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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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**Running head** Cardiomyopathy in rabbit fed a high-fat diet

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## Abstract

2 The role of oxidative stress in the activating of downstream signalling leading to structural and
3 functional changes in the left ventricle of rabbits fed a high-fat diet for 12 wks and the effect of
4 dietary dehydroepiandrosterone supplementation, were analyzed. The animals showed
5 hyperglycemia, insulin-resistance, dyslipidemia and early features of cardiomyopathy, i.e. altered
6 myosin heavy-chain isoforrns, tissue degeneration and reduced contractility of the papillary muscle.
7 Dietary supplementation with dehydroepiandrosterone (0.02%,wt/wt) in these animals reduced
8 oxidative stress evaluated as levels of reactive oxygen species and hydroxynonenal levels, without
9 affecting high plasma glucose level or insulin resistance. Dehydroepiandrosterone supplementation
lO also counteracted both the activation of redox-sensitive transcription factor and of nuclear
Il trascription factor- kB, and the expression of tumor necrosis factor-alpha and of C-reactive protein-
12 mRNA. Improvement of the oxidative balance counteracted the shift to myosin heavy chain
13 isoforrns, ameliorating the heart contractility. A high-fat diet induced oxidative stress and metabolic
14 syndrome in this rabbit model, and that dehydroepiandrosterone, by restoring oxidative balance,
15 lowering lipid levels and down-regulating inflammation, prevented molecular and functional
16 aIterations of the cardiac muscle, thus exeterting a beneficaI effect and delaying the onset of diabetic
17 cardiomyopathy.
18

18 **Introduction.**

19 Diabetic cardiomyopathy is characterized by systolic and diastolic dysfunction and has been reported
20 in diabetic patients with no ischemic, vaIvuIar or hypertensive heart disease. Its development
21 includes metabolic disturbances, small-vessei diseases, autonomic dysfunction, insuIin-resistance and
22 myocardiai fibrosis (1,2). RecentIy, an important role in its pathophysiology has been attributed to
23 the generation of reactive oxygen species, which activates a number of secondary-messenger
24 pathways, finally Ieading to cardiac dysfunction (3-5). We recently showed recently that free radical
25 overproduction appears early in human type 2 diabetes (6) and that, in a rat model oftype l diabetes,
26 oxidative damage plays a key role in the early development of cardiomyopathy (7). Antioxidants
27 might counteract insulin resistance associated with type 2 diabetes and cardiovascular diseases (8-
28 lO). We reported elsewhere that in streptozotocin rats, the treatment with dehydroepiandrosterone
29 (DHEA), a compound that possesses multi-targeted antioxidant properties (11-15), prevents
30 myocardial damage induced by oxidative stress, while avoiding impairment of cardiac myogenic
31 factors and the switch to myosin heavy chain expression (16). DHEA also counteracts oxidative
32 imbalance and advanced glycation end-product formation in type II diabetic patients (7).
33 The study targets diabetic cardiomyopathy, in a model oftype 2 diabetes induced by a high-fat (HF)
34 diet in rabbits, an animaI species that is highIy susceptible to cardiovascular damage (17). Data show
35 that DHEA treatment counteracted biochernicai changes in the Ieft ventricle induced by oxidative
36 stress, improving myocardial function.

## 37 Materials and methods

38 **Experimental procedure.**

39 Male New Zealand White rabbits, 15 wks oid (Harlan-ItaIy, Udine, Italy) weighing 3.0 -3.5 Kg were
40 cared for in compliance with the Declaration of Helsinki as revised in 1983, the Italian Ministry of
41 Health Guidelines (no. 86/609/EEC) and with the *Princip!es 01 Laboratory Anima! Care* (NIH no.
42 85-23, revised 1985). The rabbits were acclimatized for 2 wks prior to the experiment in a room with
43 12h 1ight dark cycle, individually housed in stainless stee1 cages in a temperature- and humidity-

44 controlled room (23 ± 3°C, 50 ± 5%) and fed 100 g per day of standard rabbit non-purified diet,

45 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

46 moisture, added mineraI 2.1, 40U /kg vitamin E) with appropriate certificate from the Association of

47 Official Agricultural Chemists, 1975, and relative procedure analyses (Laboratorio Dottori Piccioni,

48 Gessate Milanese, Italy). Rabbits were randomly distributed into four groups. The control group *(C)*

*49* *(n=4)* received standard rabbit diet; the DHEA-alone treated group (C-D) *(n=4)* received the same

50 diet supplemented with 0.02% DHEA (Sigma Aldrich, Milan, Italy); the high-fat group (HF) *(n=4)*

51 received a high-fat diet, consisting of standard rabbit non purified diet, composition of 100g: 16.2 g

52 crude protein, 7.3 g crude ash, 14.8 g fiber, 12 g moisture, added mineraI 2.1, 40U/kg vitamin E)

53 plus lO g added fat (6.7g com oil and 3.3 g lard) (18); the HF plus DHEA group (HF-D) *(n=4)*

54 received the high-fat diet supplemented with 0.02% DHEA. Two days before being killed, the

55 rabbits were fasted'ovemight and the glucose tolerance test was performed. The rabbits were killed at

56 3 mo from the start ofthe experiment, by aortic exsanguination after anesthetization with Zoletill00

57 (Tiletamine-Zolazepam, Virbac, Carros, France). Blood was collected and plasma and serum were

58 isolated. The heart was rapidly excised and weighed, and portions of left ventricle were taken to

59 obtain nuclear and total extracts. Other heart portions were utilized for histological microscopy. The

60 papillary musc1es were immediately removed for functional parameter detection.

61 **Generai parameters.**

62 Body weight and length of rabbits were measured at time zero and prior to death. The body mass

63 index (BMI) was calculated as body weight in kilograms divided by body length in meters, squared

64 (kglm$^2$).

65 **Oral glucose tolerance test**

66 After a fasting peri od of 12h, a 50% glucose solution was orally administered to the rabbits at 1.5

67 g/Kg. Blood samples were collected via the auricular artery before 15, 30, 45, 60, 90, 120 and 240

68 min after glucose loading. Glucose levels were tested using an Accu-Check Compact kit (Roche

69 Diagnostics Gmbh, Mannheim, Germany)

70 **Parameters in plasma.**

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

79 **Tissue extracts (cytosolic, nuclear and total extracts).**

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

91 **Oxidative biochemical parameters.**

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

96 between GSSG content and GSH is considered a good parameter of antioxidant status. 4-

97 hydroxynonenal (HNE) was detected by an HPLC procedure. The extract sample was directly

98 injected for HPLC (Waters Assoc., Milford, MA, USA) using an RP-18 column (Merck, Darmstadt,

99 Germany). The mobile phase used was 42% acetonitrilelbidistilled water (v/v). Serial concentrations

100 ofHNE (0.5-10 Ilmol/L) were used to prepare a standard curve (23). Catalase activity was evaluated

101 in the cytosolic fraction following Aebi's method (24). Total superoxide dismutase (SOD) activity

102 was assayed by the method described by Flohè and Otting (25). Since the activity of xanthine-

103 oxidase may vary, sufficient enzyme was used to produce a rate of cytochrome c reduction of at least

104 0.025 absorbance units/min in the assay without SODo

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

106 Cytosol fractions were treated with 6 mol/L hydrochloric acid for 2 h at 40°C and then centrifuged

107 (1860 g) (26); only the supernatant was utilized. A Thermo-Finnigan Surveyor instrument (Thermo

108 Electron, Rodano, Milan, Italy) equipped with autosampler and PDA-UV 6000 LP detector was used.

109 Mass spectrometry analyses were performed using an LCQ Deca XP plus spectrometer, with

110 electrospray interface and ion trap as mass analyzer. The chromatographic separations were run on a

111 Varian Polaris CI8-A column (150 x 2 mm, 3 /lm particle size) (Varian, Leinì, Turin, Italy). Flow

112 rate 200 IlL min-l. Gradient mobile phase composition was adopted: 95/5 to 0/100 v/v 5 *mmol/L*

113 heptafluorobutanoic acid in water/methanol in 13 min. The LC column effiuent was delivered to a

114 UV detector (200-400 nrn) and then to the ion source, using nitrogen as sheath and auxiliary gas

115 (Claind Nitrogen Generator apparatus, Lenno, Como, Italy). The source voltage was set to 4.5 kV in

116 the positive mode. The heated capillary was maintained at 200°C. The acquisition method used had

117 previously been optimized in the tuning sections for pentosidine quasi-molecular ion (capillary,

118 magnetic lenses and collimating octapole voltages) to maximize sensitivity. Collision energy (CE)

119 was chosen to maintain about lO % of the precursor ion. The tuning parameters adopted for the ESI

120 source were: source current 80.00 */lA,* capillary voltage 3.00 V, tube lens offset 15 V; for ion optics,

121 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

149 was normalized for the intensity of the corresponding GAPDHband before comparison between

150 control and treated samples.

151 **Histological staining.**

152 For standard histology, portions of left ventric1e were fixed in 4% neutralized formalin. Fixed

153 materia1 was processed for hematoxylin and eosin staining. Six-micron paraffin-wax sections of left

154 ventric1e were used.

155 **Isolated papillary muscle and contractility determination.**

156 Papillary muscles were driven at constant frequency (120 beats/min) with a pair of electrodes

157 connected to a 302 T Anapulse Stimulator via a 305-R Stimu1us Isolator (W.P. Instruments, New

158 Haven, CT, USA) operating in constant current mode. Isometric twitches were evaluated by a

159 Harvard transducer (60-2997), visualized on a Tektronix 2211 digital storage oscilloscope and

160 continuously acquired and recorded in a Power Mac computer, using Labview Software (Nationai

161 Instruments Corp., Austin, Texas, USA). The same software was used to measure developed peak

162 mechanical tension (T max), maximum rate ofrise and faH ofdeveloped mechanical tension (+dT/dt

163 max and *-dT/dt* max), time-to-peak mechanical tension (ttp) and duration of contraction.

164 **Statistical analysis.**

165 Results are expressed as means ± SD. ANOV A and post-hoc ana1yses inc1uded Bonferroni's test

166 were used (28). The SPSS 14.0 package for Windows (SPSS Inc., Chicago, IL) was used for the

167 statistical analysis. A value of$P<0.05$ was viewed as statisticaHy significant.

168 **Results.**

169 **Generai features.** Body weight, abdomen length, glucose and insulin were significantly increased in

170 rabbits fed a HP diet versus control values ($P<0.05$) after three months' treatment. DHEA

171 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

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

229 **Discussion**

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

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

249 significant increases in ROS, end-products of lipid peroxidation (HNE) and activation of
250 transcription factor NFkB. Activation of NFkB, which triggers in the inflammatory cascade, was
251 demonstrated by the increased expression of TNF -alpha and CRP in cardiac tissue. Here we show
252 that DHEA, by decreasing oxidative stress and reducing activationofNFkB transcription factor; may
253 could determine a reduced expression of TNF and of CRP. Moreover, the anti-inflammatory effects
254 of DHEA, due to cytokine reduction caused by decreased NFkB activation, might be amplified by
255 the reduction of cholesterollevels, which in tum directly decreases CRP secretion from the tissues
256 (40). CRP has been shown to predict morbidity and mortality from coronary heart disease (41).
257 Moreover, it has been shown that high dietary cholesterol intake can increase the production of
258 atherogenic inflammatory cytokines, such as IL-6 and TNF-a, and that reducing dietary cholesterol
259 concentration leads to a reduction in CRP production (42).
260 We suggest that the pro-oxidant and inflammatory states, observed in HF rabbits, cause activation of
261 intracellular signaling responsible for impaired myocardial function, as been reported in cases of
262 heart failure (43). *HP* rabbits showed a switch of cardiac heavy-chain myosin from the alpha to the
263 beta isoform: this event comprises the heart's "molecular motor" because contractile properties
264 depend to a great extent on the isoform composition of MHC proteins. A switch in MHC isoforrn
265 composition has been reported to cause reduced contractile velocity and energy expenditure (44). In
266 man as in animals, a reduced content of a-MHC, which is expressed exclusively in the myocardium,
267 has been reported to be responsible for the reduced myocardial contractility during heart failure (45)
268 and in diabetes (46). In a previous model of type I diabetes cardiomyopathy (7), we reported that
269 DHEA treatment, by avoiding impairment of cardiac myogenic factors and producing a switch in
270 MHC gene expression, protected against tissue damage, an early event in diabeti c cardiomyopathy.
271 DHEA's modulatory effects on MHC expression were also observed in *HP* rabbits: expression of
272 two isoforms, alpha and beta, in DHEA treated rats were not significantly different from those ofthe
273 controi group.

274 Rabbits on a HF diet clearly showed alterations in papillary muscle contractility. This was shown by
275 altered basaI contractility, including reduced maximal developed tension, maximum rate ofrise, and
276 maximum rate of fall of developed tension, which are signs of diastolic dysfunction. As in the case of
277 rat cardiac muscle (7), these alterations ofthe mechanisms controlling intracellular calcium handling
278 within cardiac myocytes are probably related to the structural damage caused by the HF diet.
279 Histological analysis of tissue ftom HF rabbits showed extensive and diffuse lipid deposition, as
280 reported in other animaI models (47). The increased release ofLDH and AST in the plasma ofH.F.
281 rabbits confirmed myocardial damage. Treatment with DHEA protects the cardiac tissue ftom this
282 altered basaI contractility, as well as minimizing histological changes and reducing cells damage
283 caused by the HP diet.
284 The role of DHEA in the cardiovascular system has been highlighted by the recent finding, in the
285 human heart, of DHEA production and CYP-17 gene expression, a key factor in DHEA synthesis
286 (48,49) and it has recently been proposed that the vascular protective effect of DHEA might be
287 dependent on G-a-GTP-binding protein mediated activation ofthe phosphatidil-inositoI3-kinase/ Akt
288 signaling pathway (50). Several explanations have been put forth for the multi-targeted antioxidant
289 effects of DHEA, including its effect on catalase expression (51), and its up-regulation of the
290 thioredoxin system (52), of the fatty-acid composition of cellular membranes and of cytokine
291 production. However, the precise mechanisms remain to be clarified, and additional non-antioxidant
292 effects cannot yet be ruled out. Whether the effect ofDHEA is due to DHEA itself, to its metabolites,
293 or to a combination of both remains unclear. DHEA is also considered to be a pre-hormone, and it
294 has been speculated that, through hormonal effects it might modulate several metabolic pathways
295 which have nothing to do with antioxidant function. However, we found negligible variations of
296 either 17 ~-estradiol or testosterone concentrations in rats treated with 4 mg DHEA. Nevertheless,
297 we report elsewhere that DHEA, but not a variety of other steroids including ~-estradiol, ADIOL and
298 dihydrotestosterone, protects bovine retinal capillary pericytes against glucose-induced lipid
299 peroxidation (53).

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