# Anabolic Steroid Nandrolone Augments Hepatic Regenerative Response in Rats

P. Gentileschi, G. Palmieri, A. Perfetti, P. Sileri, G. Sica, M. Venza, D. Benavoli, and A.L. Gaspari

## ABSTRACT

The success of recovery after liver resection depends on the regeneration and functions of the remnant liver. In this study we investigated whether liver regeneration was facilitated by nandrolone decaonate after two-thirds partial hepatectomy in rats. Study animals were pretreated with nandrolone (5 mg/kg), while control animals received a placebo. Animal were sacrificed at 12, 24, 48, and 72 hours. We compared the survival rates, liver function tests as well as the amount of apoptosis by terminal deoxynucleotidyl transferase-mediated deoxyuridine-biotin nick end labeling assay, and regeneration, which was expressed as ratio of proliferating cell nuclear antigen and restoration ratio. A significant increase in hepatocyte regeneration at 24 and 48 hours in partially hepatectomized rats treated with nandrolone decaonate was observed compared to controls. This observation was confirmed by the significant acceleration of the liver restoration rate, which was 1/5 faster than in partially hepatectomized controls. The results of this study indicate that liver regeneration in rats treated with nandrolone show a prompt, faster regeneration after partial hepatectomy.

ANABOLIC-ANDROGENIC STEROIDS (AAS) are used for the treatment of several conditions, such as male hypogonadism, anemia associated with end-stage renal disease, retarded growth, impotence, infertility, eunuchoidism, cryptoorchism, panmyelopathy, and paroxysmal nocturnal hemoglobinuria.[1–4] AAS, such as nandrolone decaonate (NDR), have also been used by athletes to build muscle mass and enhance weight-lifting performance. Liver side effects are the most common and serious ones associated with their use, including pelios hepatis, subcellular changes, hyperplasia, and adenoma or hepatocellular carcinoma.[3,5,6] Although some of these conditions have been supported by a few animal studies, the clinical findings have often been represented only by case histories and small uncontrolled studies in the absence of any clear underlying mechanisms of damage.[7,8] It has been postulated that AAS may speed liver regeneration, leading to hyperplasia and eventually to the development of tumors.[7] It is conceivable that the hepatic toxicity of NDR abuse may enhance or stimulate hepatic regeneration following an injury, thereby having obvious implications for post major liver surgery or transplantation. In order to investigate this important question, we evaluated the effects of an AAS, NDR, on liver regeneration in hepatectomized rats.

## MATERIALS AND METHODS

Male Lewis rats (Sprague-Dawley Inc, Indianapolis, Ind, USA) of 150 to 200 g were randomly divided into four groups and treated as follows: group I or sham-operated control animals (eight rats); group II or NDR-treated sham operated animals (eight rats), treated with a single dose of 1 mg/kg of nandrolone decaonate (Deca-Durabolin, NV Organon, NL) injected intramuscularly and repeated weekly for 3 weeks; group III, or placebo-treated hepatectomized animals (24 rats), received the same amount of saline solution (placebo) at the scheduled times of group II and underwent a standard 2/3 hepatectomy after 3 weeks; and group IV, or NDR-treated hepatectomized animals (24 rats), treated with a single dose of 5 mg/kg NDR injected intramuscularly and repeated weekly for 3 weeks followed by a standard 2/3 partial hepatectomy.

Briefly, rats were kept in accordance with the "Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals" and anesthetized using inhalation of ether (Etere Etilico, New Fadem s.r.l. Farmaceutici e Chimici, Giuliano Napoli, Italy) for induction and intraperitoneal injection of 25 mg/kg sodium pentobarbital (Abbott Lab, Chicago, Ill, USA) for maintenance. Through a midline incision, the liver was dissected free of its ligaments. All of the branches of the hepatic artery, portal vein, and biliary duct were identified; a small vascular clamp was used to induce total liver ischemia for 15 minutes. During this

From the Department of Surgery (P.S., G.S., P.G., M.V., D.B., A.L.G.) and Pathological Anatomy Chair (G.P., A.P.), University of Rome Tor Vergata, Rome, Italy.

Address reprint requests to Pierpaolo Sileri, MD, Universita' di Roma Tor Vergata, Policlinico Tor Vergata, Viale Oxford 81, 00133 Rome, Italy. E-mail: piersileri@yahoo.com



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time, immediately before the reperfusion, the median and left lateral lobes were removed and hemostasis was achieved using 2-0 silk ties. The removed lobes were processed for pathology as internal controls. Six animals from each study group (groups III and IV) were sacrificed at 12, 24, 48, or 72 hours after hepatectomy. Two animals of groups I and II sacrificed at each time point were used as controls. The tissue was divided with one part immediately fixed for light microscopy, while the rest was used to evaluate terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) and proliferating cell nuclear antigen (PCNA) tests. Blood was also collected to estimate serum levels of ALT, AST and LDH using standard laboratory techniques.

### Measurement of Liver Restoration Ratio

The liver restoration ratio was calculated as previously described.[9] Briefly the total liver weight was estimated assuming that the resected liver was 70% of the total liver. The restoration ratio was expressed as percentage of the restored liver weight/total liver before surgery.

### Histology

Samples obtained at the indicated time points were fixed in 10% buffered formalin and embedded in paraffin. Sections of 5-$\mu$m thickness were stained with hematoxylin and eosin. Semithin sections (1 $\mu$m thick) were stained with methylene blue.

### TUNEL

Liver biopsies were fixed in 4% phosphate-buffered paraformaldehyde for a few hours and then embedded in paraffin. Four-micrometer sections were collected on poly-L-lysine-coated glass slides. The nuclear DNA fragmentation of apoptotic hepatocytes was labeled in situ by the TUNEL assay as follows: Sections were deparaffinized with xylene and rehydrated with progressive decreasing alcohol concentrations followed by phosphate-buffered saline (PBS). Then each section was treated with 20 $\mu$g/mL proteinase K (Sigma) in 0.1 mol/L Tris/HCL buffer (pH 7.4) for 15 minutes. After rinsing with PBS, endogenous peroxidase activity was blocked with 3% $H_2O_2$ for 5 minutes. Sections were rinsed again with PBS and incubated with 0.5 U/$\mu$L terminal deoxynucleotidyl transferase (Boerhinger Mannheim, Germany) plus 0.05 nmol/$\mu$L biotinylated deoxyuridine triphosphate in terminal deoxynucleotidyl transferase buffer (Boerhinger Mannheim) in a humidified chamber (37°C 60 min). Each slide was then observed with a light microscope to check the staining quality.

Five liver sections for each rat were analyzed by counting cells in five fields randomly chosen. After images were electronically captured we counted apoptotic positive nuclei.

### PCNA

To localize proliferating cells, we utilized mouse monoclonal anti-PCNA antibody (Dako, Glostrup, Denmark) diluted 1:200 and incubated overnight in a humidity chamber at 4°C. As secondary antibody we used biotinylated LSAB2 solution (Dako). Subsequent development was performed with diaminobenzidine to yield a brown color reaction. Sections were counterstained with Mayer's hematoxylin to facilitate nuclear identification. Each slide was then observed by light microscopy to check the staining quality and for image acquisition. PCNA activity was expressed as ratio per observed field.

### Statistical Analysis

Student $t$ test and Kaplan-Meier survival curves were used for statistical analyses.

## RESULTS

All control animals (group I and II) survived. Overall survival rates following hepatectomy were significantly different between the two study groups, namely, 71% for the placebo-treated hepatectomized rats (group III) and 87.5% for NDR-treated hepatectomized rats (group IV) ($P < .05$). The majority of the animals died early (within 24 hours) after hepatectomy: 71% of group III and 100% of group IV animals. Increased enzyme levels confirmed the liver damage in all groups that underwent hepatectomy compared to control animals. There were no significant differences between placebo and NDR-treated hepatectomized animals.

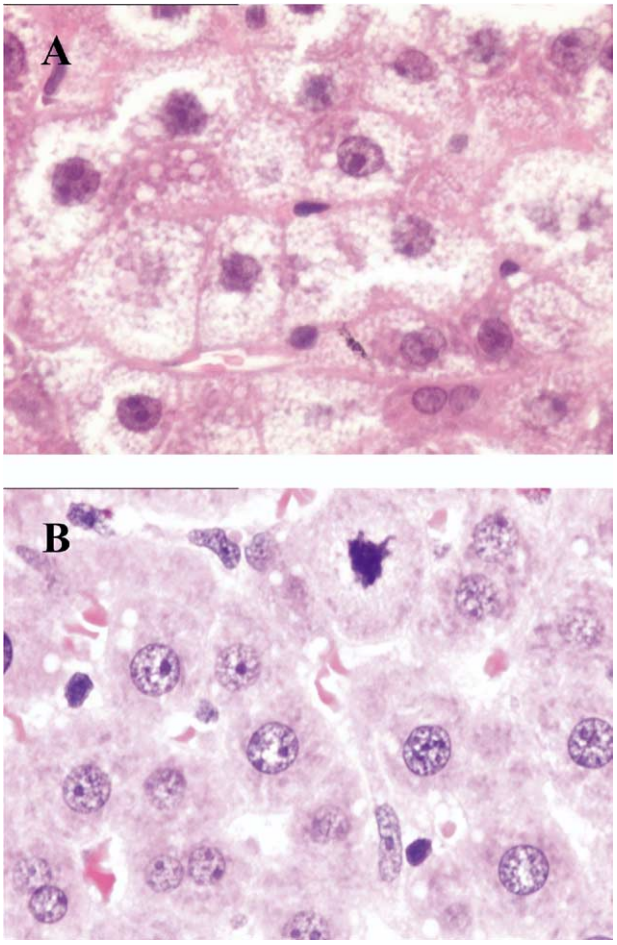


**Fig 1.** Diffuse hepatocyte vacualization and edema was more evident in placebo-treated hepatectomized animals **(A)** compared to NDR-treated hepatectomized animals **(B)** at 72 hours (hematocylin and eosin, ×400). NDR-treated hepatectomized animal showed increased mitotic hepatocytes compared to placebo-treated animals.

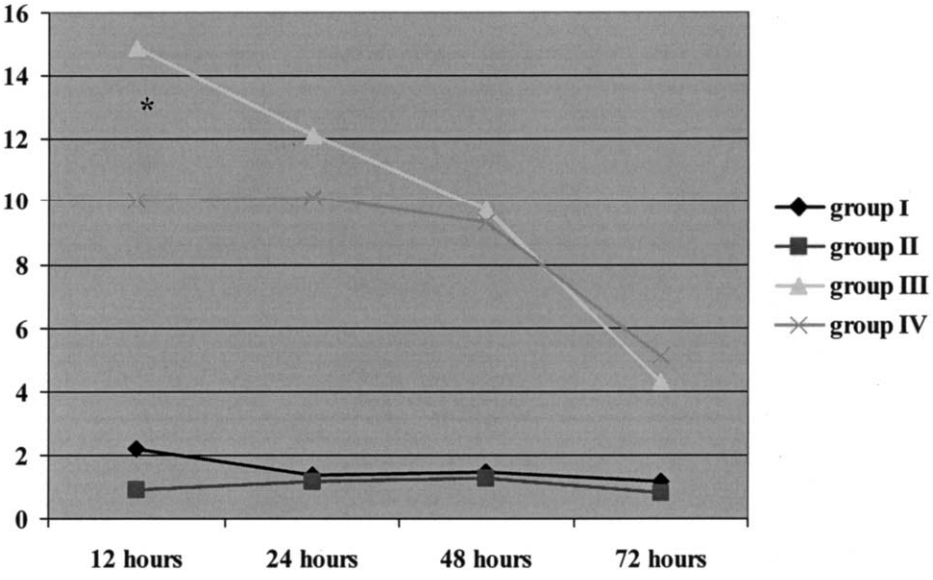


**Fig 2.** Apoptotic nuclei count (expressed as mean positive nuclei per field) among groups. *Significant difference.

Livers from treated unhepatectomized rats appeared to have normal architecture at all times. Although ischemia was protracted for only 15 minutes during the procedure, all hepatectomized animals showed mild sinusoidal congestion and edema. Liver steatosis was observed in all animals that underwent hepatectomy as represented by diffuse micro- and macrovesicular steatosis. Steatosis was significantly less pronounced in NDR-treated hepatectomized animals compared to group III animals ($P < .05$). Hepatocyte cytoplasmic vacuolization was evident at all times in both groups. However, NDR-treated hepatectomized animals showed significantly less hepatocyte vacuolization compared to placebo hepatectomized animals, where scattered necrotic and apoptotic cells were also observed. As witnessed in Fig 1(B), at 72 hours group IV animals still showed focal hepatocyte vacuolization with partial cell involvement in contrast with diffuse and considerable vacuolization in group III animals (A).

Twelve hours after resection the number of apoptotic nuclei was significantly increased in NDR-treated hepatectomized animals (14.9 ± 6.4; data expressed as mean positive nuclei per field ± SD) compared to placebo-treated hepatectomized animals (10 ± 4.9; data expressed as mean positive nuclei per field ± SD). This difference was less evident at 24 and 48 hours, when both hepatectomized groups (III and IV) showed similar patten of apoptotic nuclei counts, returning to baseline at 72 hours after surgery. This trend is summarized in Fig 2. Control animals (group I and II) showed similar grades of apoptosis, namely, 1.2 ± 0.7 for group I and 0.8 ± 0.7 for group II, which were significantly lower than the study groups at all times ($P < .001$).

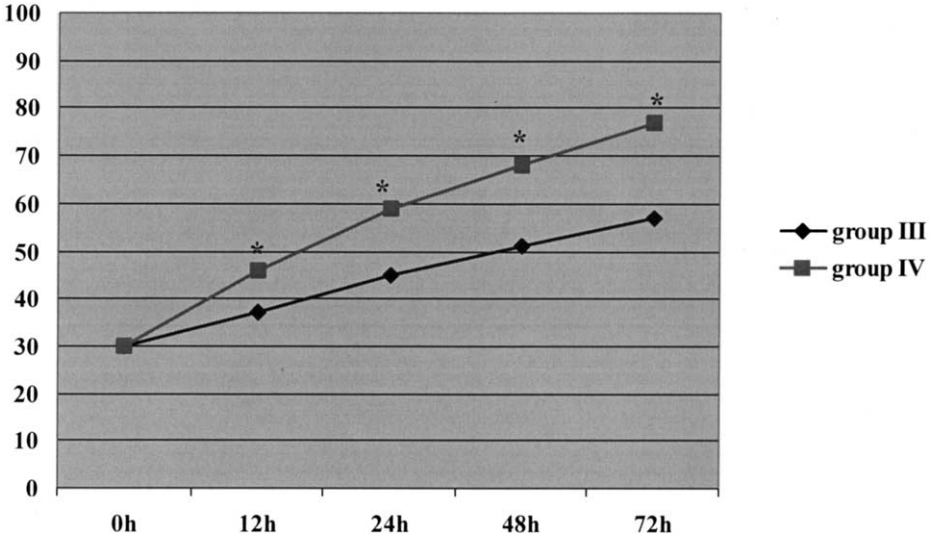


**Fig 3.** Liver restoration rate in hepatectomized animals. *Significant difference.

**Table 1. PCNA Expression Ratio Distribution Among the Study Groups at Different Time Points**

| Group | 12 h | 24 h | 48 h | 72 h |
|---|---|---|---|---|
| Sham placebo-treated (I) | 0.2 ± 0.2 | 0.3 ± 0.2 | 0.4 ± 0.2 | 0.2 ± 0.2 |
| Sham NDR-treated (II) | 0.3 ± 0.2 | 0.3 ± 0.1 | 0.1 ± 0.1 | 0.5 ± 0.2 |
| Placebo-treated resected (III) | 42 ± 11 | 62 ± 19 | 43 ± 14 | 36 ± 7 |
| NDR-treated resected (IV) | 52 ± 9 | 81 ± 12 | 64 ± 5 | 44 ± 7 |

Liver restoration rate measurement showed increased restoration in the NDR-treated group: a 77% regeneration after 72 hours versus 57% in the placebo-treated resected animals (Fig 3).

In sham-operated rats, the PCNA expression ratios were less than 1% without differences among the two control groups (I and II). On the other hand, PCNA expression ratios showed increased regeneration in hepatectomized animals, being significantly higher among the NDR-treated group compared with placebo animals at all times (<1%; $P < .05$; Table 1).

## DISCUSSION

Sex hormones, including androgenic, estrogenic, and progestinic steroids, have all been implicated to affect liver metabolism and morphology.[6,9,10] Over the last decade, the most serious complication linked to AAS is the development of hepatocellular carcinoma, liver cell adenoma, and focal nodular hyperplasia.[7,8,10,11] Epidemiological and clinical observations suggest that an androgenic environment favors development of liver tumors in chronic and high-doses "users" of AAS.[10–12] However, the experimental data are scant and the underling pathogenetic mechanisms are poorly understood.[13–16] It has been postulated that AAS may selectively speed the growth of preexisting liver adenomas or cancers because of an increased expression of sex and androgenic receptors on these cells compared to surrounding tissue.[17,18] Other authors suggest that the hyperplastic condition might be a reactive phenomenon related to initial damage induced by AAS, such as pelios hepatis or cholestasis. Interestingly most of the conditions regress on cessation of androgen use, showing a hormone-dependence of these lesions. Furthermore, in the absence of any primary liver disease or damage, the prolonged use of AAS may cause cell hyperplasia predisposing to malignant transformation. On the basis of these observations, it has been proposed that AAS may play a role in the initiation or facilitation of liver regeneration after liver surgery or transplantation.

Data obtained from our study suggested that nandrolone exerts a protective effect after liver resection hastening liver regeneration. Such protection seems to be shown by a better survival rate in the NDR-treated animals than that in placebo-treated animals following an approximate 70% liver resection. As a matter of fact, it has been shown that NDR might help to regularize the catabolic events during the postoperative period. In NDR-treated animals, structural damage was less and hepatocyte regeneration occurred early and was more pronounced. However, the underlying mechanisms of this phenomenon are still unclear.

In conclusion, nandrolone administration after partial hepatectomy promoted liver regeneration in rats, suggesting its potential application after surgical resection or liver transplant.

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