Eur J Nutr (2008) 47:349–356
DOI 10.1007/s00394-008-0734-7

ORIGINAL CONTRIBUTION

Horacio A. Peredo
Marcos Mayer
Ileana R. Faya
Ana M. Puyó
Andrea Carranza

Dehydroepiandrosterone (DHEA) prevents the prostanoid imbalance in mesenteric bed of fructose-induced hypertensive rats

Received: 29 January 2008
Accepted: 31 July 2008
Published online: 25 August 2008

H.A. Peredo
Dept. of Pharmatechnology I
Faculty of Pharmacy and Biochemistry, INFIBIOC
University of Buenos Aires
Buenos Aires, Argentina

H.A. Peredo · M. Mayer · A. Carranza
CONICET
Buenos Aires, Argentina

M. Mayer · A. Carranza
Dept. of Physiopathology
Faculty of Pharmacy and Biochemistry, INFIBIOC
University of Buenos Aires
Buenos Aires, Argentina

I.R. Faya · A.M. Puyó
Dept. of Macro and Microscopy Anatomy
Faculty of Pharmacy and Biochemistry, INFIBIOC
University of Buenos Aires
Buenos Aires, Argentina

A. Carranza (✉)
Facultad de Medicina, Instituto de Neurociencias
Universidad de Buenos Aires
Paraguay 2153, Piso 1
Sector Uriburu (C1121ABG)
Buenos Aires, Argentina
Tel.: +54-11/5950-9500
Fax: +54-11/4508-3645
E-Mail: carranza.ma@gmail.com

Abstract *Background* In previous studies we reported an altered prostanoid (PR) release-pattern in mesenteric vessels in fructose (F)-overloaded rats, an experimental model of insulin resistance and hypertension. Dehydroepiandrosterone (DHEA) and its precursor Dehydroepiandrosterone sulfate (DHEA-S) are the most abundant circulating steroid hormones produced by the adrenal and recent studies in both cells and animals suggest that DHEA may have acute non-genomic actions that mimic both metabolic and vascular actions of insulin. *Aim of the study* This study was to analyze in F-overloaded rats, the effects of DHEA treatment on arterial blood pressure and the PR production in mesenteric vessels and aorta. *Methods* Male 6 week-old Sprague–Dawley rats were randomly divided in four groups: a control group (C), a DHEA (30 mg/kg/sc/48 h)-treated group (D), a fructose (10% w/v in drinking water)-fed group (F), and both treatments simultaneously group (FD). The systolic blood pressure (SBP) was measured by tail cuff method and glycemia and triglyderidemia were measured by enzymatic assays. The mesenteric beds of all groups were dissected, and incubated in Krebs solution. The PR released were measured by HPLC. *Results* F overload increased SBP and triglyceridemia and decreased the mesenteric vasodilatory PR release. DHEA treatment prevented the increment in SBP and triglyceridemia and decreased vasoconstrictor PR in F-treated rats. *Conclusion* DHEA normalize the PGI_2/TX ratio, diminished in F-overloaded rats, through the decrease in thromboxane (TX) production and this could be one of the mechanisms by which DHEA prevented the slight hypertension in F-animals.

Key words DHEA – insulin resistance – hypertension – fructose – prostanoids

Introduction

Type 2 diabetes is an epidemic multi-factorial disease, frequently associated with a cluster of pathologies including hypertension, hypertriglyceridemia, obesity, impaired glucose tolerance and insulin resistance, collectively referred to as the metabolic syndrome [39]. Recent studies have linked soft drink consumption with increased risk for hypertension and

type 2 diabetes. In this regard, F has a low glycemic index but may be causally linked with the epidemic of obesity and cardiovascular disease [42]. Male rats fed a high F diet (F-rats) develop mild degree of hypertension, hypertriglyceridemia, insulin resistance but not fasting hyperglycemia, a pathologic status resembling type 2 diabetes mellitus [17, 40, 46]. An elevation of peripheral resistance has been postulated as one of the mechanisms that trigger the blood pressure increase in this model. The development of increased vascular tone could be derived from the activation of several vasoconstrictor and antinatriuretic systems as the renin-angiotensin [47] and sympathetic nervous systems [26]. We also found alterations in vascular contractility [8], increased thickness and area of mesenteric vessels and prostaglandin (PG) production [37] in F-fed rats.

PR, metabolites of arachidonic acid through the cyclooxygenase (COX) pathway, include the vasodilator substances prostacyclin (PGI_2) (measured by its stable metabolite 6-keto $PGF_1\alpha$), PGE_2, and the vasoconstrictor substances TXA_2 (measured by its stable metabolite TXB_2) and $PGF_2\alpha$. These vasoactive substances, synthesized and released by the vascular wall, may diffuse across the vascular smooth muscle and have been implicated in the modulation of vascular tone. In previous studies we have reported an altered PR release-pattern in mesenteric vessels in an experimental model of type 2 diabetes in rats [34] and F-overloaded rats [35–37]. It has also been reported that a TX synthesis inhibitor prevents blood pressure increase in F-overloaded rats [13]. Recently, an imbalance between endothelium-derived relaxing and contracting factors in mesenteric arteries in a model of experimental type 2 diabetes has been reported [25].

DHEA and its precursor DHEA-S are the most abundant circulating steroid hormones produced by the adrenal gland [4, 32]. The plasma concentrations of DHEA and DHEAS progressively decline with age [5, 18]. Moreover, several epidemiological studies have shown an inverse correlation between DHEA/DHEAS plasma concentrations and mortality, particularly due to cardiovascular disease in aged men [3], and postmenopausal women [20]. In addition, DHEA replacement (50 mg daily for 12 weeks) in hypoadrenal women improves insulin sensitivity [9]. DHEA is widely available as a nutritional supplement without prescription that is touted for its putative anti-aging properties and beneficial effects on metabolic and cardiovascular health. However, molecular mechanisms mediating DHEA action are poorly understood.

Steroid hormones regulate vascular function indirectly through metabolic modifications, but mostly through actions exerted directly on the vessel wall [45]. Recent studies in both cells and animals models suggest that DHEA may exert acute non-genomic actions that mimic both metabolic and vascular actions of insulin. Interestingly, specific binding of DHEA to the plasma membrane of endothelial cells has been reported [22], and recently it has been demonstrated that DHEA caused an acute increased in nitric oxide production through the activation of PI 3-kinase (PI3-K) and phosphorylation of Akt (PKB) and endothelial NO synthase (eNOS) in vascular endothelial cells [12]. Taken together, these studies suggest that DHEA mimics acute actions of insulin.

Insulin resistance causes decreases in plasma DHEA levels [49] and on the other hand, acute hyperinsulinemia in humans causes a decline in serum DHEA-S concentrations [33]. Interestingly, F-enriched diets could induce decreased levels of plasma DHEAS [15]. We propose that DHEA treatment could prevent the altered PR release described in mesenteric vessels of F-induced hypetension. Therefore, the aim of the present study was to analyze the effects of DHEA treatment on arterial blood pressure and PR production in mesenteric vessels and aorta of F-overloaded rats.

Materials and methods

Animals and experimental design

Twenty four male 6 week-old Sprague–Dawley rats weighing 180–240 g at the beginning of the study were randomly divided in four groups of six rats each: a control (tap water ad libitum plus 0.5 ml of sunflower oil/sc, every alternate day)-group (C), a DHEA (30 mg/kg/sc in 0.5 ml of sunflower oil every alternate day)-treated group (D). Rats treated with DHEA (30 mg/kg, every alternate day) induces a circulating DHEA sulfate level of ≈0.2 μM (60 ng/ml) after 3 weeks [6], a F (10% w/v in tap water plus 0.5 ml of sunflower oil/sc, every alternate day)-fed group (F) [8], and both treatments-group (FD). All treatments were administrated during 9 weeks. Animals were maintained in a room at 22 ± 2°C and the air was adequately recycled and were fed with standard rodent diet (Asociación Cooperativas Argentinas) in pellets, with the following composition (w/w): 20% proteins, 3% fat, 2% fiber, 6% minerals and 69% starch and vitamins supplements, containing the same amount of calories. In addition, rats were weighed before dietary manipulation and weekly during the study for the DHEA- doses calculations.

The experiments were approved in advance by the local ethics committee on animal research. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with international laws (Guide

for the care and use of laboratory animals, US National Research Council, 1996).

Measurement of systolic blood pressure and metabolic parameters

The rats were trained to the procedure of SBP measurement at 10.00 A.M., twice a week, and the SBP was registered during 2 weeks prior to the sacrifice by decapitation. The mean of three to four consecutive readings was used as the reported value of the SBP for each rat. Indirect SBP was measured by means of a tail-cuff using a microphone connected to a Grass D.C. driver amplifier (model 7DAC, Grass Instruments Co. Quincy, MA, USA) in series with a Grass polygraph (model 79D, Grass Instruments Co. Quincy, MA, USA).

At week 8 of treatment, all animals were fasted overnight and subjected to an oral glucose tolerance test (OGTT) as described [24]. At time 0 min, a blood sample (0.5 ml) was collected by cutting the tail tip, and then a glucose solution (2 g/ml/kg body weight) was immediately administrated by oral gavage. Five more blood samples were collected from the tail tip at 15, 30, 60, 90, and 120 min after glucose administration. All blood samples were collected in tubes pre-rinsed with heparin solution and centrifuged (3,500×*g* at 4°C for 10 min). The plasmas were immediately assayed for glycemia (Color GPO/GP, enzymatic methods, Wiener Labs, Rosario, Santa Fe, Argentina) and insulinemia (Rat insulin enzyme immunoassay kit, A05105—96 wells, SPI-BIO).

After 9 weeks, all groups were fasted for 12 h, weighed and sacrificed by decapitation. Blood samples collected from the bleeding trunk were centrifuged (3,500×*g* at 4°C for 10 min). Plasma glucose and triglyceride levels were measured by means of spectrophotometry (Automatic Analyzer Abbott Spectrum CCX, Abbot Diagnostics, Abbott Park, IL, USA) and commercial kits for glycemia and triglyceridemia (Color GPO/PAP AA, enzymatic methods, Wiener Labs, Rosario, Santa Fe, Argentina).

Prostanoid release measurement

The mesenteric beds from all groups were dissected, transferred to a Petri dish with saline solution at 4°C in order to wash the blood and incubated in Krebś solution with the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, NaH_2PO_4 1.0, $CaCl_2$ 2.6, $NaHCO_3$ 25.0, glucose 11.1 during 60 min at 37°C. In order to measure the PR release, at the end of the incubation period, the media were acidified to pH 3.5 with 1 M formic acid and extracted three times with two volumes of chloroform. The chloroform fractions were pooled and evaporated to dryness. Reversed-phase HPLC was carried out on a C_{18} column (Ultropack Lichrosorb E. Merck, Darmstadt, Germany). The solvent system was 1.7 mM H_3PO_4 67.2: acetonitrile 32.8 v/v. The flow rate was 1 ml min^{-1} and UV absorption was measured at 218 nm. Dried samples were resuspended in 0.15 ml of the mobile phase and injected into the HPLC system. Standards PR: 6-keto $PGF_1\alpha$, PGE_2, $PGF_2\alpha$ and TXB_2 (Sigma Chemical Co., Saint Louis, MO, USA) were run along with the samples and a bracket assay was performed to determine the amount of PR in the samples. All values were corrected for recovery loss as determined by parallel standards. Results were expressed as nanograms of PR per milligram of wet tissue weight.

Statistical analysis

All data are expressed as mean ± SEM. Intergroup comparisons were made by one-way analysis of variance (ANOVA) and a P value of 0.05 or less was considered statistically significant. When necessary the Tuckey post test was applied.

Results

Chronic administration of *F* as a 10% in tap water during 9 weeks was able to induce some of the alterations included in the cluster of risk factors called metabolic syndrome. There was no significant difference in the body weight (Table 1) or in the epididymal fat pads among groups at the end of the experiment (data not shown). Plasma triglycerides were increased in F-rats (*F* vs. C, $P < 0.01$) and diminished by DHEA treatment in control animals (D vs. C, $P < 0.01$). Hipertriglyceridemia in F-rats was prevented by DHEA treatment (*F* vs. FD, $P < 0.05$)

Table 1 Physical and biochemical parameters of the rats in the *C*, *D*, *F* and FD groups at the end of the experiment

	C	*D*	*F*	FD
Body weight (g)	459.7 ± 19.9	436.0 ± 20.1	474.5 ± 30.2	488.5 ± 20.9
Plasma glucose (mg dl^{-1})	88.5 ± 6.0	78.6 ± 1.2	87.7 ± 2.6	96.84 ± 5.6
Plasma triglycerides (mg dl^{-1})	93.7 ± 13.6	36.6 ± 5.25*	191.1 ± 35.2*†	106.6 ± 11.2‡

Mean values with their standard deviations, $n = 6$
C control, *D* DHEA; *F* fructose, *FD* fructose and DHEA
*$P < 0.01$ vs. *C*; †$P < 0.01$ vs. *D*; ‡$P < 0.05$ vs. *F*

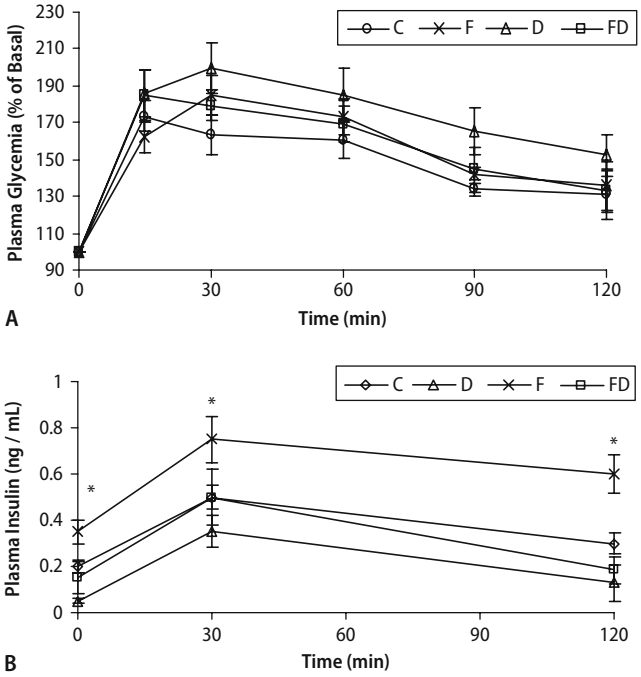

Fig. 1 Changes in plasma glucose (% of basal) (**a**) and insulin (**b**) in an oral glucose tolerance test (OGTT) (2 g glucose/kg BW) performed after 9 weeks of treatment from control (*C*), DHEA (*D*), Fructose (*F*) and Fructose and DHEA (FD) ($n = 6$ in all groups). Values are shown as the mean ± SD $^{*}P < 0.05$ compared to group C

(Table 1). Fasting serum glucose levels were similar among groups at the end of the study (Table 1). The analysis of the OGTT during 120 min showed that *F* overload during 9 weeks did not produced glucose intolerance, and that DHEA treatment did not modify it as shown in Fig. 1. Fasting hyperinsulinemia was only observed in the F-group (Fig. 1).

Although blood pressure in F-rats at the end of the treatment did not reach those levels corresponding to a severe hypertension, there was a significant difference compared to control levels (*F* vs. C, $P < 0.01$). DHEA treatment had no effect in control animals (*D*

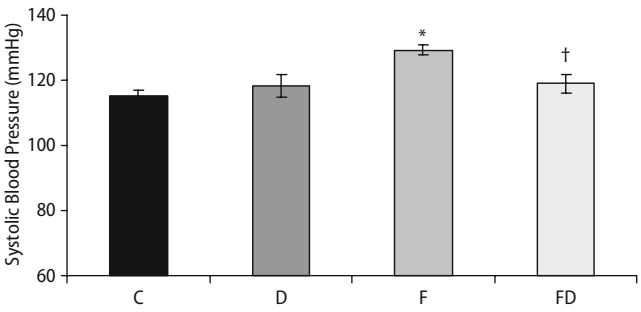

Fig. 2 Systolic blood pressure (mmHg) after 8 weeks of treatment from control (*C*), DHEA (*D*), Fructose (*F*) and Fructose and DHEA (FD) ($n = 6$ in all groups). Values are shown as the mean ± SD. $^{*}P < 0.01$ vs. *C*, $^{\dagger}P < 0.05$ vs. *F*

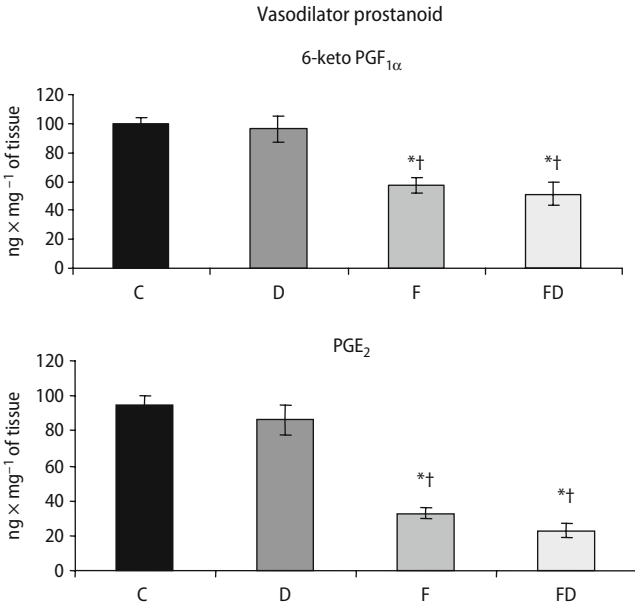

Fig. 3 Release of vasodilator PR (6-keto $PGF_{1\alpha}$ and PGE_2) by mesenteric vascular beds from control (*C*), DHEA (*D*), Fructose (*F*) and Fructose and DHEA (FD) ($n = 6$ in all groups). Values are shown as the mean ± SD (ng.mg tissue^{-1}) $^{*}P < 0.01$ vs. *C*; $^{\dagger}P < 0.01$ vs. DHEA

vs. *C*, n.s.), however DHEA prevented the increase in SBP levels of *F*-treated animals (*F* vs. FD, $P < 0.05$) (Fig. 2).

To evaluate the possible mechanisms by which DHEA treatment prevented the slight increase in SBP in F-animals, we measured the PR release from the mesenteric vascular bed in all experimental groups. As shown in Fig. 3, F-overload diminished 6-keto $PGF_1\alpha$ and PGE_2 release (*F* vs. *C* $P < 0.01$) and the treatment with DHEA in control and *F*-animals did not affect these vasodilator PR (*D* vs. *C* n.s.; FD vs. *F* n.s.).

On the other hand, in spite of the fact that *F* treatment did not modify vasoconstrictor PR release in the vascular bed, DHEA treatment decreased TXB_2 and $PGF_{2\alpha}$ in *F*-rats (FD vs. *F* $P < 0.01$) (Fig. 4). Moreover, the ratio PGI_2/TX is a parameter of endothelial dysfunction and F-feeding caused a significant decrease in this indicator which was prevented by DHEA treatment (Fig. 5).

Discussion

It has been reported that *F* feeding lowers plasma DHEAS levels in rats, a potentially important side effect of diabetes [15]. Although several studies have investigated the effects of DHEA supplementation in other experimental models [6, 14, 28, 44, 50], no

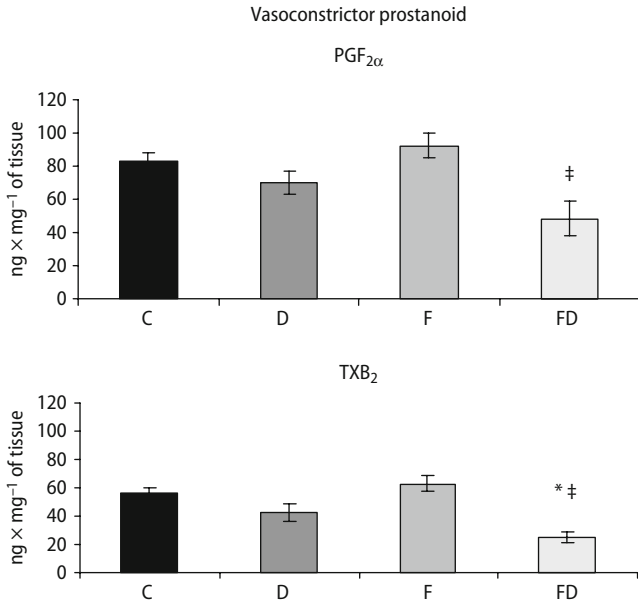

Fig. 4 Release of vasoconstrictor PR ($PGF_{2\alpha}$ and TXB_2) by mesenteric vascular beds from control (*C*), DHEA (*D*), Fructose (*F*) and Fructose and DHEA (FD) at the end of the treatment ($n = 6$ in all groups). Values are shown as the mean ± SD (ng mg tissue^{-1}) *$P < 0.01$ vs. *C*; ‡$P < 0.01$ vs. *F*

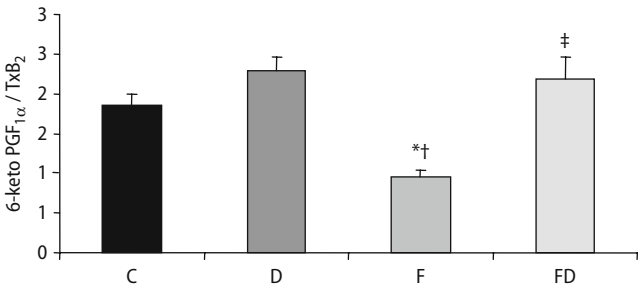

Fig. 5 Ratio PGI_2/TX from control (*C*), DHEA (*D*), Fructose (*F*) and Fructose and DHEA (FD) at the end of the treatment ($n = 6$ in all groups). Values are shown as the mean ± SD *$P < 0.01$ vs. *C*; †$P < 0.01$ vs. DHEA; ‡$P < 0.01$ vs. *F*

experiments were conducted so far looking in parallel the vascular and metabolic actions of DHEA supplementation in conscious, chronically overloaded F-rats, an experimental model of insulin resistance. The vascular and metabolic profiles associated with this animal model could provide information about the possible mechanisms underlying the etiology of hypertension in the metabolic syndrome and the possible role of DHEA in vascular production of PR. Our results demonstrate for the first time that treatment with DHEA prevents the development of F-induced hypertriglyceridemia, insulin resistance and hypertension in male rats.

It has been reported that rats treated with DHEA (30 mg/kg, every alternate day) induces a circulating DHEA sulfate level of ≈0.2 μM (60 ng/ml) after 3 weeks [6]. The baseline level of DHEA in humans is ≈10 nM. However, when DHEA supplements ranging from 50 to 2,250 mg/day are taken, the DHEA level in serum has been shown to increase to as high as 230 nM. Even thought the amount of DHEA required in rats was high to achieve an effect on vascular function, the amount of DHEA in the serum of the rats after the treatment, is achievable in humans when oral supplements are taken [50]. In the same paper, Yorek et al has reported that when serum DHEA levels achieved 100≈ ng/ml (0.35 μM), the estrogen level is ≈150 pg/ml (0.5 nM). However, the increase in estrogen level in the serum was not significant compared with the level in control rats. They demonstrated that estrogen, at a concentration similar to estrogen levels found in rats fed 0.25% DHEA (≈0.5 nM), did not induced vasodilatation in epineurial vessels in vitro [50]. It is unlikely that the effect of DHEA in improving insulin resistance-induced impairment in vascular relaxation was due to an increase in estrogen levels.

Pérez de Heredia et al. [38] showed that a high fat diet supplemented with DHEA (0.5% w/w) induced, in female and aged (77 weeks old) Sprague Dawley rats, significantly higher DHEA-S concentrations than control rats (829.0 ± 93.3 and 71.8 ± 26.0 ng/ml respectively). They found significant changes in food intake and in total weight and body fat in aged rats treated with DHEA during 12 weeks. We could not find any change in body and epididymal fat weight. These results are in accordance with other studies [14]. These differences could be a consequence of the length of the treatment, the way of administration, the age and sex of the animals, and the doses of DHEA. In our experimental conditions, male and young rats, treated with DHEA during 9 weeks, had circulating DHEA sulfate level of ≈0.2 μM (60 ng/ml) after 3 weeks [6].

Nagal and cols. have demonstrated that the expression of PPARα was downregulated in the liver of F-fed rats and in hepatocytes incubated in a high *F* condition, indicating a direct effect of *F* or its metabolites on PPARα expression [31]. DHEA has been characterized as a peroxisome proliferator [23] that increases the rate of lipolysis, increases flux of fatty acids through the *β*-oxidation pathway, and reduces the lypogenesis "de novo" in adipose tissue, accompanied by an increase in energy expenditure [21]. The reduced expression of PPARα in the liver reported in the liver of F-rats promotes the idea that stimulation of fatty acid oxidation and lypolysis by DHEA through the activation of PPARα and PPARγ is

other way by which DHEA prevents the increase in triglycerides found in F-rats.

In agreement with previous reports [35–37], the vascular mesenteric bed of F-rats showed a diminished release of PGE_2 and PGI_2, as well as an increased peripheral resistance associated to the inhibition of vasodilator PR release. This alteration could be one of the mechanisms that lead to the elevation of SBP levels in F-treated rats. DHEA treatment in F-rats diminished the release of vasoconstrictor PR such as $PGF_{2\alpha}$ and TXB_2 without any change in vasodilator PR. The PR release of DHEA treated animals tended to be lower than that of controls, but the difference was not statistically significant. Probably the action of DHEA is different between normal and insulin resistant rats. Metabolic insulin resistance is typically associated with selective impairment in NO production in the vasculature, predisposing to imbalanced intracellular signaling pathways that favor prohypertensive and proaterogenic effects of endothelin-1 [12]. As DHEA stimulates the same vasoactive substances as insulin, then the net vasoactive effect of DHEA may depend upon the balance between vasodilator and vasoconstrictor substances as PRs. Therefore, in animals with metabolic syndrome, DHEA treatment prevents the increases in SBP, normalizing the prostacyclin/tromboxane (PGI_2/TX) ratio, an indicator of endothelial dysfunction found diminished in F-rats. This could be one of the mechanisms by which DHEA prevents the mild increase of SBP in F-rats.

Although we did not investigate the regulatory mechanisms of PR production, we have previously reported that F-overloaded rats showed an impairment in the NO-dependent vascular contractility [8] which is the effector molecule in the insulin induced vasodilatation [29, 30]. Decreased eNOS activity was shown in aorta and mesenteric arteries from F-fed rats [27]. On the other hand, it has been recently demonstrated that DHEA caused an acute increase in NO production in vascular endothelial cells through PI3-K activation and PKB and eNOS phosphorylation [12] by a putative G protein coupled receptor [22]. NO acts mainly by two pathways: activation of guanylyl cyclase to yield cGMP and stimulation of COX 1-2 activities to produce PG [41]. Therefore, it is possible to speculate that the F-treated animals have an impaired insulin-dependent endothelial NO release which could also affect the vasodilator PR production and that DHEA increased NO production in F-rats, which induces the COX 1-2 activation and preventing the SBP increase.

Abnormalities in the arachidonic acid cascade pathways have been observed in diabetic animals [16, 43] and such disturbances disappeared when rats were treated with DHEA [2]. Platelet activation has been implicated in the atherogenesis and DHEA exert antiatherogenic and cardioprotective actions through the inhibition of platelet aggregation [19]. Mitogen-activated protein kinase (MAPK) is a central component of the growth factor stimulated protein kinase cascade. Yoshimata et al. [51] reported that DHEA significantly lessened platelet derived growth factor (PDGF) induced MAPK activation in human male aortic smooth muscle cells, probably via PGE_2 overproduction. Although in our experimental conditions DHEA treatment did not significantly modify the PGE_2 production, this regulation of MAPK cascade regulation could be involved.

We did not identify any change in PR production in the aorta. The mesenteric bed is a resistance vascular bed that contributes actively to the blood flow control during altered demands [7], and seems to be more sensitive than aorta, a conductance vessel, to the effects of F and DHEA regarding PR production. The important role of resistance vessels in the regulation of the peripheral resistance and consequently blood pressure could justify these differences.

We confirmed the development of insulin resistance and the elevation of triglycerides, already found in this model [11, 48]. DHEA treatment did not modify the insulin levels in C rats and impaired the insulin resistance without increasing insulin concentrations in F rats. DHEA is known to improve the effectiveness of insulin [9]. As insulin per se has some vasodilatory effects and an increase in insulin sensitivity would increase the vasodilatory effects of insulin per se, we can not conclude whether the prevention of endothelial dysfunction by DHEA, detected by impairment of mesenteric PGI_2 release in *F* rats, was directly related to insulin, DHEA, or both of them. However, Galipeau et al. had demonstrated that treatment with dazmegrel, an inhibitor of TX synthase, prevents the development of F-induced hypertension in male rats without any change in hyperinsulinemia or insulin resistance [13].

Estrogen (E_2) increases COX-2 expression in vascular tissues and augments PGI_2 production in vitro [1]. Egan et al had demonstrated that E_2–mediated COX-2-derived PGI_2 confers atheroprotection on female LDLR mice [10]. We cannot rule out the endogenous conversion of DHEA to E_2 but under our experimental conditions we were not able to detect any change in mesenteric vascular production of PGI_2 in animals treated with DHEA.

We reported here that DHEA has vasodilatory properties through the inhibition of TX production in a mimetic model of acquired insulin resistance characterized by diminished PGs production. In this way, our work contribute the original finding that DHEA normalize the PGI_2/TX ratio, diminished in F-overloaded rats, through the decrease in TX production and this could be one of the mechanisms by which

DHEA prevented the slight hypertension in F-animals. Therefore, in pathophysiological situation of underproduction of vasodilatory PR, DHEA could regulate a compensatory mechanism diminishing the production of vasoconstrictor PR. On the basis of the present data and others reports [25], alterations in COXs-dependent modulation of vasomotor function should be taken into consideration in future investigations of type 2 diabetes.

Acknowledgments This study was suppored by grants from the Secretaría de Ciencia y Técnica of the Universidad de Buenos Aires (Code B 109) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), PIP 2109/00 and PIP 5497. We gratefully acknowledge the valuable assistance of Dr. Belisario Fernandez in discussing and revising the manuscript.

References

1. Akarasereenont P, Techatraisak K, Thaworn A, Chotewuttakorn S (2000) The induction of cyclooxygenase-2 by 17-beta-estradiol in endothelial cells is mediated through protein kinase C. Inflamm Res 49:460–465
2. Argano M, Parola S, Brignardello E, Manti R, Betteto S, Tamagno E, Danni O, Boccuzzi G (2001) Oxidative stress and eicosanoids in the kidneys of hyperglycemic rats treated with dehydroepiandrosterone. Free Radical Biol Med 31:935–942
3. Barret-Connor E, Kaw KT, Yen SS (1986) A prospective study of dehydroepiandrosterone sulphate, mortality, and cardiovascular disease. N Engl J Med 315:1519–1524
4. Baulieu EE, Corpechot C, Dray F, Emiliozzi R, Lebeau MC, Mauvais-Jarvis P, Robel P (1965) An adrenal secreted androgen: dehydroepiandrosterone sulfate. Its metabolism and a tentative generalization on the metabolism of other steroid conjugates in man. Recent Prog Horm Res 21:411–500
5. Belanger A, Candas B, Dupont A, Cusan L, Diamond P, Gomez JL, Labrie F (1994) Changes in serum concentrations of conjugated and unconjugated steroids in 40- to 80-year-old men. J Clin Endocrinol Metab 79:1086–1090
6. Bonnet S, Dumas-de-la-Roque E, Begueret H, Marthan R, Fayon M, Dos Santos P, et al (2003) Dehydroepiandrosterone (DHEA) prevents and reverses chronic hypoxic pulmonary hypertension. PNAS 100:9488–9493
7. Chistensen KL, Mulvany MJ (2001) Location of resistance arteries. J Vasc Res 38:1–12
8. Damiano PF, Cavallero S, Mayer M, Rosón MI, de la Riva I, Fernández B, Puyó AM (2002) Impaired response to insulin associated to protein kinase C in chronic fructose-induced hypertension. Blood Press 11:345–351
9. Dhatariya K, Bigelow ML, Nair KS (2005) Effect of dehydroepiandrosterone replacement on insulin sensitivity and lipids in hypoadrenal women. Diabetes 54:765–769
10. Egan KM, Lawson JA, Fries S, Koller B, Rader DJ, Smyth EM, FritzGerald GA (2004) COX-2-derived prostacyclin confers atheroprotection on female mice. Science 306:1954–1956
11. Erdos B, Miller AW, Busija DW (2002) Impaired endothelium-mediated relaxation in isolated cerebral arteries from insulin-resistant rats. Am J Physiol 282:H2060–H2065
12. Formoso G, Chen H, Kim JA, Montagnani M, Consoli A, Quon MJ (2006) DHEA mimics acute actions of insulin to simulate production of both NO and ET-1 via distinct PI 3-kinase- and MAP-kinase- dependent pathways in vascular endothelium. Mol Endocrinol 20:1153–1163
13. Galipeau D, Arikawa E, Sokivov J, McNeill SH (2001) Chronic thromboxane synthase inhibition prevents fructose-fed hypertension. Hypertension 38:872–876
14. Hansen PA, Han DH, Nolte LA, Chen M, Holloszy JO (1997) DHEA protects against visceral obesity and muscle insulin resistance in rats fed a high-fat diet. Am J Physiol 273:R1704–R1708
15. Harati M, Ani M (2004) Vanadyl sulfate ameliorates insulin resistance and restores plasma dehydroepiandrosterone-sulfate levels in fructose-fed, insulin-resistant rats. Clin Biochem 37:694–697
16. Holman RT, Johnson SB, Gerrard JM, Mauer SM, Kupcho-Sandberg S, Brown DM (1983) Arachidonic acid deficiency in streptozotocin-induced diabetes. Proc Natl Acad Sci USA 80:2375–2379
17. Hwang JS, Ho H, Hoffman BB, Reaven GM (1987) Fructose induced insulin resistance and hypetension in rats. Hypertension 10:512–516
18. Iamberts SW, van den Beld AW, van der Lely AJ (1997) The endocrinology of aging. Science 278:419–424
19. Jesse RL, Loesser K, Eich DM, Qian YZ, Nestler JE (1995) Dehydroepiandrosterone inhibits human platelet aggregation in vitro and in vivo. Ann NY Acad Sci 774:281–290
20. Johannes CB, Stellato RK, Feldman HA, Longcope C, McKinlay JB (1999) Relation of dehydroepiandrosterone and dehydroepiandrosterone sulfate with cardiovascular disease risk factors in women: longitudinal results from the Massachusetts women's health study. J Clin Epidemiol 52:95–103
21. Karbowska J, Kochan Z (2005) Effect of DHEA on endocrine functions of adipose tissue, the involvement of PPARγ. Biochem Pharm 70:249–257
22. Liu D, Dillon JS (2002) Dehydroepiandrosterone activates endothelial cell nitric oxide synthase by a specific plasma membrane receptor coupled to $G_{\alpha i2,3}$. J Biol Chem 277:21379–21388
23. Mastrocola R, Argano M, Betteto S, Brignardello E, Catalano MG, Danni O, Boccuzzi G (2003) Pro-oxidant effect of dehydroepiadrosterone in rats is mediated by PPAR activation. Life Sci 73:289–299
24. Matsuda M, DeFronzo R (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing. Diab Care 22:1462–1470
25. Matsumoto T, Kakami M, Noguchi E, Kobayashi T, Kamata K (2007) Imbalance between endothelium-derived relaxing and contracting factors in mesenteric arteries from aged OLEFT rats, a model of type 2 diabetes. Am J Physiol Heart Circ Physiol 293:41480–41490
26. Mayer MA, Höcht C, Opezzo JA, Taira CA, Fernández BE, Puyó AM (2007) High fructose diet increases anterior hypothalamic alpha 2-adrenoceptors responsiveness. Neurosci Lett 423:128–132
27. Miatello R, Risler N, Gonzalez S, Castro C, Ruttler M, Cruzado M (2002) Effects of enalapril on the vascular wall in an experimental model of syndrome X. Am J Hypertens 15:872–878
28. Mohan PF, Ihnen JS, Levin BE, Cleary MP (1990) Effects of dehydroepiandrosterone treatment in rats with diet-induced obesity. J Nutr 120:1103–1114

29. Montagnani M, Chen H, Barr VA, Quon MJ (2001) Insulin-stimulated activation of eNOS is independent of Ca^{2+} but requires phosphorylation by Akt at Ser(1179). J Biol Chem 276:30392–303928
30. Muniyappa R, Montagnani M, Koh KK, Quon MJ (2007) Cardiovascular actions of insulin. Endocr Rev 28:463–491
31. Nagal Y, Nishio Y, Nakamura T, Maegawa H, Kikkawa R, Kasiwagi A (2002) Amelioration of high fructose-induced metabolic derangements by activation of PPARα. Am J Physiol 282:E1180–E1190
32. Nestler JE, Clore JN, Blackard WG (1991) Metabolism and actions of dehydroepiandrosterone in humans. J Steroid Biochem Mol Biol 40:599–605
33. Nestler JE, Usiskin KS, Barlascini CO, Welty DF, Clore JN, Blackard WG (1989) Suppression of serum dehydroepiandrosterone sulfate levels by insulin: an evaluation of possible mechanisms. J Clin Endocrinol Metab 69:1040–1046
34. Peredo HA (2001) Prostanoid release, constrictor responses to noradrenaline in the mesenteric vascular bed in non-insulin-dependent diabetes mellitus. J Auton Pharmacol 21:131–137
35. Peredo HA, Mayer MA, Carranza A, Puyó AM (2008) Pioglitazone and losartan prevent hypertension and hypertrigliceridemia and modify vascular prostanoids in fructosa-overloaded rats. Clin Exp Hypertens (in press)
36. Peredo HA, Mayer MA, Rodríguez-Fermepín M, Grinspon D, Puyó AM (2006) Oral treatment and in vitro incubation with fructose modify vascular prostanoid production in the rat. Auton Autacoid Pharmacol 26:15–20
37. Puyo AM, Mayer MA, Cavallero S, Donoso AS, Peredo HA (2008) Fructose overload modifies vascular morphology and prostaglandin production in rats. Clin Exp Hypertens 30:159–169
38. Pérez de Heredia F, Cerezo D, Zamora S, Garaulet M (2007) Effect of dehydroepiandrosterone on protein and fat digestibility, body protein and muscular composition in high-fat-diet-fed old rats. Br J Nutr 97:464–470
39. Reaven GM (1988) Banting lecture 1988: role of insulin resistance in human disease. Diabetes 37:1507–1597
40. Reaven GM, Ho H (1991) Sugar-induced hypertension in Sprague–Dawley rats. Am J Hypertens 4:610–614
41. Rettori V, Gimeno M, Lyson K, McCaan S (1992) Nitric oxide mediates norepinephrine-induced prostaglandin E_2 release from the hypothalamus. Proc Natl Acad Sci USA 89:11543–11546
42. Segal MS, Gollub E, Johnson RJ (2007) Is the fructose index more relevant with regards to cardiovascular disease than the glycemic index? Eur J Nutr 46(7):406–417
43. Setty B, Stuart M (1986) 15-Hydrixy-5,8,11,13-eicosatetraenoic acids inhibits its human vascular cyclooxygenase, potential role in diabetic vascular disease. J Clin Invest 77:202–211
44. Shafagoj Y, Opoku J, Qureshi D, Regelson W, Kalimi M (1992) Dehydroepiandrosterone prevents dexamethasone-induced hypertension in rats. Am J Physiol 263:E210–E213
45. Simoncini T, Hafezi-Mogham A, Brazil DP, Ley K, Chin WW, Liao JK (2000) Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-kinase . Nature 407:538–541
46. Thornburn W, Storlien LH, Jendius AB, Khouri S (1989) Fructose-induced insulin resistance and elevated plasma triglycerides in rats. Am J Clin Nutr 49:1155–1163
47. Verma S, Bhanot S, Yao J, McNeill JH (1996) Defective endothelium-dependent relaxation in fructose-hypertensive rats. Am J Hypertens 9:370–376
48. Xi L, Qian Z, Xu G, Zheng S, Sun S, Wen N, Sheng L, Shi Y, Zhang Y (2007) Beneficial impact of crocetin, a carotenoid from saffron, on insulin sensitivity in fructose-fed rats. J Nutr Biochem 18:64–72
49. Yamaguchi Y, Tanaka S, Yamakawa T, Yamakawa T, Kimura M, Ukawa K et al (1988) Reduced serum dehydroepiandrosterone levels in diabetic patients with hyperinsulinaemia. Clin Endocrinol (Oxf) 49:377–383
50. Yorek JA, Coppey LJ, Gellett JS, Davidson EP, King X, Lund DD, Dillon JS (2002): Effect of treatment of diabetic rats with dehydroepiandrosterone on vascular and neural function. Am J Physiol Endocrinol Metab 283:E1067–E1075
51. Yoshimata T, Yoneyama A, Jin-no Y, Tamai N, Kamiya Y (1999) Effects of dehydroepiandroseterone on mitogen-activated protein kinase in human aortic smooth muscle cells. Life Sci 65:431–440