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Cardiac impairment induced by oxidative stress in rabbits fed a high-fat diet is counteracted by dehydroepiandrosterone supplementation

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Abstract

The role of oxidative stress in the activating of downstream signalling leading to structural and functional changes in the left ventricle of rabbits fed a high-fat diet for 12 wks and the effect of dietary dehydroepiandrosterone supplementation, were analyzed. The animals showed hyperglycemia, insulin-resistance, dyslipidemia and early features of cardiomyopathy, i.e. altered myosin heavy-chain isoforrns, tissue degeneration and reduced contractility of the papillary muscle. Dietary supplementation with dehydroepiandrosterone (0.02%,wt/wt) in these animals reduced oxidative stress evaluated as levels of reactive oxygen species and hydroxynonenal levels, without affecting high plasma glucose level or insulin resistance. Dehydroepiandrosterone supplementation also counteracted both the activation of redox-sensitive transcription factor and of nuclear trascription factor- kB, and the expression of tumor necrosis factor-alpha and of C-reactive protein-mRNA. Improvement of the oxidative balance counteracted the shift to myosin heavy chain isoforrns, ameliorating the heart contractility. A high-fat diet induced oxidative stress and metabolic syndrome in this rabbit model, and that dehydroepiandrosterone, by restoring oxidative balance, lowering lipid levels and down-regulating inflammation, prevented molecular and functional aIterations of the cardiac muscle, thus exeterting a beneficaI effect and delaying the onset of diabetic cardiomyopathy.

Introduction.

Diabetic cardiomyopathy is characterized by systolic and diastolic dysfunction and has been reported in diabetic patients with no ischemic, vaIvuIar or hypertensive heart disease. Its development includes metabolic disturbances, small-vessei diseases, autonomic dysfunction, insuIin-resistance and myocardiai fibrosis (1,2). RecentIy, an important role in its pathophysiology has been attributed to the generation of reactive oxygen species, which activates a number of secondary-messenger pathways, finally Ieading to cardiac dysfunction (3-5). We recently showed recently that free radical overproduction appears early in human type 2 diabetes (6) and that, in a rat model oftype l diabetes, oxidative damage plays a key role in the early development of cardiomyopathy (7). Antioxidants might counteract insulin resistance associated with type 2 diabetes and cardiovascular diseases (8-lO). We reported elsewhere that in streptozotocin rats, the treatment with dehydroepiandrosterone (DHEA), a compound that possesses multi-targeted antioxidant properties (11-15), prevents myocardial damage induced by oxidative stress, while avoiding impairment of cardiac myogenic factors and the switch to myosin heavy chain expression (16). DHEA also counteracts oxidative imbalance and advanced glycation end-product formation in type II diabetic patients (7).

The study targets diabetic cardiomyopathy, in a model oftype 2 diabetes induced by a high-fat (HF) diet in rabbits, an animaI species that is highIy susceptible to cardiovascular damage (17). Data show that DHEA treatment counteracted biochernicai changes in the Ieft ventricle induced by oxidative stress, improving myocardial function.

Materials and methods

Experimental procedure.

Male New Zealand White rabbits, 15 wks oid (Harlan-ItaIy, Udine, Italy) weighing 3.0 -3.5 Kg were cared for in compliance with the Declaration of Helsinki as revised in 1983, the Italian Ministry of Health Guidelines (no. 86/609/EEC) and with the *Princip!es 01 Laboratory Anima! Care* (NIH no. 85-23, revised 1985). The rabbits were acclimatized for 2 wks prior to the experiment in a room with 12h 1ight dark cycle, individually housed in stainless stee1 cages in a temperature- and humidity-

controlled room (23 ± 3°C, 50 ± 5%) and fed 100 g per day of standard rabbit non-purified diet, composition of 100 g: 16.2 g crude protein, 3.0 g crude fat, 7.3 g crude ash, 14.8 g fiber, 12 g moisture, added mineraI 2.1, 40U /kg vitamin E) with appropriate certificate from the Association of Official Agricultural Chemists, 1975, and relative procedure analyses (Laboratorio Dottori Piccioni, Gessate Milanese, Italy). Rabbits were randomly distributed into four groups. The control group *(C) (n=4)* received standard rabbit diet; the DHEA-alone treated group (C-D) *(n=4)* received the same diet supplemented with 0.02% DHEA (Sigma Aldrich, Milan, Italy); the high-fat group (HF) *(n=4)* received a high-fat diet, consisting of standard rabbit non purified diet, composition of 100g: 16.2 g crude protein, 7.3 g crude ash, 14.8 g fiber, 12 g moisture, added mineraI 2.1, 40U/kg vitamin E) plus lO g added fat (6.7g com oil and 3.3 g lard) (18); the HF plus DHEA group (HF-D) *(n=4)* received the high-fat diet supplemented with 0.02% DHEA. Two days before being killed, the rabbits were fasted'ovemight and the glucose tolerance test was performed. The rabbits were killed at 3 mo from the start ofthe experiment, by aortic exsanguination after anesthetization with Zoletill00 (Tiletamine-Zolazepam, Virbac, Carros, France). Blood was collected and plasma and serum were isolated. The heart was rapidly excised and weighed, and portions of left ventricle were taken to obtain nuclear and total extracts. Other heart portions were utilized for histological microscopy. The papillary musc1es were immediately removed for functional parameter detection.

Generai parameters.

Body weight and length of rabbits were measured at time zero and prior to death. The body mass index (BMI) was calculated as body weight in kilograms divided by body length in meters, squared ($kglm^2$).

Oral glucose tolerance test

After a fasting peri od of 12h, a 50% glucose solution was orally administered to the rabbits at 1.5 g/Kg. Blood samples were collected via the auricular artery before 15, 30, 45, 60, 90, 120 and 240 min after glucose loading. Glucose levels were tested using an Accu-Check Compact kit (Roche Diagnostics Gmbh, Mannheim, Germany)

Parameters in plasma.

Triglyceride (TG), total cholesterol (Te), low density-lipoprotein (LDL)- and high-density-lipoprotein (HDL)-cholesterol were determined by standard enzymatic procedures using reagents kits (Hospitex Diagnostics, Florence, Italy). Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were determined using an enzymatic kit (DiaSys Diagnostic Systems GmbH, Holzheim, Germany). Plasma insulin was measured with an ultrasensitive insulin enzyme-linked immunosorbent assay kit rrom DRG Diagnostics (Marburg, Germany). Insulin sensitivity was calculated using the homeostasis model assessment (HOMA): fasting glucose (mmol/L) x fasting insulin (U/L)/22.5.

Tissue extracts (cytosolic, nuclear and total extracts).

Cytosolic and nuclear rractions rrom rabbit left ventricle were prepared by the Meldrum *et al.* modified method (19). Briefly, left ventricle (100 mg) was homogenized at 10% (w/v) in a Potter Elvehjem homogenizer (Wheaton Science Products, Millville, NJ, USA) using a homogenization buffer. Homogenates were centrifuged at 1,000 g for 5 min at 4°C. Supematants were removed and centrifuged at 15,000 g at 4°C for 40 min to obtain cytosolic rraction. The pellets were resuspended in extraction buffer and incubated on ice for 30 min for high-salt extraction, followed by centrifugation at 15,000 g for 20 min at 4 C. The resulting supematants containing nuclear proteins were carefully removed and samples were stored at -80°C until use.

Total extract was obtained by homogenizing at 10% (w/v) directly with extraction buffer and were centrifuged at 1,000 g for 5 min at 4°C. Supematants (total extract) were stored at -80°C until use. Protein content was determined using the Bradford assay (20).

Oxidative biochemical parameters.

Reactive oxygen species (ROS) were measured in total extracts using 2',7' -dichlorofluorescein diacetate (DCFH-DA) as a probe (21). Reduced and oxidized glutathione content was measured in cytosolic fractions by Owens's method (22). The difference between total glutathione and GSH content represents the oxidized glutathione (GSSG) content (expressed as *flglmg* proteins.); the ratio

between GSSG content and GSH is considered a good parameter of antioxidant status. 4-hydroxynonenal (HNE) was detected by an HPLC procedure. The extract sample was directly injected for HPLC (Waters Assoc., Milford, MA, USA) using an RP-18 column (Merck, Darmstadt, Germany). The mobile phase used was 42% acetonitrilelbidistilled water (v/v). Serial concentrations ofHNE (0.5-10 Ilmol/L) were used to prepare a standard curve (23). Catalase activity was evaluated in the cytosolic fraction following Aebi's method (24). Total superoxide dismutase (SOD) activity was assayed by the method described by Flohè and Otting (25). Since the activity of xanthine-oxidase may vary, sufficient enzyme was used to produce a rate of cytochrome c reduction of at least 0.025 absorbance units/min in the assay without SODo

Advanced glycation end-products (AGE) **detection with HPLC-MS.**

Cytosol fractions were treated with 6 mol/L hydrochloric acid for 2 h at 40°C and then centrifuged (1860 g) (26); only the supernatant was utilized. A Thermo-Finnigan Surveyor instrument (Thermo Electron, Rodano, Milan, Italy) equipped with autosampler and PDA-UV 6000 LP detector was used. Mass spectrometry analyses were performed using an LCQ Deca XP plus spectrometer, with electrospray interface and ion trap as mass analyzer. The chromatographic separations were run on a Varian Polaris CI8-A column (150 x 2 mm, 3 /lm particle size) (Varian, Leinì, Turin, Italy). Flow rate 200 IlL min-l. Gradient mobile phase composition was adopted: 95/5 to 0/100 v/v 5 *mmol/L* heptafluorobutanoic acid in water/methanol in 13 min. The LC column effiuent was delivered to a UV detector (200-400 nrn) and then to the ion source, using nitrogen as sheath and auxiliary gas (Claind Nitrogen Generator apparatus, Lenno, Como, Italy). The source voltage was set to 4.5 kV in the positive mode. The heated capillary was maintained at 200°C. The acquisition method used had previously been optimized in the tuning sections for pentosidine quasi-molecular ion (capillary, magnetic lenses and collimating octapole voltages) to maximize sensitivity. Collision energy (CE) was chosen to maintain about lO % of the precursor ion. The tuning parameters adopted for the ESI source were: source current 80.00 */lA,* capillary voltage 3.00 V, tube lens offset 15 V; for ion optics, multipole 1 offset -5.25 V, inter multipole lens voltage -16.00 V, multipole 2 offset -9.00 V. Mass

122 spectra were collected in tandem MS mode: MS^2 of (+) 379 *m/z* with 33 % CE in the range 100-400

123 *m/z.*

124 **Western blotting.**

125 NFkB-p65 on cytosol and nuclear extracts ofleft ventricle were detected by Laemmli's method (27).

126 Anti-a-actinin antibodies served as loading contro l for cytosolic proteins and anti-Lamin-Bl for

127 nuclear NFkB. Specific bands were quantified by densitometry using analytic software (Bio-Rad,

128 Multi-Analyst, Munchen, Germany) and the net intensity of bands in each experiment was

129 normalized for the intensity of the corresponding a-actinin or lamin-Bl bands, before comparison

130 between control and treated samples.

131 **RNA isolation and RT -PCR .**

132 Total RNA was isolated using the RNA fast kit (Molecular Systems, San Diego, CA, USA). Total

133 DNA was amplified using sense and antisense primers specific for the C-reactive protein (CRP) gene

134 (sense 5'-AGGATCAGGATTCGTTTG-3' and antisense 5'-CACCACGTACTTGATATGTC-3'),

135 the tumor necrosis factor alpha (TNFa) gene (sense 5'-AGGAAGAGTCCCCAAACAACCT-3' and

136 antisense 5'-GGCCCGAGAAGCTGAT CTG), the myosin heavy-chain a (MHCa) gene (sense 5'-

13 7 GCCAAGGTGAAGGAGA TGAA-3' and antisense 5' -CTCTCCTGGGTCAGCTTCAG-3 '), the

138 myosin heavy-chain ~ (MHC~) gene (sense 5'-GGTCGAATACGTTACCATCTG-3' and antisense .

139 5' -AA TCGCTGTCCACAGTGGTCG-3') or far the glyceraldehyde-3-phosphate dehydrogenase

140 (GAPDH) gene (sense 5'-CGCCTGGAGAA AGCTGCTA-3' and antisense 5'-

141 CCCCAGCATCGAAGG TAGA-3').

142 The PCR reaction system contained lJlL ofRT product, 200 jlillol/L dATP, dGTP, dCTP and dTTP

143 (Finnzymes, Espoo, Finland), 1.25 units of *Taq* DNA polymerase (Finnzymes, Espoo, Finland) and

144 50 pmol of sense and antisense primers in a total volume of 50 JlL. AH experiments were performed

145 on at least three independent cDNA preparations.

146 PCR products were e1ectrophoresed on 2% agarose gels and amplification products were stained with

147 GelStar nucleic acid gel stain (FMC BioProducts, Rockland, ME, USA). Ge1s were photographed

148 and ana1yzed with Kodak 1D Image Analysis software. The net intensity ofbands in each experiment

was normalized for the intensity of the corresponding GAPDHband before comparison between control and treated samples.

Histological staining.

For standard histology, portions of left ventric1e were fixed in 4% neutralized formalin. Fixed materia1 was processed for hematoxylin and eosin staining. Six-micron paraffin-wax sections of left ventric1e were used.

Isolated papillary muscle and contractility determination.

Papillary muscles were driven at constant frequency (120 beats/min) with a pair of electrodes connected to a 302 T Anapulse Stimulator via a 305-R Stimu1us Isolator (W.P. Instruments, New Haven, CT, USA) operating in constant current mode. Isometric twitches were evaluated by a Harvard transducer (60-2997), visualized on a Tektronix 2211 digital storage oscilloscope and continuously acquired and recorded in a Power Mac computer, using Labview Software (Nationai Instruments Corp., Austin, Texas, USA). The same software was used to measure developed peak mechanical tension (T max), maximum rate ofrise and faH ofdeveloped mechanical tension (+dT/dt max and *-dT/dt* max), time-to-peak mechanical tension (ttp) and duration of contraction.

Statistical analysis.

Results are expressed as means ± SD. ANOV A and post-hoc ana1yses inc1uded Bonferroni's test were used (28). The SPSS 14.0 package for Windows (SPSS Inc., Chicago, IL) was used for the statistical analysis. A value of$P<0.05$ was viewed as statisticaHy significant.

Results.

Generai features. Body weight, abdomen length, glucose and insulin were significantly increased in rabbits fed a HP diet versus control values ($P<0.05$) after three months' treatment. DHEA supplementation did not modify these value **(TABLE** 1). Body mass index (BM1) and HOMA were

] 72 also significantly higher in HP rabbits than in controls *(P<0.05)* but not different rrom those of HF
] 73 plus DHEA rabbits. The ratio heartlbody weight ratio was the same in all groups, whereas the heart
] 74 weight of rabbit fed the HF increased vs controls; DHEA supplementation reduced this increase.
175 The results ofthe glucose tolerance test are in **FIG. 1.** After oralloading, glucose levels remained a
176 high level for up to 240 min, in the HP and DHEA treated HF groups. TG, TC and plasma LDL-
] 77 cholesterol concentrations were significantly higher in HF rabbits versus controls *(P<O.OI)* and were
] 78 lower in HF plus DHEA rabbits than in the HP alone group. (P<0.05). HDL-cholesterol was also
] 79 significantly lower *(P<O.O* l) in the HF group versus controls; in the HF plus DHEA group its value
]80 was similar to that of control animals *(P<0.05).*

] 81 **Oxidative parameters in the left ventricle.**

] 82 Rabbits fed a HP diet for 3 mo showed a significant increase in ROS levels in total extract of left
183 ventricle vs the contro 1 group *(P<O.OI).* 4-hydroxynonenal, an end-product ofIipid peroxidation, also
184 significantly increased in HP rabbits *(P<O.OI)* **(FIGURE** 2). In rabbits fed with HF plus DHEA, the
185 ROS and HNE levels were significantly lower than in the HP rabbits *(P<0.05).* Moreover, total SOD
186 and catalase activities were also increased in the HP rabbits, and DHEA supplementation partially
187 restored these activities to control levels. No significant difference in the GSSG/GSH ratio was
188 observed among groups (data not shown). The leve l ofpentosidine was significantly lower in the HF
189 plus DHEA than in the HF alone group *(P<0.05)* **(TABLE** l). In samples rrom both control and
190 DHEA groups, gas-mass-HPLC analysis failed to detect any peak for pentosidine, apparently
191 indicating its absence in these animals.

192 **NFkB-p65.**

193 Westem Blot analysis detected NFkB-p65 protein in the nuclear and the cytosolic rractions of left
194 ventricle **(FIGURE 3, panel A and panel** B). Nuclear p65-NFkB of HF rabbits was increased
195 versus the contro l groups *(P<0.05)* **(panel** A) while p65-NFkB protein was reduced in the cytosolic
196 rraction of HF rabbits *(P<0.05)* **(panel** B). In the rabbits fed the HF plus DHEA diet, cytosolic p65
197 protein content was less markedly reduced than it was in the HF group, corresponding to an increase

198 of the nuclear level of p65, and thus indicating 10wer activation of the NFkB transcription factor
199 *(P<0.05).*
200 Inflammatory parameters.
201 Both indices ofthe proinflammatory state, TNF-a and CRP, weresignificantIy increased in the heart
202 ofrabbits fed the HF diet *(P<O.OI)* (TNFa-: FIGURE 3, panel C and CRP: FIGURE 3, panel D).
203 The HF plus DHEA diet significantly decreased expression of both TNF-a. and CRP, vs the HF
204 group *(P<0.05).*
205 Myosin expression.
206 PCR analysis was used to evaluate expression of two isofonns (a and ~) of the myosin heavy-chain
207 (MHC) protein (FIGURE 4) in the Ieft ventricle of control, DHEA, HF and HF plus DHEA rabbits.
208 The HF diet detennined a significantly *(P<O.OI)* decreased expression of a-MHC and an increased
209 expression of ~-MHC. When DHEA was added to the HF diet, a-MHC was brought closer to the
210 controi value, and ~-MHC was reduced versus the contro 1 value *(P<0.05).*
211 Necrosis markers
212 LDH and AST release were evaIuated in the plasma (FIGURE 5). Both LDH (panel A) and AST
213 (panel B) significantly increased in HF rabbits *(P<O.OI).* In the HF plus DHEA rabbits, the levels of
214 LDH and AST were significantly lower than in the HF alone rabbits *(P<0.05).*
215 HistoIogicaI anaIysis.
216 In the Ieft ventricle of the HF rabbits, histological preparations clearly showed extensive and diffuse
217 lipid deposition (FIGURE 6, panel C). This lipid infiltration was not observable in either the control
218 or the DHEA alone group (panel A and panel B). Tissues obtained from HF plus DHEA rabbits
219 (panel D) showed rare areas of slight lipid deposition.
220 Cardiac function.
221 The contractile force developed by electrically-driven papillary muscles was evaluated in basaI
222 conditions (TABLE 2). BasaI contractility was weaker in papillary muscles from HF rabbits versus

controls; this was evident not only for maximal developed mechanical tension (Tmax: *P<O.OI* by ANOV A), but also for maximum rate of rise (+dT/dtmax; *P<O.OI)* and maximum rate of fall of developed mechanical tension (-dT/dtmax; *P<0.01).* In contrast, no significant difference was found between papillary muscles rrom control and HF rabbits in regard to time to peak mechanical tension (TPT) or duration of contraction. Treatment with DHEA significantly reduced the effects of the HF diet *(P<0.05).* However, DHEA did not per se affect contractile properties ofthe papillary muscles.

Discussion

Male New Zealand rabbits fed a HF diet for 3 mo developed abdorninal obesity, hyperglycemia, insu1in-resistance, dyslipedernia, and cardiac dysfunction. DHEA supplementation did not affect the high plasma glucose levels induced by the HF diet, nor did it protect against hyperinsulinemia or the HOMA index. There is conflicting evidence about the effect of supplemental DHEA on glucose metabolism and insulin-sensitivity in healthy human subjects (29,30): our results are in agreement with several human studies showing only slight or no effect of DHEA on glucose homeostasis (31-33). As far as the plasma lipid profile is concemed, when DHEA was added to the HF diet, HDL increased in the plasma while triglycerides, total cholesterol and LDL were reduced. These resu/ts are in agreement with studies reporting that DHEA decreases serum triglycerides and the body weight in hyperlipidemic rats and diabetic mice (34,35). The mechanisms whereby DHEA exerts its anti-lipid effects are incompletely understood. However, it has been reported that the anti-obesity effect ofDHEA may in part be related to changes in lipase activity and in beta-adrenergic receptor density (36,37). Moreover, DHEA accelerates lipid catabolism by direct regulation of hepatic lipid metabolism (38) and also has antiglucocorticoid activity, caused by direct modulation of the hepatic glucocorticoid receptor (39).

However, alongside its effects on lipid metabolism and on insulin axis, we believe that DHEA's key action is against oxidative imbalance (6,7). Indeed, DHEA counteracted the increase in glycoxidative products observed in the plasma ofrabbits fed a HF diet, in agreement with a previous rat study (13-16). Oxidative stress was also observed in the cardiac tissue of HF rabbits, in which there were

significant increases in ROS, end-products of lipid peroxidation (HNE) and activation of transcription factor NFkB. Activation of NFkB, which triggers in the inflammatory cascade, was demonstrated by the increased expression of TNF -alpha and CRP in cardiac tissue. Here we show that DHEA, by decreasing oxidative stress and reducing activationofNFkB transcription factor; may could determine a reduced expression of TNF and of CRP. Moreover, the anti-inflammatory effects of DHEA, due to cytokine reduction caused by decreased NFkB activation, might be amplified by the reduction of cholesterollevels, which in tum directly decreases CRP secretion from the tissues (40). CRP has been shown to predict morbidity and mortality from coronary heart disease (41). Moreover, it has been shown that high dietary cholesterol intake can increase the production of atherogenic inflammatory cytokines, such as IL-6 and TNF-a, and that reducing dietary cholesterol concentration leads to a reduction in CRP production (42).

We suggest that the pro-oxidant and inflammatory states, observed in HF rabbits, cause activation of intracellular signaling responsible for impaired myocardial function, as been reported in cases of heart failure (43). *HP* rabbits showed a switch of cardiac heavy-chain myosin from the alpha to the beta isoform: this event comprises the heart's "molecular motor" because contractile properties depend to a great extent on the isoform composition of MHC proteins. A switch in MHC isoforrn composition has been reported to cause reduced contractile velocity and energy expenditure (44). In man as in animals, a reduced content of a-MHC, which is expressed exclusively in the myocardium, has been reported to be responsible for the reduced myocardial contractility during heart failure (45) and in diabetes (46). In a previous model of type I diabetes cardiomyopathy (7), we reported that DHEA treatment, by avoiding impairment of cardiac myogenic factors and producing a switch in MHC gene expression, protected against tissue damage, an early event in diabeti c cardiomyopathy. DHEA's modulatory effects on MHC expression were also observed in *HP* rabbits: expression of two isoforms, alpha and beta, in DHEA treated rats were not significantly different from those ofthe controi group.

Rabbits on a HF diet clearly showed alterations in papillary muscle contractility. This was shown by altered basaI contractility, including reduced maximal developed tension, maximum rate ofrise, and maximum rate of fall of developed tension, which are signs of diastolic dysfunction. As in the case of rat cardiac muscle (7), these alterations ofthe mechanisms controlling intracellular calcium handling within cardiac myocytes are probably related to the structural damage caused by the HF diet. Histological analysis of tissue ftom HF rabbits showed extensive and diffuse lipid deposition, as reported in other animaI models (47). The increased release ofLDH and AST in the plasma ofH.F. rabbits confirmed myocardial damage. Treatment with DHEA protects the cardiac tissue ftom this altered basaI contractility, as well as minimizing histological changes and reducing cells damage caused by the HP diet.

The role of DHEA in the cardiovascular system has been highlighted by the recent finding, in the human heart, of DHEA production and CYP-17 gene expression, a key factor in DHEA synthesis (48,49) and it has recently been proposed that the vascular protective effect of DHEA might be dependent on G-a-GTP-binding protein mediated activation ofthe phosphatidil-inositoI3-kinase/ Akt signaling pathway (50). Several explanations have been put forth for the multi-targeted antioxidant effects of DHEA, including its effect on catalase expression (51), and its up-regulation of the thioredoxin system (52), of the fatty-acid composition of cellular membranes and of cytokine production. However, the precise mechanisms remain to be clarified, and additional non-antioxidant effects cannot yet be ruled out. Whether the effect ofDHEA is due to DHEA itself, to its metabolites, or to a combination of both remains unclear. DHEA is also considered to be a pre-hormone, and it has been speculated that, through hormonal effects it might modulate several metabolic pathways which have nothing to do with antioxidant function. However, we found negligible variations of either 17 ~-estradiol or testosterone concentrations in rats treated with 4 mg DHEA. Nevertheless, we report elsewhere that DHEA, but not a variety of other steroids including ~-estradiol, ADIOL and dihydrotestosterone, protects bovine retinal capillary pericytes against glucose-induced lipid peroxidation (53).

In conclusion, we show that DHEA supplementation can prevent molecular and functional alterations of the cardiac muscle, restoring oxidative balance and lowering lipid levels, in rabbit fed a high-fat diet. Since similar results have been reported in a model of diabetes type I diabetes, we suggest that heart damage is chiefly dependent on impaired glucose metabolism, and not on insulin-resistance. These data, together with our recent observations on type Il diabetes patients (6), suggest that DHEA treatment might prevent many events that lead to the cellular damage induced by hyperglycemia, thus delaying the onset or progression of cardiac complications in type Il diabetes.