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Concerning the Mechanism of Increased Thermogenesis in Rats Treated with Dehydroepiandrosterone

Valentina Bobyleva,[1] Nancy Kneer,[2] Monica Bellei,[1] Daniela Battelli,[1] and Henry A. Lardy[2]

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Dehydroepiandrosterone (DHEA) treatment of rats decreases gain of body weight without affecting food intake; simultaneously, the activities of liver malic enzyme and cytosolic glycerol-3-P dehydrogenase are increased. In the present study experiments were conducted to test the possibility that DHEA enhances thermogenesis and decreases metabolic efficiency via trans-hydrogenation of cytosolic NADPH into mitochondrial $FADH_2$ with a consequent loss of energy as heat. The following results provide evidence which supports the proposed hypothesis: (a) the activities of cytosolic enzymes involved in NADPH production (malic enzyme, cytosolic isocitrate dehydrogenase, and aconitase) are increased after DHEA treatment; (b) cytosolic glycerol-3-P dehydrogenase may use both NAD^+ and $NADP^+$ as coenzymes; (c) activities of both cytosolic and mitochondrial forms of glycerol-3-P dehydrogenase are increased by DHEA treatment; (d) cytosol obtained from DHEA-treated rats synthesizes more glycerol-3-P during incubation with fructose-1,6-P_2 (used as source of dihydroxyacetone phosphate) and $NADP^+$; the addition of citrate *in vitro* further increases this difference; (e) mitochondria prepared from DHEA-treated rats more rapidly consume glycerol-3-P added exogenously or formed endogenously in the cytosol in the presence of fructose-1,6-P_2 and $NADP^+$.

KEY WORDS: Dehydroepiandrosterone: glycerol 3-phosphate production and consumption; $NADP^+$-dependent enzymes; thermogenesis; liver.

INTRODUCTION

Cellular thermogenesis has been studied using different models such as cold exposure of animals (Guernsey and Don Stevens, 1977; Goglia *et al.*, 1988), feeding animals a cafeteria diet (Rothwell and Stock, 1988), and treatment with catecholamine and thyroid hormones (Gale, 1973). The adrenal steroid dehydroepiandrosterone (DHEA)[3] has attracted the attention of different scientific groups for its numerous pharmacological effects (for review, see Gordon *et al.*, 1987), one of which is increased thermogenesis (Tagliaferro *et al.*, 1986). This causes DHEA to decrease gain of body weight of animals without affecting dietary intake (Yen *et al.*, 1977). To explain the increase of thermogenesis in the case of DHEA treatment, several possibilities have been proposed: the increase of peroxisomal β-oxidation of fatty acids not coupled with ATP generation (Leighton *et al.*, 1987; Cottam *et al.*, 1989; Frenkel *et al.*, 1990; Bellei *et al.*, 1992) and the increase of liver mitochondrial oxidation as a consequence of increased mitochondrial mass and change of their configuration (Miller *et al.*, 1988; Mohan and Cleary, 1988, 1989, 1991; Frenkel *et al.*, 1990; Mohan *et al.*, 1990; Bellei *et al.*, 1992). Recently McIntosh and Berdanier (1991) proposed that treatment with DHEA diverts energy-yielding substrates from mitochondrial oxidative phosphorylation toward microsomal xenobiotic

[1]Institute of General Pathology, University of Modena, Italy.
[2]Institute for Enzyme Research, University of Wisconsin-Madison, Madison, Wisconsin.

[3]The abbreviations used are: DHEA, dehydroepiandrosterone; G-3-P, *sn*-glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate; *p*-CMB, the sodium salt of *p*-chloromercuribenzoic acid; OMA, oxalomalate.

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detoxification and peroxisomal activities via NADPH generated by malic enzyme whose activity is increased many times during this treatment.

In the present study another possibility was investigated. Previous studies (Tepperman *et al.*, 1968; Cleary *et al.*, 1983; Mohn and Berdanier, 1984; Casazza *et al.*, 1986; Lardy *et al.*, 1989) demonstrate that treating animals with DHEA induces some modifications similar to those induced by thyroid hormones (Lee *et al.*, 1959; Tepperman and Tepperman, 1964; Lee and Lardy, 1965; Richert and Westerfeld, 1970; Guernsey and Edelman, 1983; Lardy *et al.*, 1989), e.g., increase of activity of liver malic enzyme and mitochondrial *sn*-glycerol-3-phosphate (G-3-P) dehydrogenase. The increased activity of the latter would permit electron transfer from cytosolic NADH to oxygen by a pathway that bypasses the NADH-ubiquinone sequence of nonheme iron enzymes. This bypass would increase heat generation and decrease metabolic efficiency. However, this bypass cannot be responsible for all of the decreased metabolic efficiency observed in rats fed DHEA if it dealt only with the NADH generated in cytosol during glycolysis (Lardy *et al.*, 1989). It has been postulated that electrons might shuttle from mitochondria to cytosol via malate or citrate (Lardy *et al.*, 1965, 1989; Su and Lardy, 1991), two metabolites that generate NADPH in the cytosol, and this NADPH might be utilized to reduce dihydroxyacetone phosphate to *sn*-glycerol-3-phosphate. Support for this hypothesis was obtained in the present study.

MATERIALS AND METHODS

Male Sprague-Dawley strain rats were fed 0.2% DHEA (w/w) in a standard chow diet for a period of 7–10 days before sacrifice (Bellei *et al.*, 1992). Unless otherwise indicated, fed animals have been used for all experiments.

Liver mitochondria were isolated in 0.25 M sucrose as described by Bobyleva-Guarriero *et al.* (1982). Mitochondrial G-3-P dehydrogenase (EC 1.1.99.5) was measured at 37°C as described previously (Gardner, 1974; Wernette *et al.*, 1981).

Cytosol was obtained by centrifuging 10% liver homogenate at 105,000 × *g* for 60 min. The activities of cytosolic enzymes were measured in a total volume of 1 ml at 20°C. Malic enzyme (EC 1.1.1.40) was assayed by the procedure of Launay *et al.* (1974) in the presence of 10 mM malate and 2 mM $NADP^+$. The activity of cytosolic G-3-P dehydrogenase (EC 1.1.1.8) was measured in the presence of 0.2 mM NADPH or NADH and various concentrations of dihydroxyacetone phosphate (DHAP), recording the change of absorbance at 340 nm. Cytosolic isocitric dehydrogenase ($NADP^+$-dependent) (EC 1.1.1.42) activity was measured by following the increase in absorbance at 340 nm in the presence of 10 mM citrate and 2 mM $NADP^+$. Aconitate hydratase (EC 4.2.1.3) activity was measured following the increase in absorbance at 240 nm (Anfinsen, 1955) in the presence of 10 mM citrate. A unit of enzyme activity is defined as the amount that transforms 1 μmol of substrate in 1 min under given experimental conditions.

For measurement of G-3-P produced in cytosol, 0.5 ml of cytosolic fraction was incubated in the presence of 13.3 mM fructose-1,6-P_2, 1.33 mM $NADP^+$, and substrate in the standard buffer indicated below at 30°C for the times indicated in Table I with or without mitochondria. Fructose, 1,6-bisphosphate was used as a source of DHAP in most experiments with cytosol because fructose-1,6-P_2 (Beisenherz *et al.*, 1953) and its metabolites (Borrebaek *et al.*, 1965) may affect the activity of G-3-P dehydrogenase, and using DHAP directly might have obscured some of the regulatory phenomena. Incubation was terminated by addition of 0.06 ml of 60% perchloric acid, the tubes were

Table I. Effect of DHEA Treatment on *sn*-glycerol-3-P Dehydrogenase Activities in Rat Liver[a]

	Cytosol		Mitochondria
	NADH	NADPH	
	munits/mg protein		
Control	72 ± 13	12 ± 3.4	6.5 ± 1.2
DHEA	140 ± 52[c]	25 ± 11[c]	23 ± 6.0[b]

[a]The activities of the cytosolic enzyme were determined as follows: 0.8 ml of cytosol from 10% rat liver homogenate was incubated at 30°C with 0.2 ml of 100 mM fructose-1,6-P_2 for 10 min in order to generate DHAP via the aldolase reaction. Then 0.3 ml of the incubated mixture was added to a cuvette containing 0.2 mM NADH or NADPH and the standard buffer in a total volume of 1 ml. The decrease in absorbance at 340 nm was followed at 20°C and the initial rate of reaction was taken to calculate enzyme activity. The activity of the mitochondrial enzyme was measured as described in the Materials and Methods section. Data are the means ± SD from 3 experiments for the cytosolic enzyme and from 14 experiments for the mitochondrial enzyme. DHEA, dehydroepiandrosterone.
[b]$p < 0.001$ with respect to control.
[c]$p < 0.05$ with respect to control.

centrifuged at 5,000 × *g* for 20 min, and G-3-P was determined in neutralized supernatant (Gerhard and Lang, 1974) using G-3-P dehydrogenase. In order to standardize experimental conditions as much as possible, the medium used for enzyme activity determinations and incubations was always the same and is referred to as the standard buffer. It had the following composition: 100 mM KCl, 10 mM $MgCl_2$, 10 mM Na,K phosphate buffer, and 10 mM Tris(hydroxymethyl)aminomethane-HCl, pH 7.4.

Hepatocytes were isolated and washed in Krebs–Henseleit bicarbonate medium without added Ca^{2+} as described by Kneer *et al.* (1979) and then washed twice in a medium containing 250 mM mannitol, 70 mM sucrose, and 3 mM Hepes, at pH 7.4. The cells were resuspended in the mannitol–sucrose–Hepes medium (approximately 6–8 × 10^7 cells in a total volume of 5 ml) and homogenized with a polytron fitted with a PT-10 generator (Brinkman Instruments) at setting 5 for 15 sec. Cytosolic fractions were obtained from the 15,000 × *g* × 10 min supernatant fractions by centrifugation at 100,000 × *g* for 30 min.

Protein concentrations were measured by the Biuret method (Layne, 1957).

Reagents were of analytical grade. Enzymes and coenzymes were purchased from Boehringer-Mannheim, Indianapolis; DHEA, oxalomalate (OMA), and the sodium salt of *p*-chloromercuribenzoic acid (*p*-CMB) were from Sigma, St. Louis, Missouri.

The obtained data were processed by Student's *t* test.

RESULTS

We searched for possible pathways by which electrons might move from mitochondria to the cytosol and in turn be passed to the electron transport chain via mitochondrial *sn*-glycerol-3-phosphate dehydrogenase. Electrons are shuttled from mitochondria to cytosol via malic and citric acids (Lardy *et al.*, 1965), permitting cytosolic malic enzyme and isocitric dehydrogenase to generate NADPH. Therefore, we sought possible mechanisms by which NADPH could affect the production of G-3-P from DHAP. Casazza *et al.* (1986) found that the cytosolic NADH/NAD ratio is not changed by DHEA treatment. But because DHEA treatment enhances malic enzyme activity manyfold (Cleary *et al.*, 1983; Casazza *et al.*, 1986; Lardy *et al.*, 1989), the potential exists for increased amounts of NADPH being formed and for the possibility that it might reduce NAD^+ and thus provide a means of generating G-3-P for the mitochondrial dehydrogenase. To test this hypothesis, the cytosol obtained from DHEA-treated rats was incubated with NADPH and NAD^+ for 10 min, then 0.2 mM acetoacetate and 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) were added to reveal NADH formed during the previous incubation period (Fig. 1). As shown, no reaction occurred, but when NADH was added to the mixture (dashed line), a decrease in absorbance was observed. These data indicate that there was no transhydrogenation from NADPH to NAD^+ in the cytosol.

In a standard assay system, where a low concentration of DHAP was used, the reaction with NADPH was negligible; however, when the concentration of DHAP was increased, NADPH was oxidized at an appreciable rate (Fig. 2). Moreover, because of an unusually strong inhibition of the enzyme by high DHAP concentration in the presence of NADH, the ratio between the rates of oxidation of NADH and NADPH was progressively decreased with increase of DHAP concentration (at 2 mM it was approximately 90 and at 5 mM, approximately 8; see Fig. 2). When G-3-P was produced in more physiological conditions, i.e., incubating cytosol obtained from control rats for 10 min in the presence of 20 mM fructose-1,6-P_2 as a source of DHAP, and adding 0.8 mM NADPH or 0.8 mM NADH, the amounts of G-3-P produced were equal with each of the coenzymes, i.e., 0.53 ± 0.10 (n = 4) and 0.55 ± 0.23 (n = 7) μmol/ml of cytosol corresponding to 100 mg wet weight of liver. Additional evidence that NADPH may be used in the reaction catalyzed by cytosolic G-3-P dehydrogenase is presented in Fig. 3. In preliminary experiments it was found that 5 mM *p*-CMB does not inhibit the activity of glucose-6-P dehydrogenase (EC 1.1.1.49), but strongly inhibits cytosolic G-3-P dehydrogenase. As is shown in Fig. 3, no change of optical density occurred when cytosol that was not preincubated with fructose-1,6-P_2 was added to the cuvette containing NADPH (trace A) or when cytosol that was preincubated with fructose-1,6-P_2, in order to form DHAP, was added to the cuvette containing both NADPH and *p*-CMB (trace B). When preincubated cytosol was added to the cuvette containing 0.2 mM NADPH, a rapid decrease in absorbance occurred; the reaction ended after about 4 min. When glucose-6-P and *p*-CMB were added to the same cuvette, a slow increase in absorbance was observed

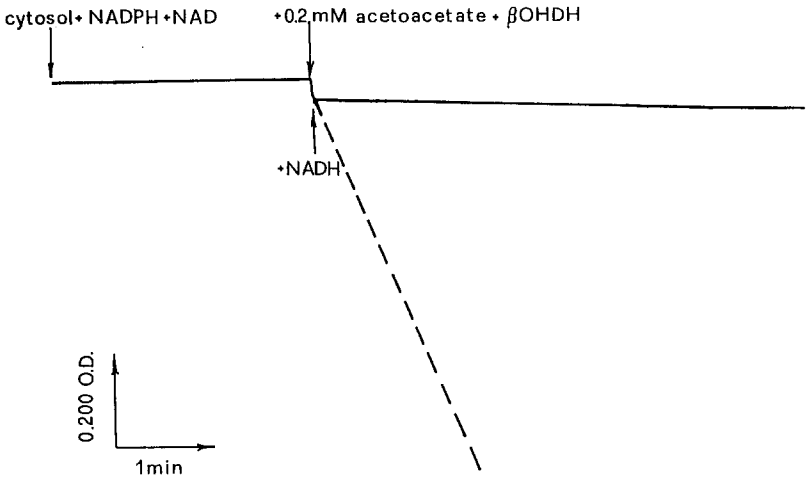

Fig. 1. Representative experiment indicating the lack of NADPH/NAD transhydrogenation in liver cytosol. Cytosolic fractions were prepared from hepatocytes isolated from DHEA-treated rats as described in the Materials and Methods section. Aliquots (0.2 ml of a fraction containing 12 mg of protein/ml) were incubated with 0.2 mM NADPH and 0.2 mM NAD^+ in the standard buffer in a total volume of 1 ml. After approximately 4 min. 0.2 mM acetoacetate and 3-hydroxy-butyrate dehydrogenase (β-OHDH) were added to the reaction mix (upper arrow). Subsequent addition of NADH (lower arrow; recorder reset to origin) resulted in a decrease in absorbance (dashed line), indicating that reduction of acetoacetate could proceed under the conditions described.

(trace C). The increase was much faster when glucose-6-P dehydrogenase was added along with glucose-6-P (trace D). The amount of NADPH produced by the oxidation of glucose-6-P was that consumed before *p*-CMB addition. Trace E shows that similar results have been obtained when liver cytosol from a DHEA-treated rat was incubated in the presence of 10 mM DHAP. Because the reaction catalyzed by glucose-6-P dehydrogenase is exclusively $NADP^+$-dependent, the reactions presented in traces D and E provide evidence for the reduction of DHAP by NADPH with the production of $NADP^+$ in the liver cytosolic fraction. Borrebaek *et al.* (1965) found similar activity with the rabbit muscle enzyme.

Once it was established that DHAP could be reduced by NADPH, the attempt was made to determine whether or not DHEA treatment induces some change in components of the above postulated mechanism. The activities of cytosolic and mitochondrial rat liver G-3-P dehydrogenase in control and DHEA-treated animals are given in Table I. The activity of the cytosolic form was measured in the presence of either NADH or NADPH under conditions where DHAP was produced from fructose-1,6-P_2. Activities of both cytosolic and mitochondrial forms of the enzyme were increased after DHEA treatment. Moreover, the activity of the cytosolic enzyme was increased with both NADH and NADPH. Table II shows that DHEA treatment resulted in enhancement of the activities of cytosolic enzymes connected with NADPH production, i.e., malic enzyme, isocitric dehydrogenase, and aconitase (cf. Su and Lardy, 1991).

G-3-P production by cytosol incubated in the presence of fructose-1,6-P_2 and $NADP^+$ was also increased by DHEA treatment (Table III). The increase was observed both in the presence or absence of exogenously added $NADP^+$-dependent enzyme substrates. The addition of 10 mM malate did not enhance G-3-P production above that observed in systems without exogenous substrate, whereas the presence of 10 mM citrate enhanced G-3-P production both in control and in DHEA-treated samples. When the metabolism of citrate was inhibited by the addition of oxalomalate (OMA) (Ruffo and Adinolfi, 1963; Adinolfi *et al.*, 1971), the production of G-3-P was decreased both in control and DHEA-treated samples in comparison with samples without inhibitor. However, in DHEA samples the amount of G-3-P was higher than in controls The addition of malate to incubation medium containing citrate and OMA did not cause any change.

In preliminary experiments incubating mitochondria for 9 min in the presence of 10 mM pyruvate and 10 mM $NaHCO_3$, it was found that about 98% of the total citrate and malate formed from pyruvate moved into extramitochondrial space and their concentrations corresponded to 0.3 and 0.6 mM, respectively (data not shown). Since these concentrations are very near to physiological concentrations of malate and citrate (Bergmeyer, 1974) and in order to avoid as much as possible the effects of endogenous substrates, the experiments presented in Table IV were performed with rats fasted for 24 h. In the presence of fructose-1,6-P_2 and $NADP^+$, 0.3 and 10 mM citrate significantly increased G-3-P production by cytosol of control rats, whereas no significant change was observed with the same concentrations of malate. In the case of cytosol obtained from treated rats the increase of G-3-P production was observed either with citrate or malate

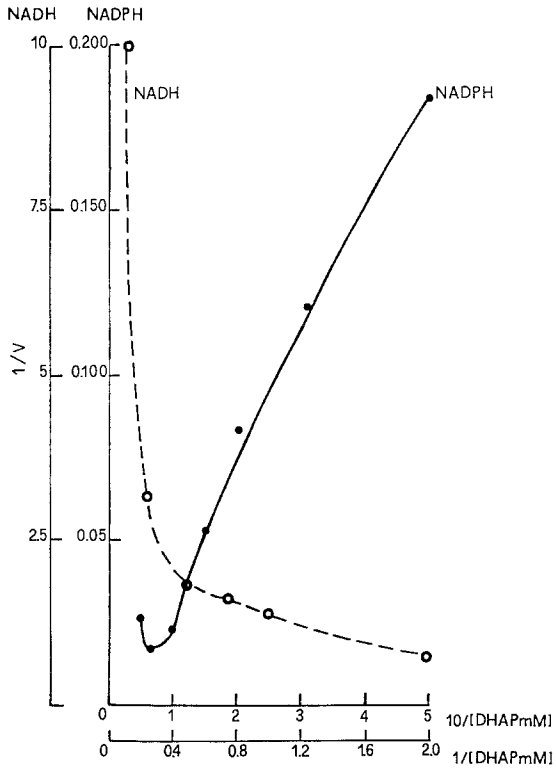

Fig. 2. Double reciprocal plot of *sn*-glycerol-3-P dehydrogenase activity of cytosolic liver enzyme. Cytosolic fractions were prepared from hepatocytes isolated from control rats as described in the Materials and Methods section. Enzyme activity was measured in aliquots of cytosol (containing 15 mg of protein/ml) in the standard buffer in a total volume of 1 ml. Upper abscissa; 0.04 ml aliquots were assayed in the presence of 0.2 mM NADPH; lower abscissa: 0.005 ml aliquots were assayed in the presence of 0.2 mM NADH. Activity was observed as a decrease in absorbance at 340 nm and expressed as nmol of NADPH or μmol of NADH consumed in 1 min by 1 mg of protein. DHAP, dihydroxyacetone phosphate. (●) NADPH; (○) NADH.

taken in low and high concentrations. Moreover, the total amount of G-3-P produced by cytosol from treated animals was higher than that produced by cytosol from control rats.

In agreement with previous work and with the data of Table I which show that mitochondrial G-3-P dehydrogenase was increased after DHEA treatment, mitochondria prepared from DHEA-treated rats and incubated for 2 min in the presence of 0.45 mM G-3-P, 2 μM rotenone, and 2.7 mM ADP metabolized about 4 times more G-3-P than control mitochondria incubated under the same conditions [0.14 $\pm$ 0.02 and 0.031 $\pm$ 0.018 μmol/mitochondria from 100 mg liver weight, respectively (n = 7), $p < 0.001$]. The results obtained with the partially reconstructed system, when mitochondria, ADP, and rotenone were added to cytosol prepared from DHEA-treated animals preincubated for 7 min with fructose-1,6-P_2, are presented in Table IV. Also, in this case, when exogenous substrates were added to the system, mitochondria prepared from DHEA-treated rats consumed more G-3-P than control mitochondria. This difference was much higher when citrate was added to the system. The differences were not so conspicuous as those observed when mitochondria were incubated with added G-3-P in the presence of rotenone. It must be noted, however, that the results presented in Table IV were obtained in a more complex and dynamic system where simultaneous production and consumption of G-3-P were occurring.

DISCUSSION

The results of Figs. 2 and 3 indicate that rat liver cytosolic G-3-P dehydrogenase may use both NAD^+ and $NADP^+$ as coenzymes. This is not surprising for "NAD^+-dependent" dehydrogenases. Walsh and Sallach (1965) found that 3-phospho-D-glycerate dehydrogenase from chicken liver may reduce phosphohydroxypyruvate in the presence of either NADH or NADPH. Furthermore, Dr. Seung-Hee Huang found low ionic strength and low pH to increase the effectiveness of NADPH for reduction of pyruvate and DHAP in the presence of lactate and G-3-P dehydrogenases, respectively (unpublished data, this laboratory). In the present study it was found that G-3-P production by cytosol incubated with fructose-1,6-P_2 as a source of DHAP was almost the same with either NADH or NADPH added in nonlimiting amounts. These incubations were performed at pH 7.4 and in isoosmotic conditions. Therefore, it may be concluded that when both substrates (DHAP and NADPH) of the reaction catalyzed by cytosolic G-3-P dehydrogenase are not limiting, the possibility of G-3-P formation in the presence of NADPH really does exist. There is no doubt, however, that the reactions of NADPH with "NAD^+-dependent" dehydrogenases are strictly controlled *in vivo*, for the triphosphonucleotide pair is maintained at 10^5 more reduced than the NADH/NAD^+ system (Veech *et al.*, 1969) despite their identical standard redox potentials.

Previous results (Tepperman *et al.*, 1968; Cleary *et al.*, 1983; Su and Lardy, 1991) and results of this study (Table II) indicate that during DHEA treatment the activities of enzymes involved in NADPH produc-

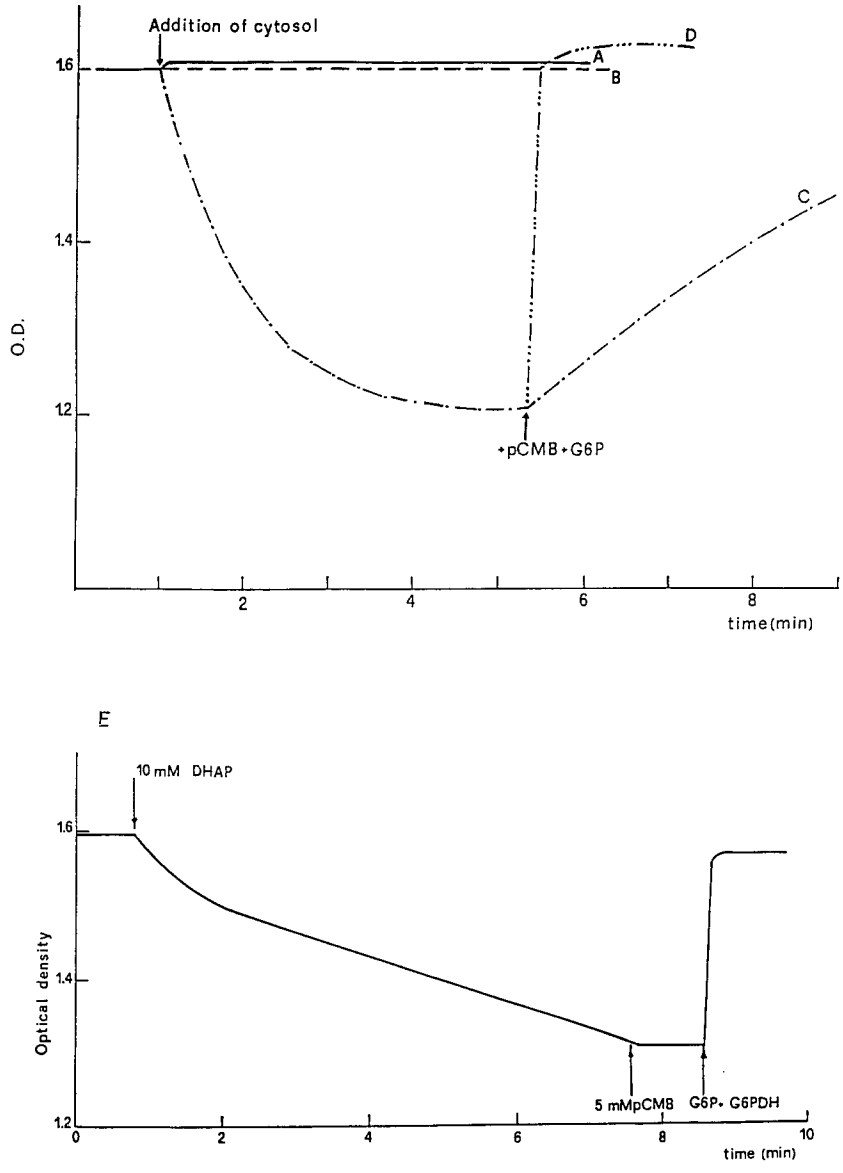

Fig. 3. Identification of NADP as a product of reaction catalyzed by cytosolic *sn*-glycerol-3-P dehydrogenase. Assays were conducted with aliquots of cytosol from control rats (traces A,B,C, and D) and from DHEA-treated rats (trace E) in the presence of 0.2 mM NADPH in the standard buffer in a total volume of 1 ml as described in the Materials and Methods section. In the control series of traces, 0.3 ml of cytosol (containing 11 mg of protein/ml) was used; for traces B,C, and D, the cytosol was incubated first at 30°C with 20 mM fructose-1,6-P_2, to produce DHAP, and then added to the assay cuvette. In trace E, 0.04 ml of cytosol (containing 12 mg of protein/ml) was used. DHAP, dihydroxyacetone phosphate; *p*-CMB, *p*-chloromercuribenzoic acid; G6P, glucose-6-P; G6PDH, glucose-6-P dehydrogenase. (A) ——, cytosol (not preincubated), no fructose-1,6-P_2; (B) – – – –, cytosol + 5 mM *p*-CMB; (C) —·—·—, cytosol + (*p*-CMB + 5 mM glucose-6-P after 5 min); (D) —···—···, cytosol + (*p*-CMB + 5 mM glucose-6-P + glucose-6-P dehydrogenase after 5 min); (E) ——, DHEA cytosol; reaction started by 10 mM DHAP.

tion are increased. These effects along with the increased NADPH-dependent cytosolic G-3-P dehydrogenase activity (Table I) may enhance G-3-P production in the cytosol. In fact, Tables III and IV show that in cytosol from DHEA-treated rat liver the amount of G-3-P synthesized during incubation is increased in comparison with controls. Citrate is far more effective than malate in enhancing G-3-P formation despite the fact that NADPH-generating enzymes for both of these substrates are enhanced. This difference cannot be explained by allosteric activation of G-3-P dehydrogenase by citrate because no increase in G-3-P was observed in the presence of malate and oxalomalate which inhibits the metabolism of citrate. The reason for the difference is that pyruvate, a product of the malic enzyme reaction, will react with any NADH present in the cytosol fraction and poise the G-3-P/DHAP pair in favor of the oxidized member. On the other hand, α-ketoglutarate, a product of the isocitrate dehydrogenase reaction, provides no means of oxidizing NADH. *In vivo*, the utilization of pyruvate by mitochondria would permit malate to be at least as effective as citrate. Thus, the first part of this study supports the previously postulated possibility (Lardy *et al.*, 1989) that the flux of electrons through the G-3-P shuttle is enhanced in cytosol from liver of DHEA-treated animals.

The experiments in which mitochondria prepared

Table II. Effect of DHEA Treatment on Activities of Cytosolic Enzymes Connected with NADPH Production[a]

	Malic enzyme	Isocitric dehydrogenase	Aconitate hydratase
	munits/mg protein		
Control	6.0 ± 2.7 [16]	7.8 ± 4.3 [5]	22 ± 4.8 [5]
DHEA	38 ± 17[b] [16]	20 ± 3.6[c] [5]	37 ± 3.8[b] [5]

[a]Procedures for obtaining liver cytosolic fractions from control and DHEA-treated rats and conditions for measuring enzyme activities are described in the Materials and Methods section. Values are the means ± SD from the number of experiments given in brackets.
[b]$p < 0.001$ with respect to control.
[c]$p < 0.01$ with respect to control.

from treated animals were added to medium containing G-3-P, either exogenously supplemented (see Results section) or formed from fructose-1,6-P_2 in the presence of malate or citrate in physiological concentrations (Table IV), indicate that G-3-P is consumed more rapidly by mitochondria prepared from DHEA-treated animals thus performing transhydrogenation of cytosolic NADPH into mitochondrial $FADH_2$ with loss of one site of phosphorylation on the respiratory chain and consequent loss of energy as heat. It has to be stressed that all the results are expressed per unit of liver weight in order to obtain information on the possible situation in whole liver and not on the potential capacity of mitochondria to consume G-3-P calculated on the basis of protein concentration in the mitochondrial fraction. This was done also for the following reason: although the amount of protein in the fraction prepared from DHEA-treated rats (49 ± 4 mg/g wet weight, $n = 10$) was higher than that in the controls (32 ± 4 mg/g wet weight, $n = 10$, $p < 0.001$), electron microscopy and biochemical study (Bellei *et al.*, 1992) revealed that liver hypertrophy, observed during DHEA treatment (Cleary *et al.*, 1983, 1984; Shepherd and Cleary, 1984) is due not only to increased amount of mitochondria as observed by Miller *et al.* (1988) and by Mohan and Cleary (1988), but also to increased number and size of peroxisomes (Frenkel *et al.*, 1990; Bellei *et al.*, 1992). Since the dimension of peroxisomes is near to that of mitochondria, they may precipitate in the same range of centrifugation force as do mitochondria. This causes an increase of total protein in the mitochondrial fraction used.

Table III. *sn*-Glycerol-3-P Synthesis by Liver Cytosol in the Presence of $NADP^+$[a]

Addition	Control	DHEA
	μmoles G-3-P/tube formed in 9 min	
None	0.36 ± 0.05	0.48 ± 0.08[b]
10 mM malate	0.37 ± 0.08	0.52 ± 0.14[c]
10 mM citrate	0.48 ± 0.08	0.68 ± 0.14[d]
10 mM citrate + 0.2 mM OMA	0.27 ± 0.06	0.44 ± 0.10[d]
10 mM citrate + 10 mM malate + 0.2 mM OMA	0.28 ± 0.03	0.48 ± 0.10[d]

[a]Cytosolic fractions (0.5 ml) from 10% rat liver homogenates were incubated with 13 mM fructose-1,6-P_2, 1.33 mM $NADP^+$, 10 mM $MgCl_2$, and 10 mM K·Na phosphate, pH 7.4, in a total volume of 1.5 ml for 9 min at 30°C. Incubations were stopped, sampled processed, and G-3-P measured as described in the Materials and Methods section. Values are the means ± SD from 5 to 8 separate experiments.
[b]$p < 0.01$ with respect to control.
[c]$p < 0.02$ with respect to control.
[d]$p < 0.05$ with respect to control.

In conclusion, the present data support the previously advanced hypothesis (Lardy *et al.*, 1989) and demonstrate a prominent role of citrate in enhancement of cytosolic G-3-P production which is further increased by DHEA treatment. Increased activities of cytosolic malic enzyme and $NADP^+$-dependent isocitrate dehydrogenase and of *sn*-glycerol-3-phosphate dehydrogenase may increase the cytosolic branch of the G-3-P shuttle, thus decreasing utilization of DHAP by the usual glycolytic pathway. G-3-P in turn is more rapidly oxidized by mitochondria. Since intramitochondrial oxidation of G-3-P proceeds via FAD^+-dependent dehydrogenase, a decrease in the efficiency of oxidative phosphorylation and consequent enhancement of heat production occurs. Moreover, the fact that a higher amount of G-3-P is produced in cytosol of treated rats in the presence of physiological concentrations (0.3 mM) of citrate and malate, which may be produced and brought out of mitochondria during their incubation with pyruvate, may contribute to the observed magnitude of thermogenesis (Tagliaferro *et al.*, 1986) and weight loss (Yen *et al.*, 1977).

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Table IV. Effect of DHEA Treatment on Production and Consumption of *sn*-Glycerol-3-P in Rat Liver[a]

Source substrate	[G-3-P] in cytosol after 9 min of incubation		Decrease produced by addition for 2 min of mitochondria to preincubated cytosol of treated samples	
	Control	DHEA-treated	Control mitochondria	Treated mitochondria
	μmoles/tube			
None	0.20 ± 0.03	0.20 ± 0.03	0.015 ± 0.010	0.047 ± 0.016[f]
0.3 mM citrate	0.43 ± 0.04[c]	0.54 ± 0.03[b,c]	0.097 ± 0.017	0.151 ± 0.016[d]
10 mM citrate	0.40 ± 0.05[c]	0.74 ± 0.09[b,c]	0.175 ± 0.023	0.252 ± 0.022[e]
0.3 mM malate	0.24 ± 0.02	0.34 ± 0.03[b,c]	0.059 ± 0.018	0.090 ± 0.007[f]
10 mM malate	0.22 ± 0.04	0.42 ± 0.05[b,c]	0.060 ± 0.030	0.110 ± 0.060

[a]Animals were fasted for 24 h. Cytosolic fractions (0.5 ml) from 10% rat liver homogenates were incubated with 13 mM fructose-1,6-P_2 and 1.33 mM $NADP^+$ in the standard buffer (total volume of 1.5 ml) for 9 min at 30°C. When the consumption of *sn*-glycerol-3-phosphate was studied, 0.1 ml of mitochondria corresponding to 100 mg of fresh tissue, 4 μmol of ADP, and 2 μM rotenone were added to the cytosol from treated rats preincubated for 7 min, and the incubations were continued for another 2 min. Incubations were terminated, samples processed, and G-3-P measured as described in the Materials and Methods section. The numbers represent mean ± SD of 5 separate experiments, where the incubations were performed in duplicate.

[b] $p < 0.001$ with respect to control cytosol.

[c] $p < 0.001$ with respect to endogenous sample.

[d] $p < 0.001$ with respect to sample with control mitochondria.

[e] $p < 0.005$ with respect to sample with control mitochondria.

[f] $p < 0.05$ with respect to sample with control mitochondria.

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