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Dehydroepiandrosterone prevents age-associated alterations, increasing insulin sensitivity☆

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Abstract

The age decline in DHEA levels has been associated with the appearance of age-related disorders such as obesity and insulin resistance. The aim of this study was to analyse the effect of chronic administration (13 weeks) of DHEA (5 g/kg diet) to old female rats fed on a high-fat diet on body weight and adiposity, and concretely on the expression of the adipokines related to obesity and insulin resistance, such as leptin, adiponectin and resistin. DHEA treatment induced a decrease in body weight, adipose tissue mass and serum insulin, adiponectin and leptin levels. Adiponectin mRNA expression in visceral fat depots decreased with aging, but this reduction was attenuated by DHEA treatment. DHEA treatment also stimulated resistin gene expression in the ovaric and renal adipose depots, which is associated with an increase in its circulating levels. In conclusion, DHEA treatment decreases body weight and adiposity in old female rats fed a high-fat diet, leading to an improvement of the HOMA index for insulin sensitivity, with decreasing circulating insulin levels, and preventing the age-associated decline of visceral-adipose adiponectin expression.

Keywords: DHEA; Adiponectin; Leptin; Resistin; Insulin resistance

1. Introduction

Obesity is closely associated with insulin resistance and is considered to be the leading risk factor for Type 2 diabetes mellitus and cardiovascular diseases [1,2]. Adipose tissue is an active endocrine organ that releases a number of cytokines, namely, leptin, adiponectin and resistin, which influence not only body weight homeostasis but also insulin resistance [3].

Leptin is released into the circulatory system by the adipose tissue in proportion to the amount of lipid stores [4,5] and acts at hypothalamic receptors [6], decreasing food intake and increasing energy expenditure [7]. Resistin and adiponectin are two adipokines with postulated opposite functions. Resistin has been related with insulin resistance in obesity, while adiponectin could be associated to higher insulin sensitivity [8]. Adiponectin is secreted by fat cells and circulates in the blood in a high concentration. Plasma adiponectin concentration is reduced in obesity and insulin resistance states [9,10] and increases with the improvement of insulin sensitivity and with body weight loss [11,12]. In initial studies, serum resistin levels were found to be elevated in obese and diabetic mice; neutralization of resistin with antibodies improved insulin sensitivity, and the treatment with thiazolidinediones down-regulated the gene expression of this cytokine in white adipose tissue (WAT) [13]. Although later studies initially failed to confirm this hypothesis, recent studies with resistin-knockout mice and transgenic mice overexpressing a fusion protein that blocks

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resistin action clearly support a role for this protein in the development of obesity and Type 2 diabetes [14,15]. In addition, mice with reduced resistin mRNA levels in adipose tissue and reduced circulating resistin levels after retinoic acid administration exhibit improved glucose tolerance [16].

The adrenal androgen dehydroepiandrosterone (DHEA) and its sulfate derivate (DHEA-S) are the most abundant adrenal steroids in humans [17]. Plasma levels of both DHEA and DHEAS decrease steadily with aging [18]. This age-related decline in DHEAS has been inversely related with a number of age-associated diseases, including atherosclerosis [19], non-insulin-dependent diabetes mellitus [20] and obesity [21]. Several studies performed in humans have suggested that higher circulating DHEA levels are associated with lower body fat accumulation (reviewed in Ref. [22]). DHEA administration has long been associated with reduced weight gain in growing animals and with weight loss in mature animals [23-28]. DHEA may reduce weight gain by inhibiting food intake [23,29] or may cause a metabolic effect, as evidenced by less weight gain despite no change in food intake [25,30]. In addition, DHEA treatment has antidiabetic effects in some strains of mice [20] and reduces serum insulin levels in hyperinsulinemic, diet-induced or genetically obese rats [25,27]. Accordingly, it has been proposed that DHEA may have an effect on adipose tissue, which results in the prevention of insulin resistance [31].

The aim of this study was to analyse the effect of the chronic administration of DHEA to high-fat (HF) diet-fed old rats on body weight and adiposity, and its effects on the expression of adipokines related to obesity and insulin resistance.

2. Methods and materials

2.1. Animals and experimental design

Female Sprague-Dawley rats housed in controlled temperature (24±2°C) and light (lights on from 0800 to 2000) conditions were used. At the age of 7 weeks rats were fed with a HF diet. The diet contained (expressed as a percentage of dry mass) 18.4% protein, 50.4% carbohydrates (50% of which was sucrose) and 19.6% fat; the energy content was 1880 kJ/100 g, and fat amounted for 39% total dietary energy [29]. Palm oil was used as source of fat, and fatty acid composition of the diet was 53.6% saturated fatty acids, 36.3% monounsaturated fatty acids, 9.5% polyunsaturated fatty acids and 0.6% *trans* fatty acids. This diet was freshly prepared by our own group once a week, gassed with nitrogen and stored at 5°C to avoid rancidity. A randomly assigned group of rats (n=6) were sacrificed at the age of 14 months (14-month-old group). The rest of the rats remained with the same HF-diet until the age of 17 months when half of the rats (n=11) were treated for 13 weeks (until the age of 20 months) with the HF-diet supplemented with DHEA (5 g/kg diet) while the rest (n=10) continued without changes. Food was freshly dispensed every 2–3 days; both the remaining and the spilled food were always removed, collected and weighed, in order to determine individual food intake

Animals were anaesthetised with diethyl ether and killed by exsanguination under fasting conditions at the beginning of the light phase (0800–1100 hours). Different WAT depots — ovaric (oWAT), renal (rWAT), mesenteric (mWAT) and total subcutaneous adipose tissue from the abdominal region (scWAT) — were rapidly removed, weighed and then the adipocytes from the different WAT were isolated. Carcasses were homogenised by mincing in a grinder, and fat contents were determined in the Soxhlet apparatus. Body fat was calculated considering carcass fat and dissected adipose depots.

Blood was also collected, stored at room temperature for 1 h and centrifuged at 3500 rpm for 15 min to collect the serum, which was stored at −80°C until analysis.

The animal protocol followed in this study was reviewed and approved by the bioethical committee of the university, and guidelines for the use and care of laboratory animals of the university were followed.

2.2. Quantification of serum adiponectin, resistin and leptin concentration

Serum adiponectin concentration was measured with a rat adiponectin enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Europe GmbH, Karlsruhe, Germany), serum resistin concentration was measured with a human enzyme immunoassay kit (Phoenix Europe) and leptin concentration in serum was measured with a mouse leptin ELISA kit (R&D Systems, Minneapolis, MN, USA).

2.3. Quantification of serum triacylglyceride, insulin and glucose levels

Serum triacylglyceride concentration was measured enzymatically using a commercial kit and following standard procedures (Triglyceride (INT)20, Sigma Diagnostics, St. Louis, MO, USA). Serum insulin was measured by RIA, using a commercial kit (Linco, St. Charles, MO, USA), and serum glucose was measured by spectrophotometry using an enzymatic kit with glucose-oxidase and peroxidase.

The homeostatic model assessment for insulin resistance (HOMA-IR) was used to assess insulin resistance. It was calculated from fasting insulin and glucose concentration using the formula of Matthews et al. [32]:

$$\text{HOMA}-\text{IR} = \text{Fasting glucose (mmol/L)} \times \text{fasting insulin (mIU/L)}/22.5.$$

2.4. Adipocyte isolation

Adipocytes from different WAT depots (ovaric, renal, mesenteric and subcutaneous) were isolated according to the Rodbell method [33] with minor modifications [34]. Tissue samples were subjected to collagenase A digestion (1 mg/ml; 37°C) (Boehringer Mannheim, Mannheim, Germany) in Krebs-Ringer bicarbonate (KRB) buffer, containing 3.5 g/

100 ml of bovine serum albumin (BSA V) (Sigma, St. Louis, MO) and 6 mmol/L of glucose at pH 7.4 (KRBA). Adipocytes were filtered through a nylon mesh and washed with the same incubation buffer (KRBA). Fat cell size was measured by optic microscopy, with the aid of a computed image analysis system (MIP 4.5 Microm Image Processing Software, Consulting Image Digital, SL, Barcelona, Spain), and mean diameter was calculated by measuring 200 cells. Adipocyte number was estimated in each depot considering average cell weight and depot weight.

2.5. RNA extraction

Total RNA from isolated adipocytes was extracted using RNeasy Mini Kit from Qiagen (IZASA SA, Barcelona, Spain) according to the instructions of the manufacturers. Isolated RNA was quantified by spectrophotometry and its integrity was confirmed using agarose gel electrophoresis.

2.6. Real-time quantitative polymerase chain reaction analysis

Real-time polymerase chain reaction (PCR) was used to measure the mRNA expression levels of adiponectin, resistin and leptin in isolated adipocytes from different WAT depots (renal, ovaric, mesenteric and subcutaneous).

0.5 μg of total RNA (in a final volume of 25 μl) was denatured at 90°C for 1 min and then reverse transcribed to cDNA using MuLV reverse transcriptase (Applied Biosystem, Madrid, Spain) at 20°C for 15 min and 42°C for 30 min, with a final step of 5 min at 99°C in a Perkin-Elmer 9700 Thermal Cycler (Perkin-Elmer, Wellesley, MA, USA).

Real-time PCR was completed using the LightCycler System with SYBR Green I sequence nonspecific detection (Roche Diagnostic Gmbh, Mannheim, Germany). Primers for the different genes are described in Table 1. All primers were obtained from Bonsai Technologies Group, SA (Madrid, Spain).

Each PCR was performed in a total volume of 10 μl, made from diluted (1/50) cDNA template, forward and reverse primers (1 μM each), and SYBR Green I master mix (1.8 μl, including Taq polymerase, reaction buffer, $MgCl_2$, SYBR Green I dye and dNTP mix). After an initial Taq activation at 95°C for 10 min, LightCycler PCR was performed using 40 cycles with the cycling conditions described in Table 1. In order to verify the purity of the products, a melting curve was produced after each run by increasing the temperature of the reaction mixtures up to 95°C, by 0.1°C/s, starting at 55°C for 10 s.

Relative quantification of a target gene was calculated based on efficiency (E) and the crossing point (CP) deviation of an unknown sample vs. a control, and expressed in comparison to a reference gene (18S) [35].

$$\text{Ratio} = \frac{(E_{target})^{\Delta CP_{target}(control-sample)}}{(E_{ref})^{\Delta CP_{ref}(control-sample)}}$$

The ratio of a target gene is expressed in a sample vs. a control in comparison to a reference gene. E_{target} is the real-time PCR efficiency of target gene transcript; E_{ref} is the real-time PCR efficiency of a reference gene transcript, $\Delta CP_{\text{target}}$ is the CP deviation of control average value minus sample of the target gene transcript and ΔCP_{ref}=CP deviation of control average value minus sample of reference gene transcript. Real-time PCR efficiencies were calculated, according to $E=10^{[-1/\text{slope}]}$ [35].

2.7. Statistical analysis

All data are expressed as the mean±S.E.M. One-way ANOVA followed by least significances difference post hoc comparison was used to assess statistical differences between the groups. Threshold of significances was defined at $P<.05$.

3. Results

3.1. Effect of DHEA body weight, WAT weight and cumulative food intake

At 20 months of age, rats that received the DHEA treatment for 13 weeks presented a lower body weight than rats with the same age that did not receive the hormonal treatment and even lower than 14-month-old rats (15.3% and

Table 1
Nucleotide sequences of primers and cycling conditions used for PCR amplification

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Cycling conditions	Amplicon size (bp)
Adiponectin	GCTCAGGATGCTACTGTTG	TCTCACCCTTAGGACCAAG	95°C for 2 s 57°C for 6 s 72°C for 10 s	247
Resistin	CTGCTCCTGTGGCTCTGGC	CAGACCCTCATCTCGTTTCCTG	95°C for 2 s 60°C for 6 s 72°C for 10 s	241
Leptin	CCAGGATGACACCAAAACCCTC	ATCCAGGCTCTCTGGCTTCTG	95°C for 2 s 58°C for 8 s 72°C for 14 s	316
18S	CGCGGTTCTATTTTGTTGGT	AGTCGGCATCGTTTATGGTC	95°C for 2 s 60°C for 6 s 72°C for 10 s	219

Table 2
Body weight and size of total WAT, rWAT, mWAT and scWAT, as well as diameter and number of adipocytes from the oWAT, mWAT and scWAT, of rats fed on a HF diet at 14 and 20 months and at 20 months after 13 weeks of treatment with DHEA

	14-month-old (n=6)	20-month-old (n=10)	20-month-old +DHEA (n=11)
Body weight	325±7[a]	336±16[a]	285±9[b]
Total body fat (g)	67.4±2.9[ab]	88.6±11.2[a]	35.5±2.9[b]
Total WAT (g)	15.79±0.52[a]	24.50±3.04[b]	9.97±0.84[a]
oWAT (g)	5.45±0.83[a,b]	7.25±1.00[a]	3.35±0.29[b]
rWAT (g)	2.84±0.32[a]	3.43±0.50[a]	1.67±0.17[b]
mWAT (g)	2.73±0.26[a]	4.80±0.71[b]	2.03±0.23[a]
scWAT (g)	4.78±0.29[a]	9.02±1.05[b]	2.92±0.22[a]
oWAT adipocyte diameter (μm)	97.42±3.57[a]	90.54±5.36[a]	73.96±2.95[b]
mWAT adipocyte diameter (μm)	70.68±1.39[a]	69.57±3.05[a]	54.70±3.25[b]
scWAT adipocyte diameter (μm)	67.65±5.56[a]	58.04±3.85[a,b]	52.69±2.42[b]
oWAT adipocyte number ($\times 10^6$)	13.11±2.61[a]	20.51±2.84[b]	17.43±1.22[a,b]
mWAT adipocyte number ($\times 10^6$)	16.12±1.31[a]	29.22±3.88[b]	26.89±2.69[b]
scWAT adipocyte number ($\times 10^6$)	39.15±8.29[a]	110.12±20.38[b]	46.10±6.85[a]

Data are mean±S.E.M. Data not sharing a common superscript are significantly different (P<.05, LSD post hoc test).

12.4% of body weight reduction, respectively) (P<.05, one-way ANOVA) (Table 2).

At 20 months of age, rats presented a greater amount of fat pads compared to 14-month-old rats, which was statistically significant in the case of m- and scWAT, but this increase was avoided by the DHEA treatment (P<.05, one-way ANOVA) (Table 2). In fact, the treatment resulted in a 59.3% reduction of the total WAT weight compared to rats of the same age (P<.05, one-way ANOVA) and a 36.8% reduction compared to 14-month-old rats. This reduction was observed in all depots, but was higher in the subcutaneous WAT (67.6% of reduction). The DHEA treatment resulted in diminished adipocyte size compared to both 14- and 20-month-old rats in the mWAT and oWAT (P<.05, one-way ANOVA) and in fewer adipocytes in scWAT compared to the 20-month-old group (P<.05, one-way ANOVA) (Table 2).

Cumulative food intake during the DHEA treatment (from 17 to 20 months of age) was significantly lower in the DHEA-treated group (6269±250 and 4180±195 kcal in 20-month-old and 20-month-old DHEA-treated group, respectively).

3.2. Effect of DHEA on serum triacylglyceride insulin and glucose levels

A slight increase in serum TG levels was observed as effect of age, but this increase seems to be avoided by the DHEA treatment (the mean values were 98.08±18.78, 133.47±40.47 and 87.86±11.04 mg/dL in the 14- and 20-month-old and 20-month-old DHEA-treated groups, respectively). However, these differences did not reach statistical significance.

At the age of 20 months, the rats that received the DHEA treatment presented lower serum insulin levels compared to those that received placebo, and showed similar levels to the younger group (14-month-old rats) (P<.05, one-way ANOVA) (Fig. 1).

On the other hand, serum glucose levels decreased with age and no differences were observed at the age of 20 months as an effect of DHEA treatment (P<.05, one-way ANOVA) (Fig. 1).

HOMA-IR index was calculated from fasting insulin and glucose concentrations in all groups. This value tended to be slightly lower in the 20-month-old DHEA-treated group (2.13±0.37) than in the 14- and 20-month-old groups (4.55±1.85 and 4.04±1.19, respectively).

3.3. Effect of DHEA on serum adiponectin levels and adiponectin expression in WAT

Under a HF diet, serum adiponectin levels remained unchanged as an effect of age, since no changes were observed between the 14- and 20-month-old groups. Of interest, the 20-month-old DHEA-treated group presented a significantly lower serum adiponectin concentration (P<.05, one-way ANOVA) (Fig. 2A).

As an effect of age, adiponectin expression in the visceral WAT depots (oWAT, rWAT and mWAT) decreased in both groups of 20 months of age (P<.05, one-way ANOVA), but this decrease was attenuated in the DHEA-treated group (P<.05, one-way ANOVA) (Fig. 2B). Compared to the

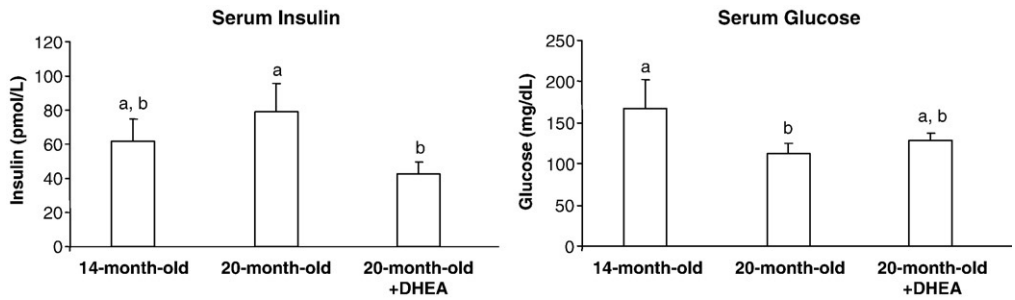

Fig. 1. Serum insulin and serum glucose concentrations of rats fed on a HF diet at 14 and 20 months and at 20 months after 13 weeks of treatment with DHEA. Data are mean±S.E.M. (n=6–11). Data not sharing a common superscript are significantly different (P<.05, LSD post hoc test).

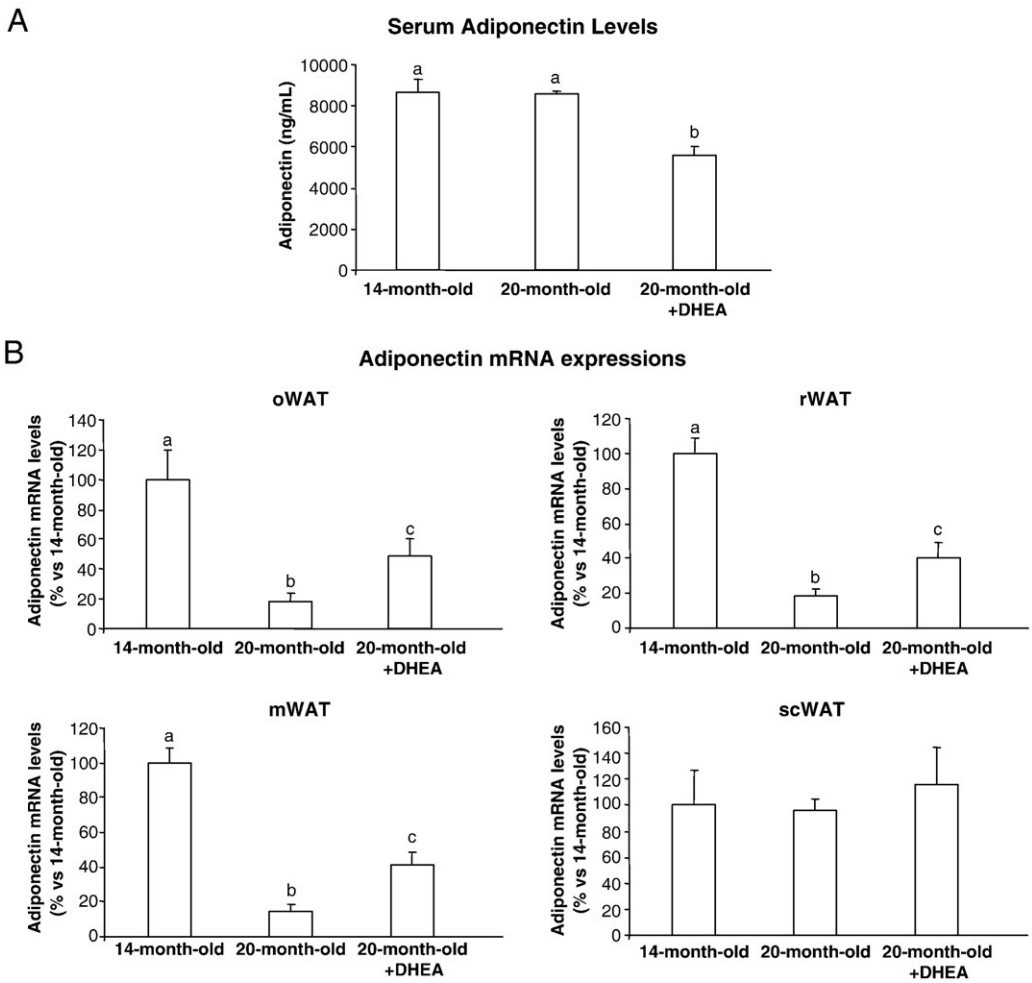

Fig. 2. Serum adiponectin levels (A) and adiponectin mRNA levels (B) in different WAT depots (oWAT, rWAT, mWAT, scWAT) in rats fed on a HF diet at 14 and 20 months and at 20 months after 13 weeks of treatment with DHEA. Adiponectin mRNA levels in WAT depots were determined by real-time transcriptase-PCR and expressed as a percentage of the mean value of 14-month-old animals. Data are mean±S.E.M. (n=6–11). Data not sharing a common superscript are significantly different (P<.05, LSD post hoc test).

14-month-old group, this reduction in adiponectin mRNA expression was 82.0%, 81.8% and 85.1% in the oWAT, rWAT and mWAT, respectively, in the 20-month-old group, and 51.1%, 60.1% and 59.0% in the oWAT, rWAT and mWAT, respectively, in the 20-month-old DHEA-treated group. On the other hand, in scWAT adiponectin mRNA expression remained unchanged by both age and DHEA treatment (Fig. 2B).

3.4. Effect of DHEA on serum resistin levels and resistin expression in WAT

No changes were observed in serum resistin levels between the 14- and 20-month-old groups, so resistin seems to remain unchanged by age. However, a significant increase in serum resistin levels was observed in the 20-month-old DHEA-treated group (51.3% rise with respect to the 20-month-old nontreated group) (P<.05, one-way ANOVA) (Fig. 3A).

There is a site-specific difference regarding resistin expression (Fig. 3B): in the oWAT, resistin mRNA expression was not affected by age, but DHEA treatment resulted in an increase in resistin expression (P<.05, one-way ANOVA); in the rWAT, resistin mRNA expression was reduced by age and this reduction was abolished by the DHEA treatment (P<.05, one-way ANOVA); in the mWAT, resistin mRNA levels tended to increase from the age of 14 to 20 months, although this increase did not reach a significant difference, and DHEA treatment did not produce any effect in this depot; and in the scWAT, resistin mRNA levels were not affected either by age or by DHEA treatment.

3.5. Effect of DHEA on serum leptin levels and leptin expression in WAT

In the same way, as serum adiponectin levels, circulating leptin concentration remained unchanged as an effect of age, since no changes were observed between the 14- and

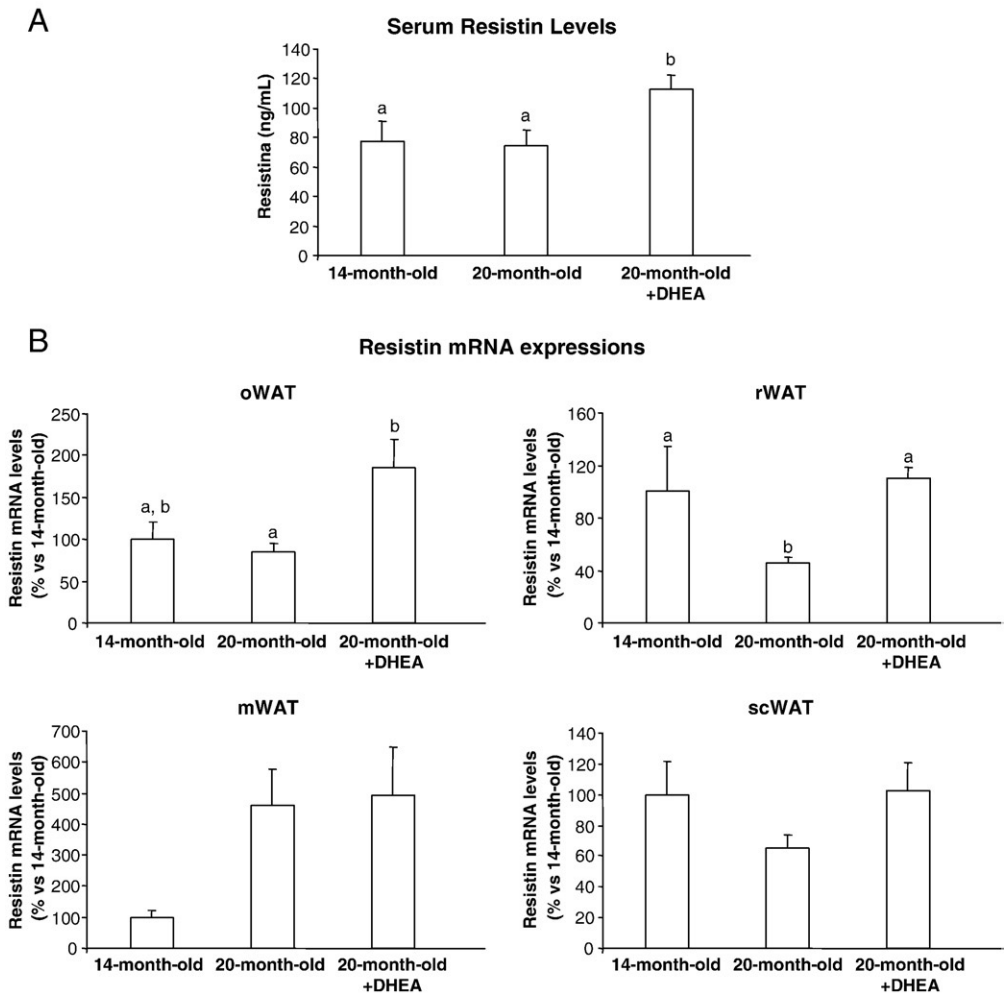

Fig. 3. Serum resistin levels (A) and resistin mRNA levels (B) in different WAT depots (oWAT, rWAT, mWAT, scWAT) in rats fed on a HF diet at 14 and 20 months and at 20 months after 13 weeks of treatment with DHEA. Resistin mRNA levels in WAT depots were determined by real-time transcriptase-PCR and expressed as a percentage of the mean value of 14-month-old animals. Data are mean±S.E.M. (n=6–11). Data not sharing a common superscript are significantly different (P<.05, LSD post hoc test).

20-month-old groups. However, in the 20-month-old DHEA-treated group a lower serum leptin concentration was observed (P<.05, one-way ANOVA) (Fig. 4A).

Leptin mRNA expression in the oWAT and in the rWAT decreased as an effect of age and this decrease was not affected by the DHEA treatment (P<.05, one-way ANOVA) (Fig. 4B): both groups of 20-month-old rats presented lower mRNA expression levels than the 14-month-old group, and at 20 months of age no differences were found between nontreated and treated groups. In the mWAT and scWAT, leptin mRNA expression remained unchanged by both age and DHEA treatment.

4. Discussion

Here we have described that DHEA administration to adult female rats under a HF diet produced a reduction in body weight and adiposity, which is in accordance to previous studies [25,27]. The low body weight in 20-month-old DHEA-treated group can be attributed to a reduction in the total body fat mass.

As an effect of age there was a great accumulation of fat, mainly in the mesenteric and subcutaneous depots, since 20-month-old rats presented an increase of total WAT compared to 14-month-old rats, but this increase was abolished by the effect of DHEA. In humans, several studies have reported an inverse relation between serum DHEA concentrations and total adiposity [22,36,37], and DHEA circulating levels are also known to decrease with age [18], so it has been postulated that the decline in DHEA may be associated with a number of age-related diseases, including obesity [21,22]. Here we have shown that DHEA prevents the body fat accumulation occurring with aging and HF-diet feeding. This DHEA-mediated reduction in fat mass may be due to a reduction in the energy intake or it may be mediated by conversion into estrogens or androgens, since DHEA is the precursor for most sexual hormones [38]. Indeed, estrogen

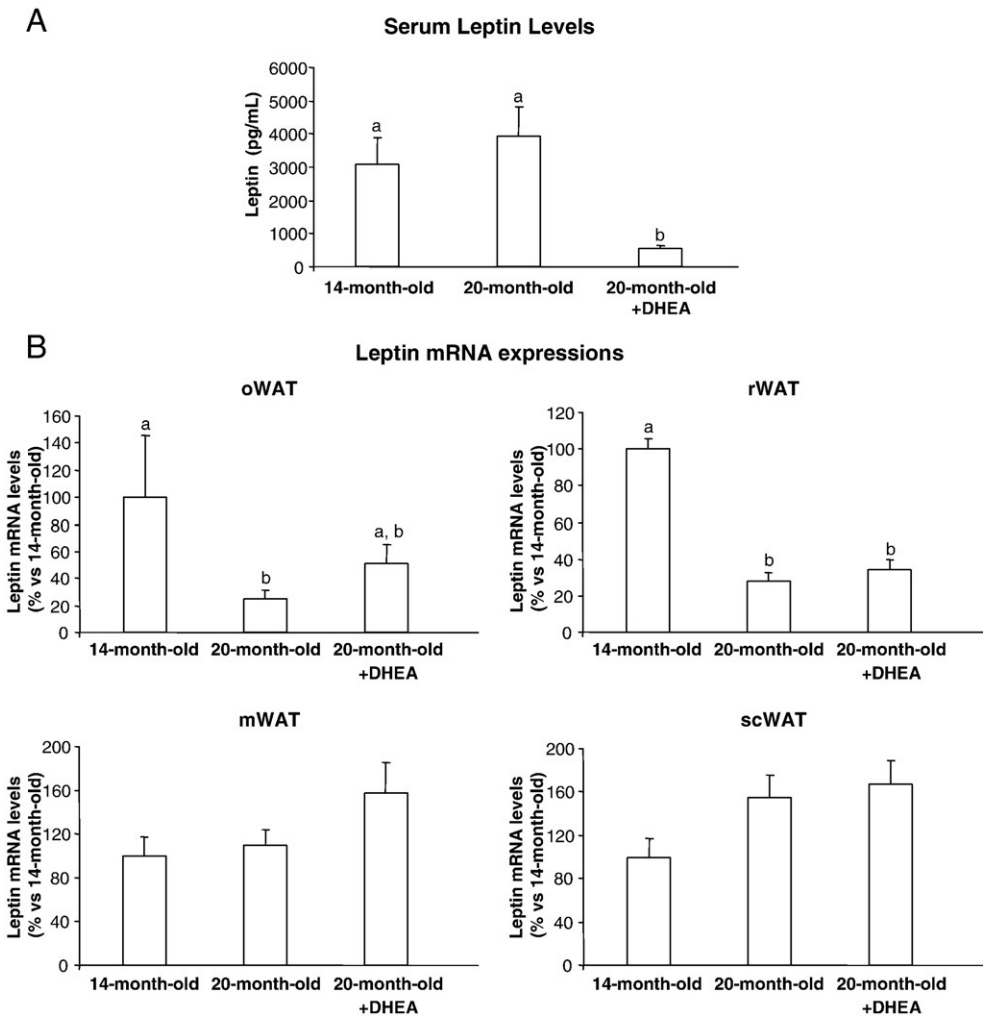

Fig. 4. Serum leptin levels (A) and leptin mRNA levels (B) in different WAT depots (oWAT, –rWAT, mWAT, scWAT) in rats fed on a HF diet at 14 and 20 months and at 20 months after 13 weeks of treatment with DHEA. Leptin mRNA levels in WAT depots were determined by real-time transcriptase-PCR and expressed as a percentage of the mean value of 14-month-old animals. Data are mean±S.E.M. (n=6–11). Data not sharing a common superscript are significantly different (P<.05, LSD post hoc test).

derivatives have been shown to induce weight loss in female rats [39], and androgens behave as antiadipogenic factors in male rat adipose tissue [40]. However, in female rats, estrogens can also act as proadipogenic agents [40] and other works have found that antiestrogenic drugs can reduce diet-induced obesity [41]. In addition, a direct action of DHEA on this tissue should not be discarded. There is evidence showing that DHEA administration may reduce adipose tissue mass by blocking adipogenesis (inhibiting proliferation and/or differentiation) [42], enhancing energy expenditure [43] and/or increasing lipid turnover, stimulating lipolysis to reduce cell size [44], among other actions. In agreement with this, in the present study we have observed a reduction in the cell number in the scWAT and a reduction in the cell size in the ovaric and mesenteric depots.

Our results show that circulating adiponectin levels were unaltered with aging similar to other observations in old Wistar male rats [45] and mice [46,47]. It has been reported that, in humans, plasma adiponectin concentration did not change significantly with age in women [48,49]. On the other hand, males over 70 years of age presented an increase in plasma adiponectin concentrations compared to younger men [49]. DHEA treatment produces a decrease in circulating adiponectin levels. Unlike most adipose-derived proteins, adiponectin mRNA expression in adipose tissue and its serum levels decreased in obesity and in insulin resistance [9,10] and increased as effect of weight loss [11,12], suggesting that the increase in adiponectin levels are accompanied by an improvement of insulin sensitivity. Thus, in principle, body weight reduction would be expected to be associated with an increase in adiponectin concentrations. However, we observed the opposite effect in the DHEA-treated rats.

Of note, adiponectin mRNA expression was reduced as an effect of age although this reduction was attenuated by DHEA treatment in all visceral adipose tissue depots studied,

but not in the subcutaneous one. This depot-specific regulation as an effect of DHEA is in agreement with a recent study performed in humans showing a significant increase in adiponectin expression in visceral adipocytes as an effect of DHEA but no significant effect on subcutaneous adipocytes was found [50]. In rats, previous results also showed an increase of adiponectin expression in epididymal WAT after 14 days of DHEA treatment [51]. The fact that DHEA up-regulates PPARγ gene expression in rat epididymal adipose tissue [51], together with the presence of a functional PPAR-responsive element in the promoter region of the gene encoding adiponectin [52], suggests that the effect of DHEA on adiponectin gene expression could be mediated by PPARγ.

There are discrepancies between adiponectin mRNA expression in the adipose tissue depots and serum protein levels similar to other studies [8,53]. A down-regulation of adiponectin gene expression as an effect of age was seen but this reduction was not reflected in the adiponectin circulating level, probably due to the significant increase in the number of adipocytes and total fat mass observed during aging. On the other hand, despite DHEA producing an increase of the visceral fat adiponectin expression, the reduction in the circulating adiponectin levels as an effect of DHEA could be associated with the high reduction produced in the overall fat mass. However, the fact that serum adiponectin is present in different molecular weight forms, not measurable in the present study, hinders this explanation.

Contrary to adiponectin, high levels of blood resistin have been related with insulin resistance in obesity [13]. In the present work, with aging, circulating resistin levels remained constant and increased as an effect of DHEA treatment. Regarding resistin mRNA expression in the different WAT depots, it remained unchanged by aging, except in the renal depot where we found a decrease in the expression. DHEA treatment increased resistin gene expression in the ovaric and renal depot. Previous results show that circulating resistin levels increase during development, probably related with the increase of body fat content, until the age of 6 months, remaining constant thereafter, while in WAT, resistin expression remains almost constant with age, except in early development, where there is a site-specific expression by the different depots [54]. In addition, administration of DHEA for 14 days to male Wistar rats increases resistin gene expression in the epididymal depot [55]. It has been proposed that DHEA effect on resistin gene expression may be exerted through a mechanism involving PPARα (the expression of which is induced by DHEA administration) [55] and that elevated resistin levels may lead to an inhibition of adipogenesis and a decrease in adipose tissue mass [56,57].

Administration of DHEA to Zucker rats, a rat model of hyperinsulinemia, has been shown to decrease insulin levels [58,59]. We have here observed that circulating insulin levels diminished with DHEA treatment. In addition, HOMA-IR tended to be lower as effect of DHEA administration. Although variations in insulin sensitivity with aging have been reported [60-62], the development of insulin resistance also appears to be associated with the development of obesity, particularly of the central-visceral type [63-65], rather than due to aging per se. In our study, in HF diet-fed old female rats, chronic DHEA treatment reduces fasting insulin levels and, consequently, HOMA-IR, and it may be one of the possible mechanisms for DHEA to improve insulin sensitivity. The reduction of fat mass and/or the increase in adiponectin expression, particularly in the visceral fat, observed in the DHEA-treated group may contribute to the improvement of insulin resistance.

Regarding leptin, it has been postulated that the increase in leptin levels with age in animals might contribute to the dysregulation of energy balance that occurs with age, which involves impairment of the fasting-induced suppression of leptin production [66]. Serum leptin levels increase during development, reaching stable levels at 7 months. Specific regional differences in leptin mRNA expression have been observed during development, revealing a site-specific influence of age that was not fully explained by changes in the tissue lipid content [67]. We have also found regional-specific differences in leptin mRNA expression: leptin expression decreased in oWAT and rWAT as an effect of age and remained unchanged by age in mWAT and scWAT.

Our results also showed a decrease in serum leptin levels in 20-month-old rats as an effect of DHEA, probably as a consequence of body weight and fat content reduction, more than a direct effect of DHEA on leptin gene expression, since no change was found in the leptin mRNA expression in the different WAT depots.

In summary, our results show that DHEA administration to old rats produces a decrease in body weight, mainly due to the decrease in the total adipose mass, leading to an increase in insulin sensitivity, decreasing circulating insulin levels and preventing the age-associated decline of visceral adiponectin mRNA expression. The major changes in gene expression, due to both ageing and DHEA treatment, were found in the visceral adipose tissue locations, highlighting the importance of these adipose depots in metabolic disturbances. Of interest, this improvement in the insulin sensitivity by DHEA treatment is better related to changes in the expression of adiponectin mRNA levels, particularly in the visceral fat, than to the variation in the concentration of adiponectin in serum.

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