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

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A B S T R A C T

Aims: The biochemical and structural cardiac oxidative-dependent damage induced by high-fat (HF) diet was examined in a rabbit model, together with the role of dehydroepiandrosterone (DHEA) in contrasting tissue damage.

Main methods: New Zealand white rabbits fed a HF diet supplemented or not with DHEA (0.02%) were utilized for 12 weeks. Oxidative stress, inflammatory and necrosis parameters, fatty deposition, heavy-chain myosin isoforms (MHC) expression and papillary muscle functionality were examined in the left ventricle of rabbits.

Key findings: Rabbits fed a HF diet that showed hyperglycemia, insulin resistance and dyslipidemia together with increased oxidative stress and of advanced end-glycation product levels have been observed. Concerning pro-inflammatory insults, there was increased p65-NFkB activation and increased tumor necrosis factor-alpha and C-reactive protein expressions. Cellular damage induced by the HF diet was detected through the switch of expression of MHC isoforms, indicating impairment of cardiac contractility, confirmed by altered of basal parameters of papillary muscle functionality. Rabbits fed the HF diet supplemented with DHEA showed a partial reduction of oxidative stress and the inflammatory state. Cardiac necrosis, the shift of MHC isoforms, and cardiac functionality, were also partially counteracted.

Significance: Rabbits fed with a HF diet showed a beneficial effect when low-dose DHEA was added to the diet. The steroid, without affecting high plasma glucose level or insulin resistance, restored oxidative balance, lowered lipid levels and inflammation insults, preventing cellular and functional alterations of cardiac tissue and thus delaying the onset of cardiac damage.

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Introduction

Diabetic cardiomyopathy is characterized by systolic and diastolic dysfunction and has been reported in diabetic patients with no ischemic, valvular or hypertensive heart disease. Its development includes metabolic disturbances, small-vessel diseases, autonomic dysfunction, insulin resistance and myocardial fibrosis (Brownlee 2005; Farhangkhoee et al. 2005). Recently, an important role in its pathophysiology has been attributed to the generation of reactive oxygen species, which activates a number of secondary-messenger pathways, eventually leading to cardiac dysfunction (Hartog et al. 2007; Masoudi and Inzucchi 2007; Wold et al. 2005).

We recently showed that free-radical overproduction appears early in human type 2 diabetes (Brignardello et al. 2007) and that, in a rat model of type 1 diabetes, oxidative damage plays a key role in the early development of cardiomyopathy (Aragno et al. 2006). Antioxidants might counteract insulin resistance associated with type 2 diabetes and cardiovascular diseases (Robertson 2006; Davì et al. 1999; Minamiyama et al. 2008).

The role of dehydroepiandrosterone (DHEA), a compound that possesses multi-targeted antioxidant properties (Simoncini and Genazzani 2007; Tchernof and Labrie 2004; Aragno et al. 2004, 2005; Brignardello et al. 2000), in the cardiovascular system has been highlighted by recent reports showing that the human heart synthesizes DHEA, that its production is suppressed in the failing heart, and that plasma levels of the sulfate conjugate of DHEA decrease in patients with chronic heart failure in proportion to the severity of the condition (Moriyama et al. 2000). We reported elsewhere that, in
from the Association of Of Declaration of Helsinki as revised in 1983, the Italian Ministry of Health.

Hospitex Diagnostics (Florence, Italy).

standard rabbit diet (sec. Sherman, cod. 57 with appropriate certi

humidity-controlled room (23±3 °C, 50±5%) and fed 100 g per day of

cycle, individually housed in stainless steel cages in a temperature- and

Animal Care (Genoa, Italy). Lipid pro

Materials and methods

Reagents

Unless otherwise indicated, all compounds were purchased from Sigma Chemical Co. (St. Louis, MO, USA), antibodies from Chemicon (Millipore, Billerica, MA, USA), and primers from Tib MolBiol s.r.l. (Genoa, Italy). Lipid profile was determined using reagents kits from Hospitex Diagnostics (Florence, Italy).

Animal treatment

Male New Zealand white rabbits, 15 weeks old (Harlan-Italy, 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/EC) and with the Principles of Laboratory Animal Care (NIH no. 85-23, revised 1985). The rabbits were acclimated for 2 weeks prior to the experiment in a room with 12 h light dark cycle, individually housed in stainless steel cages in a temperature- and humidity-controlled room (23 ± 3 °C, 50 ± 5%) and fed 100 g per day of standard rabbit diet (sec. Sherman, cod. 57 with appropriate certificate from the Association of Official Agricultural Chemists, 1975, and relative procedure analyses, Laboratorio Dottori Piccioni, Gessate Milanese, Italy) containing 16.2% (w/w) crude protein, 3.0% (w/w) crude fat, 7.3% (w/w) crude ash, 14.8% (w/w) fiber, 12% (w/w) moisture, 2.1% (w/w) added mineral, 46% (w/w) carbohydrate with water, and 40 IU kg −1 vitamin E. Rabbis were randomly distributed into four groups. The control group (C) (n = 6) received standard rabbit diet; the DHEA-alone treated group (C-D) (n = 6) received the same diet supplemented with 0.02% (w/w) DHEA (Sigma Aldrich, Milan, Italy); the high-fat group (HF) (n = 6) received a high-fat diet, consisting of standard rabbit diet, containing 16.2% (w/w) crude protein, 7.3% (w/w) crude ash, 14.8% (w/w) fiber, 12% (w/w) moisture, 2.1% (w/w) added mineral, 46% (w/w) carbohydrate with water, 40 IU kg −1 vitamin E plus 10% (w/w) added fat (6.7% w/w corn oil and 3.3% w/wlard) (Carroll and Tyagi 2005); the HF plus DHEA group (HF-D) (n = 6) received the high-fat diet supplemented with 0.02% (w/w) DHEA. Two days before being killed, the rabbits were fasted overnight and the glucose tolerance test was performed. The rabbits were killed at 3 months from the start of the experiment, by aortic exsanguination after anesthetization with Zoletil 100 (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 cytosolic, nuclear and total extracts. Other heart portions were utilized for histological microscopy. The papillary muscles were immediately removed for functional parameter detection.

General parameters

Body weight and length of rabbits were measured at time zero and immediately prior to death.

Oral glucose tolerance test

After a 12 h fasting period, a 50% (w/v) glucose solution was orally administered to the rabbits at 1.5 g/kg. Blood samples were collected via the auricular artery before, and 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 (TC), low-density-lipoprotein (LDL) and high-density-lipoprotein (HDL) cholesterol were determined by standard enzymatic procedures using reagents kits. 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 from DRG Diagnostics (Marburg, Germany). Insulin sensitivity was calculated using the homeostasis model assessment (HOMA): fasting glucose (mmol l −1 ) × fasting insulin (µg l −1 ) /22.5. Plasma DHEA was determined by specific radioimmunoassay DSL-9000 (Diagnostic System Laboratories, Oxford, U.K.).

Tissue extracts (cytosolic, nuclear and total extracts)

Cytosolic and nuclear fractions from rabbit left ventricle were prepared by the modified Meldrum et al. method (Meldrum et al. 1997). 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 containing 20 mmol l −1 HEPES, pH 7.9, 1 mmol l −1 MgCl 2 , 0.5 mmol l −1 EDTA, 1% NP-40, 1 mmol l −1 EGTA, 1 mmol l −1 DTT, 0.5 mmol l −1 PMSF, 5 µg ml −1 aprotinin, and 2.5 µg ml −1 leupeptin. Homogenates were centrifuged at 1000 g for 5 min at 4 °C. Supernatants were removed and centrifuged at 15,000 g at 4 °C for 40 min to obtain cytosolic fraction. The pellets were resuspended in extraction buffer containing 20 mmol l −1 HEPES, pH 7.9, 1.5 mmol l −1 MgCl 2 , 300 mmol l −1 NaCl, 0.2 mmol l −1 EDTA, 20% (v/v) glycerol, 1 mmol l −1 EGTA, 1 mmol l −1 DTT, 0.5 mmol l −1 PMSF, 5 µg ml −1 aprotinin, 2.5 µg ml −1 leupeptin 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 supernatants 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 1000 g for 5 min at 4 °C. Supernatants (total extract) were stored at −80 °C until use. Protein content was determined using the Bradford assay (Bradford 1976).

Oxidative biochemical parameters

The redox state was determined in the cytosolic fraction by monitoring hydrogen peroxide (H 2 O 2 ) generation adding peroxidase from horseradish and acetylated ferrocyanochrome c to cytosolic fractions. H 2 O 2 release was evaluated as the increase of the acetylated ferrocyanochrome c oxidation rate and was monitored at 550 nm minus 540 nm using an absorption coefficient of 19.9 mmol l −1 cm −1 , as described by Zoccorato et al. (1989). Reduced and oxidized glutathione contents were measured in cytosolic fractions by Owens’ method (Owens and Belcher 1965). 4-Hydroxynonenal (HNE) was detected with an HPLC procedure: the total 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 4% (v/v) acetonitrile/bidistilled water. Serial concentrations of HNE (0.5–10 µmol l −1 ) were used to prepare a standard curve (Esterbauer et al. 1991). Catalase activity was evaluated in the cytosolic fraction following Aebi’s method (Aebi 1984). Total superoxide dismutase (SOD) activity was assayed in the cytosolic
fraction by the method described by Flohé and Otting (1984). 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 IU/min in the assay without SOD.

**AGE in term of pentosidine detection with HPLC-MS**

Cytosolic fractions were treated with 6 mol l\(^{-1}\) hydrochloric acid for 2 h at 40 °C and then centrifuged (18 000 g) (Miyata et al. 2001); 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 C18-A column (150 × 2 mm, 3 μm particle size) (Varian, Leini, Turin, Italy). Flow rate 200 μl min\(^{-1}\). Gradient mobile phase composition was adopted: 95/5 to 0/100 v/v 5 mmol l\(^{-1}\) heptfluorobutanoic acid in water/methanol in 13 min. The LC column effluent was delivered to a UV detector (200–400 nm) and then to the ion source, using nitrogen as sheath and auxiliary gas (Clain 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 (capillary, magnetic lenses and collimating octapole voltages) to optimize in the tuning sections for pentosidine quasi-molecular ion then to the ion source, using nitrogen as sheath and auxiliary gas (Claind Insulin (μg l\(^{-1}\))

**RNA isolation and semi-quantitative RT-PCR**

Total RNA was isolated using the RNA fast kit (Molecular Systems, San Diego, CA, USA). Total DNA was amplified using sense and antisense primers specific for the C-reactive protein (CRP) gene (sense 5′-AGGATCAGTCTCGCTTG-3′ and antisense 5′-CACCACGTACCTGATGTC-3′), the tumor necrosis factor-alpha (TNF-α) gene (sense 5′-AGGAAGAGTCCCCAAACAACCT-3′ and antisense 5′-GCCCGAGAAGCTCATGCG-3′), the alpha-myosin heavy-chain (α-MHC) gene (sense 5′-CCAGAAGATGAA-3′ and antisense 5′-CTCCTCGGTCCAGCTTCG-3′), the beta-myosin heavy-chain (β-MHC) gene (sense 5′-GGTCAATACCTGATCAGTCAC-3′ and antisense 5′-AACCCCGTCAGCAGCAAGG-3′). The PCR reaction system contained 1 μl of RT product, 200 μmol l\(^{-1}\) dATP, dGTP, dCTP and dTTP (Finnzymes, Espoo, Finland), 1.25 units of Taq DNA polymerase (Finnzymes, Espoo, Finland) and 50 pmol of sense and antisense primers in a total volume of 50 μl. All experiments were performed on at least three independent cDNA preparations.

**Histological staining**

For standard histology, portions of left ventricle were fixed in 4% (v/v) neutralized formalin. Fixed material was processed for hematoxylin and eosin staining. Six-micron paraffin-wax sections of left ventricle 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 Stimulus 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 (National Instruments Corp., Austin, Texas, USA). The same software was used to measure developed
p65-NFkB protein in the nuclear and cytosolic fractions of left ventricle was detected by Western blot analysis (Fig. 3, panel A and panel B, respectively). Nuclear p65-NFkB level in HF rabbits was increased vs the control groups (P<0.05) (panel A). The cytosolic level of p65-NFkB was reduced in HF rabbits (P<0.05) (panel B). In the HF-DHEA rabbits, cytosolic p65-NFkB protein level was less reduced than in the HF group, while the nuclear p65-NFkB level was increased vs the HF alone group, indicating a lower activation of the p65-NFkB transcription factor in HF-DHEA group vs HF (P<0.05).

Both indices of the pro-inflammatory state, TNF-α and CRP expression, were significantly increased in the left ventricle of rabbits fed the HF diet (P<0.01) (TNF-α: Fig. 3, panel C and CRP: Fig. 3, panel D). The HF-DHEA diet significantly decreased expression of both TNF-α and CRP vs the HF group (P<0.05).

Myosin expression

PCR analysis was used to evaluate expression of two isoforms (α and β) of the MHC protein (Fig. 4) in the left ventricle of control, C-DHEA, HF and HF-DHEA animals. The HF diet determined a significantly decreased expression of α-MHC and an increased expression of β-MHC (P<0.01). When DHEA was added to the HF diet, α-MHC was brought closer to the control value and β-MHC was reduced vs the HF group (P<0.05).

Necrosis markers

LDH and AST releases were evaluated in the plasma (Fig. 5). Both LDH (panel A) and AST (panel B) significantly increased in HF rabbits (P<0.01). In the HF-DHEA group, the levels of LDH and AST were significantly lower than in the HF alone group (P<0.05).

Histological analysis

In the left ventricle of the HF rabbits (Fig. 6, panel C), histological preparations clearly showed extensive and diffuse lipid deposition.

Fig. 1. Glucose tolerance test in rabbits fed with control (C), control-DHEA (C-D), high-fat (HF) and high-fat-DHEA (HF-D) diet for 12 weeks. Data are means ± SD (n = 4) and are expressed as mmol l⁻¹. Statistical significance: *P<0.05 vs C; **P<0.01 vs C.

peak mechanical tension (T max), maximum rate of rise and fall of developed mechanical tension (+ dT/dt max and − dT/dt max), time-to-peak mechanical tension (TPT) and duration of contraction.

Statistical analysis

All values were expressed as means ± SD, and were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test. P<0.05 was considered statistically significant.

Results

General features

Body weight, abdomen length, glucose and insulin were significantly increased in rabbits fed a HF diet vs control values (P<0.01) after three months’ treatment. DHEA supplementation did not modify these values (Table 1). Homeostasis model assessment (HOMA) was also significantly higher in HF rabbits than in controls (P<0.01) and not different from those of HF plus DHEA rabbits. HOMA values, which reflect whole body insulin resistance, have also been validated in other animal models (Buettner et al. 2006). The heart/body weight ratio was the same in all groups, whereas the heart weight of rabbits fed the HF diet increased vs controls (P<0.01); DHEA supplementation reduced this increase. The results of the glucose tolerance test are reported in Fig. 1. After oral loading, glucose levels remained high for up to 240 min in both the HF and HF-DHEA groups. Control and C-DHEA showed a peak of glucose concentration between 15 and 30 min after glucose loading, after which it rapidly returned to normal levels.

TG, TC and LDL concentrations in plasma were significantly higher in HF rabbits vs controls (P<0.01) and were reduced in HF plus DHEA rabbits vs the HF alone group (P<0.05). HDL was significantly lower (P<0.01) in the HF group vs controls while in the HF-DHEA group, the HDL value was similar to that of control animals (P<0.05). Blood DHEA level after 3 months of treatment was unchanged vs the control group, and reached values similar to those found in normal humans.

Oxidative parameters in the left ventricle

Rabbits fed a HF diet for 12 weeks showed a significant increase of H₂O₂ and HNE detected in the total extract of left ventricle vs the control group (P<0.01) (Fig. 2). In rabbits fed with HF-DHEA, the H₂O₂ and HNE levels (HNE being an end-product of lipid peroxidation) were significantly lower than in the HF rabbits (P<0.05). The level of pentosidine, apparently indicating its absence in these animals. Moreover, catalase and total SOD activities were increased in the HF rabbits, and HF-DHEA supplementation partially restored these activities to control levels (P<0.05). No significant difference in the GSSH/GSH ratio was observed among groups (data not shown). The GSSH/GSH ratio, in HF and HF-DHEA groups, was significantly increased (P<0.05) vs the control group, indicating a loss of GSH level and thus a decrease of antioxidant defenses. There was no statistically significant difference between the HF-DHEA and the HF group (data not shown).

Inflammatory parameters

Fig. 2. Content of H₂O₂ and HNE detected in the total extract of the left ventricle of control (C), control-DHEA (C-D), high fat (HF) and high fat-DHEA (HF-D). DHEA was given to C and HF rabbits in the diet for 12 weeks. Data are means ± SD, (n = 6), Statistical significance: **P<0.01 vs C; ***P<0.05 vs HF.
This lipid infiltration was not observed in either the control or the C-DHEA group (panel A and panel B). Tissues obtained from HF-DHEA rabbits showed rare areas of slight lipid deposition (panel D).

**Cardiac function**

The contractile force developed by electrically-driven papillary muscles was evaluated in basal conditions (Table 2). Basal contractility was weaker in papillary muscles from HF rabbits vs controls; this was evident not only for maximal developed mechanical tension (Tmax; \( P < 0.01 \)), but also for maximum rate of rise (+dT/dt max; \( P < 0.01 \)), maximum rate of fall of developed mechanical tension (−dT/dt max; \( P < 0.01 \)) and time to peak of mechanical tension (TPT; \( P < 0.05 \)). In contrast, no significant difference was found between papillary muscles from control and HF rabbits in regard to duration of contraction. Treatment with DHEA significantly reduced the effects of

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Fig. 3. Representative gel profiles of p65-NFκB content obtained by Western blot analyses on nucleus (panel A) and on cytosol (panel B) of left ventricle from control (C), control-DHEA (C-D), high fat (HF) and high fat-DHEA (HF-D). Quantitative results of these bands are indicated in the histogram, which shows the net intensity ratio with laminin1 for nucleus and with α-actinin for cytosol. Data are expressed as percentage variations vs the control value. Panel C and panel D represent the level of tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) mRNA in left ventricle from control (C), control-DHEA (C-D), high fat (HF) and high fat-DHEA (HF-D) obtained by RT-PCR analyses. Quantitative results of these bands are indicated in histograms, which show the net intensity ratio vs glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data are means±SD, (n=6) and are expressed as percentage variations vs control. Statistical significance: * \( P < 0.05 \) vs C; ** \( P < 0.01 \) vs C; *\# P<0.05 vs HF; **\# P<0.01 vs HF.

Fig. 4. Alpha-myosin heavy-chain (α-MHC) and beta-myosin heavy-chain (β-MHC) expression in left ventricle from control (C), control-DHEA (C-D), high fat (HF) and high fat-DHEA (HF-D). Quantitative results of these bands are indicated in histograms, which show the net intensity ratio vs glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and data are expressed as percentage variations vs control. Data are means±SD, (n=6). Statistical significance: ** \( P < 0.01 \) vs C; *\# P<0.05 vs HF.
the HF diet (P<0.05). However, DHEA did not per se affect contractile properties of the papillary muscles.

Discussion

In the left ventricle of male New Zealand rabbits fed a HF diet for 12 weeks we observed unbalanced oxidative status, impairment of cardiac myogenic factors and a switch in MHC gene expression and contractility dysfunction, all of which were prevented by the addition of DHEA in the HF. We believe that DHEA’s key action is against oxidative imbalance (Brignardello et al. 2007; Aragno et al. 2006), which could be critical in inducing myocardial dysfunction. A greater propensity for oxidative stress after myocardial infarction is associated with the development of heart failure (Smith et al. 2005) and a correlation between systolic and diastolic myocardial dysfunction and oxidative stress has been reported in a highly-selected group of uncomplicated type 2 diabetic patients (González-Vilchez et al. 2005). Here we show that increased H$_2$O$_2$, increased end-products of lipid peroxidation (specifically HNE) and increased activation of the transcription factor p65-NFkB in the cardiac tissue of HF rabbits, are all countered by DHEA. Moreover, in the plasma of rabbits fed a HF diet, we observed that DHEA countered the increase in glycoxidative products, in agreement with previous rat studies (Aragno et al. 2004, 2005; Brignardello et al. 2000; Aragno et al. 2008).

Here we show that, by decreasing oxidative stress, DHEA reduces activation of p65-NFkB transcription factor, determining a reduction of inflammation in cardiac tissue. Indeed, after DHEA supplementation, expression of the pro-inflammatory cytokines TNF-α and of CRP was decreased. The anti-inflammatory effects of DHEA due to cytokine reduction might also be amplified by the reduction of cholesterol levels, which in turn directly decreases CRP release from tissues (Ridker et al. 2002). It has been shown that high dietary cholesterol intake can increase the production of inflammatory cytokines and that reduction of dietary cholesterol concentration leads to a reduction in CRP production (Han et al. 2002). When DHEA was added to the rabbits’ HF diet, alongside total cholesterol, LDL and triglycerides were also reduced. Some studies have demonstrated a lowering effect of DHEA on triglyceridemia (Mauriège et al. 2003) whereas others have not done so (Igwebuike et al. 2008). However, it must be noted that in the latter studies, lipid metabolism was assessed in the fasted state, that is at a time when lipid flux to the liver is minimal. Thus, the consequences of DHEA treatment might be underestimated, since it is conceivable that they become more evident in the postprandial state, when the metabolic handling of lipids becomes fully active. It has been reported that the anti-obesity effect of DHEA may in part be related to changes in lipase activity and beta-adrenergic receptor density (Shepherd and Cleary 1984). Moreover, DHEA accelerates lipid catabolism by direct regulation of hepatic lipid metabolism (Tang et al. 2007) and our other preliminary

Fig. 5. Level of lactate dehydrogenase (LDH) (panel A) and of aspartate aminotransferase (AST) (panel B) evaluated in plasma of control (C), control-DHEA (C-D), high fat (HF) and high fat-DHEA (HF-D). Data are expressed as units/liter (U/l). Data are means±SD (n = 6). Statistical significance: *P<0.05 vs C; **P<0.01 vs C; *P<0.05 vs HF.

Fig. 6. Histochemical analysis of left ventricle sections of control (A), control-DHEA (B), high fat (C) and high fat-DHEA (D). For standard histology, portions of left ventricle were fixed in 4% v/v neutralized formalin. Fixed material was processed for hematoxylin and eosin staining. Six-micron paraffin sections of left ventricle were used. Magnification × 10.
data on rats suggest that DHEA participates in controlling expression of genes involved in both triglyceride and cholesterol synthesis and storage in the liver (data not reported here). In addition, it was hypothesized that DHEA could play a role in the inhibition of lipogenesis and adipogenesis as well as in the activation of lipolysis in the adipose tissue possibly through the its effects on adipokines (Pérez-de-Heredia et al. 2008). However, the detailed mechanisms whereby DHEA exerts its anti-lipid effects and modulates triglyceride (TG) storage and mobilization have not yet been fully elucidated. In agreement with several human studies showing only slight or no effect of DHEA on glucose homeostasis (Hunt et al. 2000; Callies et al. 2001; Christiansen et al. 2004), we found that DHEA supplementation did not affect the high plasma glucose levels induced by the HF diet, nor did it protect against hyperinsulinemia or improve the HOMA index.

The HF 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 isoform composition has been reported to cause reduced contractile velocity and energy expenditure (Ramarurthy et al. 2001). This has been reported that in man, as in animals, a reduced content of α-MHC, which is expressed exclusively in the myocardium, is responsible for the reduced myocardial contractility during heart failure (Gupta et al. 2003) and in diabetes (Razeghi et al. 2002; Wang et al. 2004). Here we show that DHEA modulates MHC expression in HF rabbits: in the DHEA treated rats, neither isoform, alpha nor beta, was significantly different from the control group levels.

Rabbits on a HF diet clearly showed alterations in basal papillary muscle contractile properties, including reduced maximal developed tension, maximum rate of rise and maximum rate of fall of developed tension, a sign of diastolic dysfunction. As in the case of rat cardiac muscle (Aragno et al. 2008), these alterations of the mechanisms controlling intracellular calcium handling within cardiac myocytes are probably related to structural damage caused by the HF diet.

Histological analysis of tissue from HF rabbits showed extensive and diffuse lipid deposition, as reported in other animal models (Wang et al. 2008; Deng et al. 2005). The increased release of LDH and AST in the plasma of HF rabbits are in keeping with the myocardial damage. Treatment with DHEA protects the cardiac tissue from this altered basal contractility, as well as minimizing histological changes and reducing cell damage caused by the HF diet.

The role of DHEA in the cardiovascular system has been highlighted by the recent finding, in the human heart, of DHEA production and cytochrome P450-17 gene expression, encoding cytochrome P450 17α-hydroxylase, a key factor in DHEA synthesis (Nakamura et al. 2004). More interestingly, it has been reported that DHEA production is suppressed in the failing human heart (Moriyama et al. 2000). The vascular protective effect of DHEA might be dependent on G-α-TPB-binding protein mediated activation of the phosphatidylinositol-3-kinase/Akt signaling pathway (Liu et al. 2007). Moreover, DHEA can regulate calcium homeostasis (Zylnska et al. 2009): it has been reported that DHEA induces an acute (nongenomically-mediated) vasorelaxing effect on the human umbilical artery which may be mediated by a decrease in external Ca2+ influx by inactivating Ca2+ channels (Perusquía et al. 2007). Several explanations have been put forward for multi-targeted antioxidant effects of DHEA, including its effect on catalase expression (Yildirim et al. 2003), and its up-regulation of the thioredoxin system (Gao et al. 2005), of the fatty-acid composition of cellular membranes and of cytokine production (Aragno et al. 2000). However, the precise mechanisms are yet to be clarified, and additional non-antioxidant effects cannot yet be ruled out. Whether the effect of DHEA is due to DHEA itself, to its metabolites, or to a combination of both remains unclear. Hayashi et al. have claimed that the athero-protective effect of DHEA may be mediated through its conversion to estrogen (Hayashi et al. 2000). However, the amount of DHEA added to the diet was many times higher than the dose we used and, furthermore, we found negligible variations of either 17β-estradiol or testosterone concentrations in rats treated with 4 mg DHEA. Nevertheless, as we report elsewhere, DHEA, but not a variety of other steroids including β-estradiol, 17 β-diol and dihydrotestosterone, protects bovine retinal capillary pericytes against glucose-induced lipid peroxidation (Brignardello et al. 1998).

Conclusion

We show that DHEA supplementation can prevent molecular and functional alterations of the cardiac muscle, restoring oxidative balance and lowering lipid levels, in rabbits fed a high-fat diet. These data, together with our recent observations on type 2 diabetes patients (Brignardello et al. 2007) 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 2 diabetes.

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References


Table 2

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<th>CD</th>
<th>HF</th>
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<tr>
<td>F max (mN mm⁻²)</td>
<td>2363±201</td>
<td>3159±335</td>
<td>751±89*</td>
<td>1605±68**</td>
</tr>
<tr>
<td>+dT/dt max (mN s⁻¹)</td>
<td>7536±1356</td>
<td>8515±1788</td>
<td>2711±407**</td>
<td>6481±778**</td>
</tr>
<tr>
<td>−dT/dt max (mN s⁻¹)</td>
<td>2864±173</td>
<td>3752±1163</td>
<td>1343±158**</td>
<td>3859±615**</td>
</tr>
<tr>
<td>TPT (ms)</td>
<td>250.6±21.0</td>
<td>217.0±24.4</td>
<td>205.0±16.7</td>
<td>240.0±19.8</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>716.7±19.6</td>
<td>705.3±70.6</td>
<td>539.0±48.2</td>
<td>646.2±58.3</td>
</tr>
</tbody>
</table>

T max: peak mechanical tension; +dT/dt max: maximum rate of rise of developed tension; −dT/dt max: maximum rate of fall of developed tension; TPT: time-to-peak tension; duration of contraction. Data are means ± SD (n=4). Statistical significance: *P<0.05 vs C; **P<0.01 vs C; #P<0.05 vs HF.