

## INCREASE IN NAD BUT NOT ATP AND GTP CONCENTRATIONS IN RAT LIVER BY DEHYDROEPIANDROSTERONE FEEDING

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Dehydroepiandrosterone (5-androsten-3 $\alpha$ -ol-17-one; DHEA), the main circulating steroid in humans, has been described to exert varied beneficial effects including antiobesity, anti-aging and anticancer action when used at pharmacological doses in experimental animals. To elucidate the mechanism of the pleiotropic effects of DHEA, we studied the effect of this steroid on concentrations of NAD and adenine and guanine nucleotides in rat liver. Administration of DHEA at 0.3% in the diet for 7 consecutive days caused an increase in liver NAD and NADP, but was without effect on NADH concentrations. This indicates a shift of the redox couple (NAD/NADH) towards oxidation in the DHEA-treated rats. Moreover, there was no change in adenine and guanine nucleotide concentrations, which disproves the hypothesis that the DHEA anticancer actions are due to a decrease in the availability of nucleosides for DNA synthesis. The findings indicate that an increase in liver NAD pool and/or altered redox status, but no changes in adenine or guanine nucleotide content, may be involved in the pleiotropic effects of DHEA.

**Key words:** *dehydroepiandrosterone (DHEA), NAD, NADP, ATP, GTP, adenine nucleotide, liver (rat)*

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## INTRODUCTION

Dehydroepiandrosterone (5-androsten-3 $\alpha$ -ol-17-one; DHEA) and DHEA sulfate (DHEA-S) are known as major secretory products of the adrenal cortex in humans and the main circulating steroids in the blood [25]. DHEA secretion is very high in fetus, drops sharply after birth, rises again before puberty reaching a maximum in young adulthood, and then declines progressively, reaching very low levels after age 70 [23]. DHEA and DHEA-S are classified into the group of steroids, named as neurosteroids, because they can be synthesized *de novo* in the central nervous system [12]. Furthermore, concentrations of DHEA and DHEA-S are considerably higher in the brain than in other organs [12]. Despite the abundance of DHEA its biological role is not clear, except that it is an intermediate in the biosynthesis of estrogens and androgens from cholesterol [25]. It has been claimed that DHEA has antiobesity, antidiabetic, antiviral, anti-aging and anticancer properties when used at pharmacological doses [11]. A positive response to DHEA treatment of Alzheimer and multiple sclerosis patients has also been reported [11]. The mechanism by which DHEA exerts these varied therapeutic effects is far from being clear.

Much interest has been focused on the anticarcinogenic effect of DHEA both in experimental models and in clinical trials [2, 8, 18, 26]. Reduction of the activity of glucose-6-phosphate dehydrogenase by DHEA is claimed to be responsible for the anticarcinogenic action of the steroid [14, 17]. Garcea et al. [7] suggested that the anticarcinogenic effect of DHEA could be due to a relative deficiency of nucleosides for DNA synthesis, caused by a substantial reduction in pentose phosphate production. However, this hypothesis was contradicted by the observation that rats treated with DHEA did not exhibit significant changes in rat liver hexose monophosphate shunt metabolites and adenine nucleotide concentrations as compared to controls [3]. To verify the hypothesis that the anticarcinogenic effect of this steroid is dependent on deficiency of nucleosides for DNA synthesis we investigated the effect of DHEA treatment on adenine and guanine nucleotide concentrations in rat liver. We also studied the effect of DHEA on NAD concentration in liver (as well as in heart and skeletal muscle) since: a) NAD is a coenzyme necessary for several dehydrogenases, the activity of which

depends upon an adequate supply of this cofactor; b) NAD is a substrate for poly(ADP-ribose)polymerase (PARP; EC 2.4.2.30) which is involved in a variety of physiological events including chromatin decondensation, DNA replication, cellular differentiation, gene expression and cellular defense against DNA damage by genotoxic compounds [6, 10]. This means that an increase in NAD-concentration in the tissue by DHEA treatment could be beneficial to the animal and may explain in part the mechanism by which DHEA exerts varied therapeutic effects.

## MATERIALS and METHODS

### Chemicals

DHEA was a generous gift from Schering AG Berlin (Germany). All other chemicals used were of the purest grade available.

### Animals and diets

Male Wistar rats weighing approximately 230 g were used for the experiments. The rats were housed in wire mesh cages at 20°C with alternating 12 h light/12 h dark (7 a.m./7 p.m.). Housing and treatment of the animals were in accordance with the guidelines of the Society for Laboratory Animal Service and the Polish animal protection law. Rats were treated for 7 days with 0.3% (w/w) DHEA mixed into the diet (1324N from Altromin, Lage/Lippe, Germany). Control animals were kept on the same diet without DHEA. Food and drinking water (tap water) were provided *ad libitum*. At sacrifice of the rats, their livers, hearts and skeletal muscles (m. gastrocnemius) were rapidly removed and frozen in liquid nitrogen. These specimens were used for assessment of the total adenine nucleotide and NAD pool. For accurate assessment of redox status and ATP, ADP, AMP, GTP, CoA, NADP, adenosine in rat liver another set of experiments was performed with two groups of rats treated or untreated with DHEA. In these experiments liver edge was frozen *in situ* with aluminium clamps pre-cooled in liquid nitrogen. There was no differences in total adenine or NAD pool between these methods of sample collection. However, NAD/NADH and ATP/ADP ratios were much lower when the first method was employed. Therefore, for the determination of NAD/NADH ratio and the content of metabolites presented in Table 1, the liver edge was

frozen *in situ* with aluminium clamps. Frozen tissues were stored in tightly closed tubes at  $-80^{\circ}\text{C}$  until determination of metabolite contents was performed.

Table 1. Nucleotide concentrations in the liver of rats fed DHEA diet

Nucleotides (mol per g dry weight)	Control rats (n = 5)	DHEA-treated rats (n = 5)
NADP	$0.69 \pm 0.07$	$0.89 \pm 0.07^*$
CoA	$0.152 \pm 0.03$	$0.549 \pm 0.194^*$
ATP	$9.85 \pm 0.96$	$11.36 \pm 1.00$
ADP	$3.66 \pm 0.47$	$3.69 \pm 0.85$
AMP	$1.25 \pm 0.28$	$1.08 \pm 0.38$
Adenosine	$0.033 \pm 0.015$	$0.039 \pm 0.006$
GTP	$2.92 \pm 0.26$	$2.85 \pm 0.85$

Experimental conditions are detailed in Materials and Methods. \* significantly different from the control ( $p < 0.05$ )

### Analysis of NAD and other metabolites

Tissue extracts were prepared from freeze-dried liver using 0.4 M perchloric acid, and they were centrifuged at  $13,000 \times g$  for 3 min at  $4^{\circ}\text{C}$ . The supernatants were neutralized with 2 M KOH and centrifuged again. Analysis of the metabolites was performed using a reversed-phase high performance liquid chromatography method (HPLC) as described previously [22]. The equipment used was a Merck-Hitachi system connected to a Hewlett Packard 1050 diode array detector. Values are presented as means  $\pm$  standard deviation (SD) of 5 rats. The statistical significance of differences between control and DHEA-treated groups was assessed by one-way analysis of variance (ANOVA) followed by Mann-Whitney test, using Systat software (Systat). Differences between groups were considered significant when  $p < 0.05$ .

## RESULTS and DISCUSSION

No significant differences in daily food intake were observed between the control and DHEA-treated groups, while body weight gain in a group of rats fed DHEA was lower than that of the control (data not shown). Total adenine nucleotide pool (ATP + ADP + AMP) in liver was approximately two-fold lower than in skeletal muscle and heart. However, no significant effect of DHEA on ade-

nine nucleotide pool was found in these tissue (Fig. 1). Liver NAD concentration was increased significantly after feeding the DHEA diet (0.3%) for 7 consecutive days (Fig. 2). In contrast, heart and skeletal muscle NAD pool was decreased after DHEA treatment (Fig. 2). It is known that adenosine 5'-diphosphoribose (ADPR) is the major product formed by acidic cleavage of NADH during the tissue extraction [9]. Moreover, under our experimental conditions NADH (added to tissue extract) is hydrolyzed quantitatively to ADPR. Therefore, the measured ADPR level reflects NADH concentration in the tissues. As shown in Figure 3, despite a significant increase in liver NAD concentration, ADPR concentration was not affected by DHEA-treatment, therefore, it is conceivable that

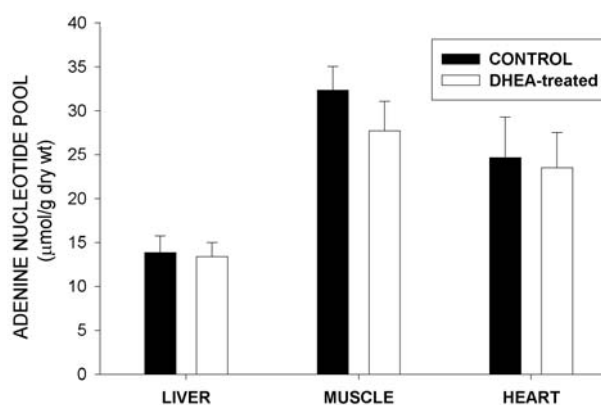


Fig. 1. Effect of DHEA feeding on adenine nucleotide pool in liver, skeletal muscle and heart. Experimental conditions are detailed in Materials and Methods

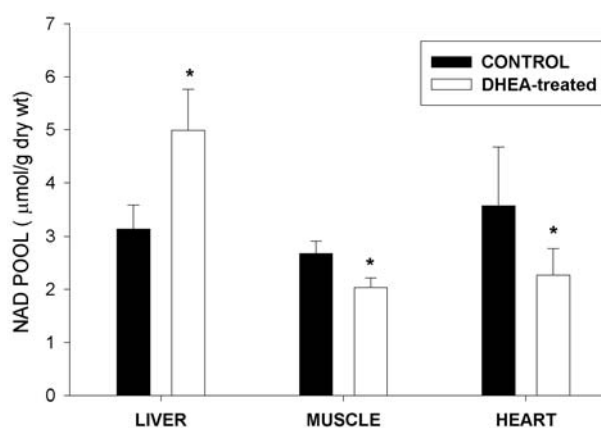


Fig. 2. Effect of DHEA feeding on NAD pool in liver, skeletal muscle and heart. Experimental conditions are described in Materials and Methods. \* significantly different from the control ( $p < 0.05$ )

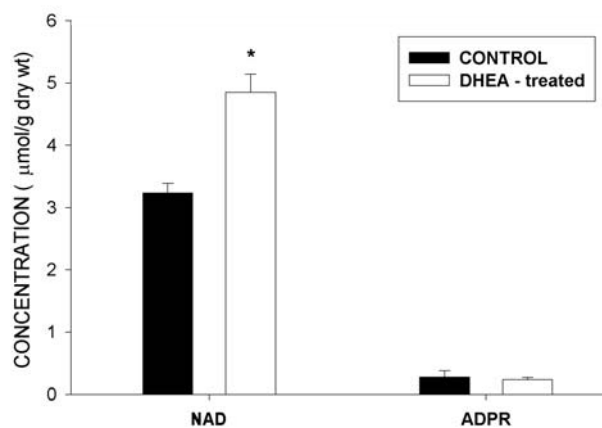


Fig. 3. Effect of DHEA feeding on NAD and adenosine 5'-diphosphoribose (ADPR) in liver. Experimental conditions are listed in Materials and Methods. \* significantly different from the control ( $p < 0.05$ )

NADH concentration is not affected by DHEA-treatment. Thus, the NAD/NADH ratio (calculated from NAD/ADPR) was increased significantly by DHEA (in control NAD/NADH = 11.5; in DHEA-treated animals NAD/NADH = 20.2). This indicates a shift of the redox couple (NAD/NADH) towards oxidation in the DHEA-treated animals. This is in contrast to the results reported by Casazza et al. [3], who described that neither cytoplasmic nor mitochondrial NAD/NADH ratio was affected by DHEA. The discrepancy between our results and those reported by Casazza et al. [3] might be due to differences in the feeding schedule. In our experiments rats were treated for 7 days with 0.3% (w/w) DHEA mixed into the diet. In Casazza et al. protocol [3] rats were treated for 16 days with 0.6% (w/w) DHEA mixed into the diet. Moreover, the discrepancy might be due to differences in the methodological approach. Casazza et al. [3] calculated free NAD/NADH ratio from near equilibrium methods based on measurements of the concentrations of oxidized and reduced substrates of lactate (pyruvate and lactate) and  $\alpha$ -hydroxybutyrate (acetoacetate and  $\alpha$ -hydroxybutyrate) dehydrogenases [27]. We measured total concentration of NAD and NADH (the last as ADPR) in liver *in situ* (liver edge was frozen *in situ* with aluminium clamps precooled in liquid nitrogen). It should be noted that the ratios found for the free NAD/NADH differ greatly from those recorded for the total NAD/NADH [27]. Thus, we believe, that our measurement reflects the true total value of NAD/NADH ratio *in situ*. The difference in the NAD/NADH ra-

tio found between the control and DHEA-treated rats could be due to an increase in NAD synthesis and/or to mitochondrial and peroxisomal proliferation [15], which both increase the capability of NADH oxidation in the liver.

We also observed significant increase in NADP in liver of DHEA-treated animals (Tab. 1). A large increase in the total amount of free CoA in the liver was also found (Tab. 1), which agrees with the data reported previously by Mohan and Clearly [16]. Significant increase in NAD and free CoA concentrations in the liver (Tab. 1) will favor fatty acid oxidation and consequently loss of body fat stores. This may explain in part the antiobesity effect of DHEA [1, 4, 5, 24]. The increase in NAD and CoA concentrations in liver may also explain the elevated rates of mitochondrial respiration [16]. Despite the elevated state 3 respiration, ATP concentration did not change after DHEA treatment (Tab. 1). This is consistent with the suggestion that some of the effects of DHEA on energy balance may be due to an influence of the steroid on futile fatty acid cycling (i.e. acylation and deacylation of fatty acid) [1]. No effect of DHEA treatment on the concentrations of adenosine, adenine nucleotides (ATP, ADP, AMP) and GTP in liver (Tab. 1) suggest that the anticarcinogenic effect of DHEA could not be due to a relative deficiency of nucleosides for DNA synthesis, as was suggested previously [7]. However, since NAD is involved in the cellular defense against DNA damage by genotoxic compounds, as a substrate for poly(ADP-ribose)polymerase [6, 10], it is very likely that the anticarcinogenic effect of DHEA could be related in part to the increase in NAD concentration in the liver caused by this steroid.

Recently, it has been shown that NAD synthesized from tryptophan in hepatocytes was significantly elevated in clofibrate-treated rats [19, 20]. Similarly to clofibrate, other peroxisome proliferators like di-2-ethylhexyl phthalate (DEHP) revealed an increase in NAD synthesis from tryptophan but not from nicotinic acid in rat hepatocytes [21]. It is likely, therefore, that DHEA, a peroxisome proliferator as well, also increases the biosynthesis of NAD from tryptophan in rat liver and consequently causes increase in NAD concentration. Increased concentration of NADP could be simply the consequence of higher concentration of NAD, since NADP is synthesized from NAD. A striking finding of the experiments presented in this paper

is that DHEA feeding caused different effects on the NAD pool in liver and muscle (both skeletal and heart). Based on the data presented above, it is difficult to explain these differences. We can only speculate that significant DHEA-evoked increase in NAD(P) synthesis in liver results in a decrease in the availability of substrate (tryptophan, nicotinate, nicotinamide) concentration necessary for NAD(P) synthesis in heart and skeletal muscle. However, it cannot be excluded that NAD increase in liver after DHEA treatment is directly related in some way to peroxisome proliferation, the phenomenon which is limited to certain tissues such as liver and kidney and can be considered both as the consequence as well as the cause of multiple biological responses [13].

It can be concluded that DHEA given at a pharmacological dose (0.3% in the diet) for 7 consecutive days increases NAD, but is without effect on NADH concentration in the rat liver. Consequently the redox couple (NAD/NADH) is more oxidized in the liver of DHEA-treated animals as compared to the controls. The obtained data may explain some of pleiotropic effects of that steroid. Moreover, the finding that DHEA treatment had no effect on liver adenosine, adenine nucleotides (ATP, ADP, AMP) and GTP concentrations refutes the hypothesis that administration of DHEA results in decreasing the availability of nucleosides/nucleotides for DNA synthesis.

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