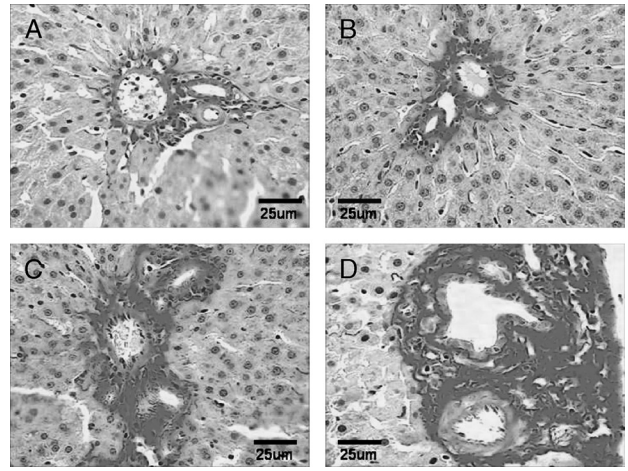

FIGURE 4—Representative photomicrographs of the collagen content in the liver portal space in the CON, CLI, INT, and SUP groups, respectively. Scale bar = 25 μm. Magnification = 400×.

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other than the liver (8). In animal studies with ND, the liver function has been evaluated only as a simple addendum to the study. For instance, Fortunato et al. (2006) demonstrated that 8 wk of ND treatment in rats leads to the increase in the weight of the thyroid gland, decrease in serum levels of T3, free T4, TSH, and increase in the liver activity of type 1 iodothyronine deiodinase, but no specific hepatotoxicity marker was studied (8). Another study by Cunha et al. (2005) demonstrated that 6 wk of ND treatment in rats alters the tissue glycogen content in some organs, such as the soleus and cardiac muscle, but it does not alter the hepatic transaminase levels (5). Generally, the human studies associating hepatotoxicity with ND are restricted to case reports (28). However, Kuipers et al. (1991) studied the effects of 8 wk of ND administration on body composition, blood pressure, lipid profile, and liver function in bodybuilders. These authors demonstrated that bodybuilders receiving ND had increased body mass and diastolic blood pressure, decreased HDL cholesterol, and no changes in liver transaminase levels (16). The authors also showed that nonhepatic changes caused by ND administration returned to the "preanabolic" levels 6 wk after ND discontinuation (16). Based on its antiwasting effects, other important human studies with ND have been carried out in HIV-infected patients. These studies have suggested the relative safety of ND treatment in these patients based on the transaminase levels, although the ND protocol in these studies was a long-term one (12 months) and was administered at high doses (100 or 300 $mg \cdot wk^{-1}$) (21). The liver structure and function of HIV-infected patients, who receive many drugs during their treatment (including ND), have been associated with some hepatic disorders, many of which show no alterations in serum transaminase levels, but with alterations in the histological evaluations (26).

In our study, we found that subchronic treatment with ND resulted in a dose-dependent increase in the serum levels of AST, ALT, and ALP, although these levels remained within the normal ranges. There is a broad variability among the results of the several human and animal studies on hepatic injury, as well as on the criteria used to categorize the severity of hepatotoxicity (23). The determination of serum transaminase levels is generally considered to be of great value to detect toxic effects on the liver (3). However, the misinterpreted idea that the increase of only one hepatic enzyme could represent liver toxicity is frequently observed, when the ideal interpretation should be made using two or more hepatic enzymes (23). In our study, we found increased levels of three important enzymatic markers of the liver toxicity, demonstrating that ND treatment can lead to a state of hepatotoxicity.

Liver proteins are decreased in many pathological conditions by the decrease in their synthesis, increase in their degradation, or even increase in their extravascular losses (29). The levels of lipoproteins can also be decreased in patients with chronic liver disease, mainly in cirrhotic malnourished patients (27). We found that the subchronic administration of a suprapharmacological dose of ND decreases serum levels of total cholesterol and fractions. However, it is not clear whether these findings are the result of a direct effect of the ND treatment on the capacity of the hepatocytes in synthesizing lipoproteins, or whether they result from an indirect effect of ND-induced liver toxicity, demonstrated by the decrease of food consumption among the animals from the SUP group.

Our results also demonstrated that subchronic ND administration leads to an increase in collagen deposition in the liver parenchyma, portal space, and centrolobular vein. However, the mechanisms underlying these effects remain to be elucidated. It would be worthy to investigate whether the expression of hepatocyte androgenic receptors is altered after ND administration, since hepatocytes have androgenic receptors and the expression and activation of these receptors are involved in collagen synthesis and deposition (18). Another possible explanation for the increased collagen deposition in the liver following subchronic ND administration is supported by our finding of increase in the number of Kupffer cells (Fig. 2C). Kupffer cells are the resident macrophages of the liver, and their primary function is to phagocyte the incoming particles, thus protecting the liver (22). Some experimental studies have demonstrated that Kupffer cells play a vital role in the development of liver fibrosis and cirrhosis (14,22). When activated, Kupffer cells produce many harmful byproducts, such as TGF-β1 (14), reactive oxygen species, NF-$\kappa\beta$, TNF-α, and IL-1β (34), which are compounds that act directly by stimulating the liver fibrosis process. Additionally, many studies demonstrate that an imbalance between matrix metalloproteinases (MMP) and their respective inhibitors, tissue inhibitors of matrix metalloproteinase (TIMP), play a central role in the process of liver fibrosis (1,15). However, the current literature is controversial about the effects of ND on the expression of MMP and TIMP, and, at the present moment, no study has evaluated its effects specifically on the liver. Triantafillopoulos et al. demonstrated that ND increased remodeling and the MMP-3 activity in bioartificial tendons (32), whereas Marqueti et al. demonstrated that ND increases tendon remodeling despite decreases in MMP-2 activity in tendons of rats (19). Therefore, further studies evaluating the activity of MMP and TIMP on the liver following ND administration could be helpful in understanding the mechanisms by which ND increases liver collagen deposition.

One limitation of our study is the fact that we did not evaluate a more prolonged period of ND administration, as well as whether these results are reversible after the discontinuation of ND. However, the present study provided initial and relevant information about the profibrotic effects of nandrolone decanoate on the liver.

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We conclude that the subchronic administration of nandrolone decanoate leads to an incipient liver fibrosis with discrete alterations of the biochemical markers of liver toxicity.

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