Dehydroepiandrosterone modifies rat fatty acid composition of serum and different adipose tissue depots and lowers serum insulin levels

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Abstract

Dehydroepiandrosterone (DHEA) is reported to exert beneficial effects, such as protection from cardiovascular risk and lowering serum insulin levels. Adipose tissue (AT) is a target for DHEA actions, and the hormone can also affect hepatic fatty acid (FA) metabolism. FAs are involved in the development of insulin resistance; thus, there might be a relationship between DHEA, FA, and insulin. However, few data are available regarding DHEA and FA composition, especially concerning AT. Seventeen-month old female Sprague–Dawley rats ($n=11$; controls: $n=10$) were treated with DHEA (0.5% w/w in the diet) for 13 weeks, after which serum, periovarian, mesenteric, s.c., and brown AT were analyzed for FA composition. DHEA treatment resulted in significant changes in FA profiles in serum and adipose depots, like reduced 16:1 $n-7$ (s.c. and brown AT; $P<0.01$), elevated $n-9$ monounsaturated FA (serum and s.c. AT;

$P<0.05$), diminished $n-6$ polyunsaturated FA (PUFA; general; $P<0.05$) and increased $n-3$ PUFA (brown AT; $P<0.01$), along with lower $n-6/n-3$ ratios (s.c. and brown AT; $P<0.05$, $P<0.01$ respectively). DHEA modified estimates of desaturase activities, decreasing stearoyl-CoA-desaturase markers in s.c., and brown AT ($P<0.05$) and increasing those of delta-6-desaturase in serum and AT ($P<0.05$). In addition, DHEA-treated rats showed lower serum insulin levels ($P<0.05$). We have demonstrated for the first time that DHEA induces significant modifications in AT fatty acid composition *in vivo*, mainly concerning unsaturated FAs, and changes occurred in a tissue-dependent manner. We propose that these changes may be related to the capacity of DHEA to lower serum insulin levels.

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Introduction

The steroid hormone dehydroepiandrosterone (DHEA) and its sulphated form (DHEA-S) are known as precursors for most sexual hormones, both androgens and estrogens (Regelson *et al.* 1994), but they have also been shown to exert several positive effects themselves, independently of their conversion into androgens or estrogens, and there is even evidence for specific receptors for DHEA (Liu & Dillon 2004).

DHEA and DHEA-S show a marked decline in their serum levels with advancing age (Arlt 2004). Besides, low serum concentrations of these hormones, they have been associated with certain pathological states that increase their prevalence during ageing. Among the beneficial effects of DHEA, we can find cancer prevention (Ratko *et al.* 1991, Kawai *et al.* 1995), amelioration of cognitive function (Yanase *et al.* 1996), or protection against cardiovascular disease (Ebeling & Koivisto 1994), atherosclerosis (Nestler *et al.* 1992), or obesity (Richards *et al.* 2000, Abadie *et al.* 2001, de Heredia *et al.* 2007). Improvement of peripheral insulin sensitivity has also emerged as one of DHEA's relevant actions; indeed, we and other authors have previously observed that DHEA

administration to rodents reduced significantly serum insulin concentrations (Richards *et al.* 2000, Abadie *et al.* 2001, Sánchez *et al.* 2008).

Adipose tissue (AT) seems to be one of the main targets in DHEA and DHEA-S actions. It has been reported that these steroids reduce AT cellularity (Ryu *et al.* 2003), inhibit preadipocyte differentiation *in vitro* (Lea-Currie *et al.* 1998), stimulate lipolysis (Tagliaferro *et al.* 1995, Hernández-Morante *et al.* 2008), and alter AT gene expression (Kajita *et al.* 2003, Sánchez *et al.* 2008). In addition, DHEA-S can modulate fatty acid (FA) metabolism, through up-regulation of hepatic enzymes involved in β -oxidation (Waxman 1996, Depreter *et al.* 2002).

The relevance of FAs is far beyond their role as energy storage molecules in AT, and they are recognized as important metabolic effectors (Yaquop 2002, Drevon 2005, Steinberg 2007). FAs act as transcriptional modulators, regulating the expression of genes involved in energy metabolism or AT function (Raclot *et al.* 1997, Wilding 2001). Of particular concern is their impact on the development of insulin resistance (Kraegen *et al.* 2001, Russell 2004, Saravanan *et al.* 2005), and research is constantly demonstrating that the

nature of FAs present in the different tissues is as important as their total amount in relation to the development of metabolic alterations (Garaulet *et al.* 2001). Consequently, the proportions in which FAs appear in AT may have a great relevance in the physiology of this tissue, in particular regarding the sensitivity to insulin stimulation.

The relationships among DHEA, FAs, and insulin sensitivity suggest that FAs could be mediators in the insulin-sensitizing actions of the hormone. Despite this fact, little is known about the effects of DHEA on FA composition in different tissues (Abadie *et al.* 2001, Imai *et al.* 2001, Gómez *et al.* 2002), and no studies to our knowledge have shown significant changes in AT fatty acid profile due to DHEA action.

Considering all this information, we aimed to study the effect of a pharmacological dose of DHEA on FA composition in serum and different AT depots in an animal model prone to metabolic disturbances, i.e., ageing rats fed on a high-energy saturated-fat diet. In the present work, we found that the hormonal treatment resulted in significant changes in the FA profiles of serum, of visceral and s.c. white AT and of brown AT, and propose that these changes can be related to the insulin-sensitizing action of DHEA.

Materials and Methods

Animals and conditions

Twenty-one female Sprague–Dawley rats, provided by the animal care facilities of the University of Murcia, were kept in a temperature-controlled room ($24 \pm 2^\circ\text{C}$) in a 12 h light: 12 h darkness schedule with lights on at 0800 h. Water and food were always provided *ad libitum*. The animal protocol followed in this study was reviewed and approved by the bioethical committee of our university and guidelines for the use and care of laboratory animals of the university were followed.

From 7 weeks of age, animals were fed on a hypercaloric diet that consisted on 200 g/kg casein (Hero, Murcia, Spain), 245 g/kg corn starch (Hero), 244 g/kg sucrose (local market), 200 g/kg palm oil (Croexsa, Barcelona, Spain), 50 g/kg cellulose (Avicel, Barcelona, Spain), 2 g/kg choline HCl (J Escuder, Barcelona, Spain), and 4 g/kg methionine (J Escuder); vitamin (10 g/kg) and mineral (45 g/kg) mixes were formulated according to AIN-93 guidelines (Reeves *et al.* 1993) and were supplied by Tegasa (Valencia, Spain) and Sigma. This diet contained (expressed as a percentage of fresh mass) 18.4% protein, 50.4% carbohydrates (50% of which was sucrose), and 19.6% fat, with an energy content of 1880 kJ/100 g; in terms of energy fat accounted for 39% total dietary energy. FA composition of dietary fat was 53.6% saturated FAs (SFA), 36.3% monounsaturated FAs (MUFA), 9.5% polyunsaturated FAs (PUFA) and 0.6% *trans* FAs (Pérez de Heredia *et al.* 2008). The diet was prepared once a week in

our laboratory and stored at 5°C in order to avoid rancidity, and food was freshly dispensed every other day.

DHEA treatment

At the age of 17 months, rats were divided into two groups: one received the diet supplemented with DHEA (Roig Farma, S.A., Terrasa, Barcelona, 99.5% purity) at a concentration of 0.5% (5 g DHEA/kg diet) (DHEA group, $n=11$), while the rest of the animals continued without changes and served as controls (control group, $n=10$). The hormonal treatment lasted for 13 weeks, when rats were 20 months old.

Sample collection

In the beginning of the light phase and following overnight fast, rats were weighed and killed under anesthesia (ether). Blood samples were collected by cardiac puncture, immediately centrifuged 15 min at 1500 g and 4°C , and serum was stored at -80°C until analyzed. White AT from visceral (periovarian and mesenteric) and s.c. (abdominal region) depots, and interscapular brown AT were removed, weighed, immediately frozen in liquid nitrogen, and stored at -80°C .

In serum, insulin levels and the homeostatic model assessment (HOMA-IR index) were determined as previously described (Sánchez *et al.* 2008). Briefly, 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 HOMA-IR index was then calculated with the following formula (Matthews *et al.* 1985): $\text{HOMA-IR} = \text{Fasting glucose (mmol/l)} \times \text{fasting insulin (mIU/l)} / 22.5$.

Determination of FA composition

Total lipids from serum and AT samples were extracted according to the method of Folch *et al.* (1957), previous addition of an internal standard (tridecanoic acid, C13:0). FA methyl esters (FAME) were prepared with methanol HCl 3 M (Supelco, Bellefonte, PA, USA) at 100°C for 1 h and dissolved in hexane. FAME were analyzed in a gas chromatograph HP-6890 (Agilent Technologies, Inc. Palo Alto, CA, USA), using a column SP-2560 of $60\text{ m} \times 0.25\text{ mm id} \times 0.15\text{ }\mu\text{m}$ (Supelco, SIGMA-Aldrich). The oven temperature was programmed at an initial temperature of 175°C and after 30 min it was increased at a rate of $7^\circ\text{C}/\text{min}$ to 230°C for 20 min. The injector and detector were set at 240°C . Helium was used as the carrier gas at a pressure of 290 kPa. Peaks were identified by comparison of their retention times with appropriate FAME standards purchased from Sigma Chemical (SIGMA-Aldrich).

The amount of each individual FA was calculated and expressed as a percentage of total lipids FAs present in the samples. Then, total SFA were calculated as the sum of

12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, and 24:0; total MUFA were calculated as the sum of 14:1 *n*-9, 16:1 *n*-9, 17:1 *n*-9, 18:1 *n*-9, 20:1 *n*-9, 22:1 *n*-9, 24:1 *n*-9, 16:1 *n*-7, and 18:1 *n*-7; *n*-6 PUFA were obtained from 18:2 *n*-6, 18:3 *n*-6, 20:2 *n*-6, 20:3 *n*-6, 20:4 *n*-6, 22:2 *n*-6, 22:4 *n*-6, and 22:5 *n*-6; *n*-3 PUFA were calculated from 18:3 *n*-3, 18:4 *n*-3, 20:3 *n*-3, 20:5 *n*-3, 22:5 *n*-3, and 22:6 *n*-3; finally, *trans* FAs were calculated as the sum of C16:1 *trans*, C18:1 *trans*, C18:2 *cis*, *trans*, C18:2 *trans*, *cis* and, C18:2 *trans*, *trans*.

Statistical analysis

Results are presented as mean $\pm$ S.E.M. Comparisons of FA proportions between control and DHEA groups were analyzed by the Student's *t*-test. To determine whether the effect of DHEA treatment on FA composition was tissue-specific, a two-way ANOVA test was also performed for AT depots, followed by Bonferroni *post-hoc* correction. Significance was always set at $P < 0.05$. All analyses were performed using the statistic software SPSS v13.0 (SPSS Inc, Chicago, IL, USA).

Results

According to the average food intake, rats in the DHEA group received an estimated average dose of 6.8 mg DHEA/rat per day. After 13 week treatment, these animals had significantly higher serum DHEA-S levels than their controls (829.6 ± 93.3 and 71.8 ± 26.0 ng/ml respectively; $P < 0.001$). In addition, DHEA-treated rats showed lower serum insulin levels than control ones (42.2 ± 7.5 and 79.3 ± 16.4 pmol/l respectively, $P = 0.047$), and a tendency to a lower HOMA-IR index (1.9 ± 0.3 and 3.5 ± 1.00 respectively, $P = 0.154$).

In serum from DHEA-treated rats, MUFA percentages were higher, while *n*-6 and *n*-3 PUFA were lower than in the control group (Table 1). *Trans* FAs were similar between groups, and so were total SFA, although with increased 16:0 and decreased 18:0 proportions in the DHEA group, which explain the apparent stability of total SFA (Table 1).

Tables 2 and 3 contain the FA profile of the periovarian and mesenteric ATs –both visceral depots– respectively. *N*-6 PUFA proportions were significantly lower in rats that received DHEA, mainly due to a reduction on 18:2 *n*-6, while 20:4 *n*-6 was increased, pointing toward a higher delta-6-desaturase activity.

In the s.c. AT, changes were more pronounced (Table 4). Total proportions of *n*-9 MUFA, *trans* FAs and 20:5 *n*-3 were higher in the DHEA group, while *n*-6 PUFA were again lower, leading to significantly diminished *n*-6/*n*-3 PUFA ratio. It is important to remark the reduction in the percentages of 16:1 *n*-7 by DHEA treatment, since it has been suggested as a potential marker of metabolic syndrome (Gil-Campos *et al.* 2008, Paillard *et al.* 2008). This decrease in 16:1 *n*-7 was also observed in the interscapular brown AT of

Table 1 Selected fatty acids in serum from experimental groups

	Control (n=10)	DHEA (n=10)	P
Fatty acids (%)			
12:0	0.21 $\pm$ 0.102	0.32 $\pm$ 0.208	NS
14:0	0.83 $\pm$ 0.452	0.48 $\pm$ 0.081	NS
16:0	18.20 $\pm$ 1.278	22.72 $\pm$ 1.089	0.015
18:0	12.97 $\pm$ 0.658	8.72 $\pm$ 0.428	<0.001
16:1 <i>n</i> -9	0.25 $\pm$ 0.016	0.42 $\pm$ 0.041	0.003
16:1 <i>n</i> -7	1.03 $\pm$ 0.096	0.78 $\pm$ 0.077	0.055
18:1 <i>n</i> -9	15.14 $\pm$ 1.000	22.15 $\pm$ 1.395	0.001
18:1 <i>n</i> -7	1.40 $\pm$ 0.101	1.51 $\pm$ 0.080	NS
18:2 <i>n</i> -6	7.31 $\pm$ 0.207	5.85 $\pm$ 0.446	0.008
18:3 <i>n</i> -6	0.48 $\pm$ 0.036	0.59 $\pm$ 0.045	0.056
20:2 <i>n</i> -6	0.19 $\pm$ 0.062	0.49 $\pm$ 0.129	0.053
20:3 <i>n</i> -6	0.33 $\pm$ 0.039	0.56 $\pm$ 0.059	0.006
20:4 <i>n</i> -6	32.74 $\pm$ 1.549	27.41 $\pm$ 1.534	0.025
22:5 <i>n</i> -6	1.93 $\pm$ 0.204	0.80 $\pm$ 0.080	<0.001
18:3 <i>n</i> -3	0.41 $\pm$ 0.179	0.44 $\pm$ 0.181	NS
18:4 <i>n</i> -3	0.11 $\pm$ 0.025	0.10 $\pm$ 0.031	NS
20:5 <i>n</i> -3	0.18 $\pm$ 0.052	0.20 $\pm$ 0.056	NS
22:6 <i>n</i> -3	2.03 $\pm$ 0.115	1.03 $\pm$ 0.091	<0.001
18:1 <i>trans</i>	0.32 $\pm$ 0.070	0.43 $\pm$ 0.103	NS
18:2 <i>trans</i> , <i>trans</i>	0.08 $\pm$ 0.024	0.01 $\pm$ 0.010	0.029
SFA	34.13 $\pm$ 1.235	34.70 $\pm$ 1.091	NS
MUFA	18.42 $\pm$ 1.130	25.48 $\pm$ 1.44	0.001
<i>N</i> -6 PUFA	43.83 $\pm$ 1.883	36.64 $\pm$ 1.450	0.007
<i>N</i> -3 PUFA	3.00 $\pm$ 0.167	2.32 $\pm$ 0.250	0.037
<i>N</i> -6/ <i>n</i> -3 PUFA	14.9 $\pm$ 0.89	17.8 $\pm$ 2.21	NS
TRANS FA	0.62 $\pm$ 0.078	0.86 $\pm$ 0.147	NS

Data are presented as mean $\pm$ S.E.M. Comparisons were performed by the Student's *t*-test. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data in bold represents statistical significance, $P < 0.05$.

DHEA-treated rats (Table 5), where we found again a lower *n*-6/*n*-3 PUFA ratio, in this case caused mainly by significantly higher *n*-3 PUFA levels.

DHEA treatment also altered the estimates of desaturase activity calculated by their product/substrate ratios (Fig. 1). The ratios calculated with serum FAs are commonly used as estimates of hepatic desaturase activities (Sjögren *et al.* 2008). In serum from DHEA-treated rats, the ratio 16:1 *n*-7/16:0 was reduced while 18:1 *n*-9/18:0 was increased (both are markers of stearoyl-CoA desaturase – SCD- activity); by contrast, in the s.c. and interscapular brown ATs both indices were lower (Fig. 1A and B). By contrast, the markers of delta-6-desaturase activity in *n*-6 PUFA were increased, with significantly higher 18:3/18:2 *n*-6 and 20:4/18:2 *n*-6 ratios (Fig. 1C and D).

When FA data from AT samples were analyzed by means of the two-way ANOVA test, the interactions DHEA $\times$ tissue were non-significant for several FAs, which means that in these cases all fat depots responded to DHEA treatment in a similar way. However, other changes were indeed tissue-specific. For instance, variations in total *n*-3 PUFA were specific for s.c. and brown AT ($P_{\text{interaction}} = 0.047$); similarly, reductions in the proportions of 16:1 *n*-7 and in the *n*-6/*n*-3 PUFA ratio were more pronounced in these two depots ($P_i = 0.001$ for both variables). Other FAs that varied in a

Table 2 Selected fatty acids in periovarian adipose tissue from experimental groups

	Control (n=10)	DHEA (n=11)	P
Fatty acids (%)			
12:0	0.32±0.090	0.30±0.034	NS
14:0	0.92±0.087	0.93±0.028	NS
16:0	30.35±0.583	30.97±0.602	NS
18:0	2.48±0.103	2.68±0.113	NS
16:1 <i>n-9</i>	0.62±0.047	0.83±0.044	0.004
16:1 <i>n-7</i>	2.67±0.232	2.41±0.245	NS
18:1 <i>n-9</i>	48.81±0.400	47.60±1.523	NS
18:1 <i>n-7</i>	3.01±0.170	4.44±1.525	NS
18:2 <i>n-6</i>	8.46±0.205	7.29±0.094	<0.001
18:3 <i>n-6</i>	0.05±0.004	0.11±0.009	<0.001
20:3 <i>n-6</i>	0.03±0.006	0.05±0.008	0.055
20:4 <i>n-6</i>	0.26±0.023	0.45±0.023	<0.001
18:3 <i>n-3</i>	0.20±0.017	0.19±0.020	NS
18:4 <i>n-3</i>	0.05±0.008	0.10±0.007	<0.001
20:5 <i>n-3</i>	0.01±0.006	0.03±0.013	NS
22:6 <i>n-3</i>	0.04±0.006	0.04±0.002	NS
18:1 <i>trans</i>	0.18±0.044	0.12±0.011	NS
18:2 <i>cis, trans</i>	0.16±0.010	0.27±0.014	<0.001
SFA	34.91±0.632	35.52±0.543	NS
MUFA	55.27±0.571	55.44±0.531	NS
<i>N-6</i> PUFA	8.99±0.189	8.07±0.083	0.001
<i>N-3</i> PUFA	0.33±0.034	0.41±0.033	NS
<i>N-6/n-3</i> PUFA	30.0±3.62	21.4±2.58	0.063
TRANS FA	0.50±0.057	0.55±0.019	NS

Data are presented as mean±S.E.M. Comparisons were performed by the Student's *t*-test. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data in bold represents statistical significance, $P<0.05$

tissue-specific manner were 14:0 ($P_1=0.048$), 20:3 *n-6* ($P_1<0.001$), 18:4 *n-3* ($P_1=0.001$), 18:2 *cis, trans* ($P_1<0.001$), and 18:2 *trans, trans* ($P_1<0.001$).

Discussion

We had previously reported that DHEA administration to our rats led to significant reductions in food intake, body weight, total body fat content, the weight of different adipose depots, and adipocyte size (de Heredia *et al.* 2007). In addition, we found that DHEA-treated rats had significantly lower serum insulin concentrations and a tendency to lower HOMA-IR index (Sánchez *et al.* 2008). The present work has revealed that DHEA treatment also induced significant changes in FA profile in serum and AT from those rats, and that these changes varied according to the tissue studied. We found elevated *n-9* MUFA in serum and s.c. AT, reduced *n-6* PUFA in serum and white adipose depots, and increased *n-3* PUFA (18:4 *n-3* in all adipose regions, 20:5 *n-3* in s.c. tissue and total *n-3* PUFA in interscapular brown AT). Besides, DHEA treatment altered the surrogate estimates of desaturase activities, again in a tissue-specific manner.

This is to our knowledge, the first work that demonstrates a significant effect of DHEA on AT fatty acid composition.

There is a previous work that reported no changes in rat AT fatty acids after 7 days of DHEA administration (Abadie *et al.* 2001). However, due to the lower lipid turn-over rate of this tissue, a 7-day period may not be long enough to appreciate variations in FA profile in AT. By contrast, our experiment spanned a 13-week period that allowed us to observe a significant effect of DHEA on AT composition. Similarly to us, other authors have reported differences in FAs in muscle (Abadie *et al.* 2001), liver (Imai *et al.* 2001), and 3T3-L1 adipocytes (Gómez *et al.* 2002) due to DHEA administration.

In our study, changes in serum 16:0 (increased) and 18:0 (decreased) observed in the DHEA group were not reflected in AT; on the contrary, individual SFA did not varied in the visceral depots, while 18:0 was higher in s.c. and brown ATs of DHEA-treated rats. The increment in serum 16:0 after DHEA administration is somehow surprising, since this FA induces peripheral insulin resistance (Chávez & Summers 2003, Powell *et al.* 2004), but we observed lower insulin concentrations in DHEA-treated rats (Sánchez *et al.* 2008). However, it seems that impairment of peripheral insulin sensitivity by 16:0 is produced by conversion of this FA into ceramides (Chávez & Summers 2003, Powell *et al.* 2004), and DHEA could be counteracting this process somehow.

Other FAs associated with insulin resistance are *trans* FAs (Saravanan *et al.* 2005). In the present work, total *trans* FAs

Table 3 Selected fatty acids in mesenteric adipose tissue from experimental groups

	Control (n=10)	DHEA (n=11)	P
Fatty acids (%)			
12:0	0.09±0.020	0.11±0.051	NS
14:0	0.76±0.039	0.87±0.055	NS
16:0	30.99±0.384	31.57±0.495	NS
18:0	3.17±0.210	3.41±0.128	NS
16:1 <i>n-9</i>	0.52±0.029	0.78±0.041	<0.001
16:1 <i>n-7</i>	2.39±0.222	2.00±0.240	NS
18:1 <i>n-9</i>	47.72±0.598	48.95±0.505	NS
18:1 <i>n-7</i>	2.59±0.082	1.89±0.076	<0.001
18:2 <i>n-6</i>	8.43±0.161	7.22±0.107	<0.001
18:3 <i>n-6</i>	0.05±0.008	0.12±0.005	<0.001
20:3 <i>n-6</i>	0.03±0.004	0.07±0.004	<0.001
20:4 <i>n-6</i>	0.27±0.043	0.52±0.035	<0.001
18:3 <i>n-3</i>	0.22±0.016	0.21±0.010	NS
18:4 <i>n-3</i>	0.09±0.012	0.12±0.007	0.012
20:5 <i>n-3</i>	0.02±0.006	0.04±0.005	NS
22:6 <i>n-3</i>	0.12±0.082	0.04±0.006	NS
18:1 <i>trans</i>	0.28±0.062	0.19±0.031	NS
18:2 <i>cis, trans</i>	0.20±0.006	0.31±0.007	<0.001
SFA	36.26±0.787	36.84±0.516	NS
MUFA	53.50±0.757	53.81±0.513	NS
<i>N-6</i> PUFA	8.98±0.179	8.12±0.476	0.001
<i>N-3</i> PUFA	0.53±0.073	0.48±0.023	NS
<i>N-6/n-3</i> PUFA	18.7±1.58	17.2±0.83	NS
TRANS FA	0.72±0.060	0.75±0.034	NS

Data are presented as mean±S.E.M. Comparisons were performed by the Student's *t*-test. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data in bold represents statistical significance, $P<0.05$

Table 4 Selected fatty acids in subcutaneous adipose tissue from experimental groups

	Control (n=10)	DHEA (n=11)	P
Fatty acids (%)			
12:0	0.34 ± 0.044	0.22 ± 0.029	0.039
14:0	1.04 ± 0.052	0.87 ± 0.030	0.010
16:0	30.07 ± 0.604	29.72 ± 1.167	NS
18:0	2.60 ± 0.272	3.45 ± 0.132	0.009
16:1 <i>n-9</i>	0.57 ± 0.044	0.73 ± 0.045	0.020
16:1 <i>n-7</i>	3.54 ± 0.524	1.33 ± 0.107	0.002
18:1 <i>n-9</i>	47.53 ± 0.601	50.08 ± 1.033	0.052
18:1 <i>n-7</i>	2.94 ± 0.207	2.37 ± 0.133	0.029
18:2 <i>n-6</i>	8.71 ± 0.082	7.75 ± 0.136	<0.001
18:3 <i>n-6</i>	0.06 ± 0.005	0.09 ± 0.008	0.001
20:3 <i>n-6</i>	0.04 ± 0.005	0.08 ± 0.006	<0.001
20:4 <i>n-6</i>	0.50 ± 0.042	0.73 ± 0.031	<0.001
18:3 <i>n-3</i>	0.17 ± 0.047	0.19 ± 0.030	NS
18:4 <i>n-3</i>	0.05 ± 0.007	0.12 ± 0.007	<0.001
20:5 <i>n-3</i>	0.03 ± 0.004	0.05 ± 0.009	0.026
22:6 <i>n-3</i>	0.05 ± 0.008	0.05 ± 0.004	NS
18:1 <i>trans</i>	0.18 ± 0.021	0.22 ± 0.030	NS
18:2 <i>cis, trans</i>	0.17 ± 0.009	0.26 ± 0.012	<0.001
SFA	35.20 ± 0.960	35.53 ± 1.247	NS
MUFA	55.46 ± 1.063	55.59 ± 1.155	NS
N-9 MUFA	48.42 ± 0.610	51.18 ± 1.073	0.043
N-6 PUFA	9.56 ± 0.097	8.93 ± 0.139	0.002
N-3 PUFA	0.35 ± 0.047	0.48 ± 0.045	0.061
N-6/n-3 PUFA	29.9 ± 2.28	19.9 ± 1.52	0.001
TRANS FA	0.54 ± 0.033	0.68 ± 0.040	0.011

Data are presented as mean ± s.e.m. Comparisons were performed by the Student's *t*-test. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data in bold represents statistical significance, *P* < 0.05

practically did not change because of DHEA in the different fat depots. We found lower proportions of 18:2 *trans*, *trans* in serum and higher percentages of 18:2 *cis*, *trans* and 18:2 *trans*, *cis* in AT of DHEA-treated rats, but these are minority *trans* isomers and even their quantification is very difficult. The main *trans* isomer in diet and tissues is 18:1 *trans* that were practically not modified by the hormonal treatment.

More remarkable were the changes in *n-9* MUFA. These FAs, of which oleic (18:1 *n-9*) is the most representative, were significantly higher in the serum and s.c. AT from the DHEA group than from the control one. Quite the opposite, palmitoleic acid (16:1 *n-7*) was reduced in s.c. and brown AT (and in serum, with marginal significance). These results are in agreement with those obtained by Imai *et al.* (2001), who observed that DHEA stimulates oleate synthesis, resulting in increased 18:1 *n-9* contents in liver, and with Gómez *et al.* (2002), who also reported reduced 16:1 *n-7* and elevated 18:1 *n-9* in 3T3-L1 adipocytes treated with DHEA.

Different authors have reported that oleic acid is negatively associated with features of the metabolic syndrome (Garaulet *et al.* 2001, Carluccio *et al.* 2007), and positively with enhanced peripheral glucose uptake (Vessby *et al.* 1994, Dimopoulos *et al.* 2006). Regarding palmitoleic acid, it also has been shown to increase glucose uptake *in vitro* (Dimopoulos *et al.* 2006), whereas other authors have

proposed it as a marker for metabolic risk (Gil-Campos *et al.* 2008, Paillard *et al.* 2008). Although it remains to be determined, whether increments in palmitoleic acid indicate causal or compensatory events for insulin resistance, higher levels of this FA are related to metabolic alterations. In light of these data, it could be suggested that the insulin-lowering effect of DHEA that we observed may be related to the higher oleic acid and the reduced palmitoleic acid proportions in the tissues of DHEA-treated rats.

PUFA were also affected by DHEA treatment in our study. In serum, the DHEA group showed a significant reduction of linoleic (18:2 *n-6*) and arachidonic (20:4 *n-6*) acids. In AT, linoleic acid was also lower while arachidonic acid was higher, in agreement with observations in rat liver (Imai *et al.* 2001). Some authors have reported negative associations between *n-6* PUFA and insulin sensitivity (Vessby *et al.* 1994, Aldámiz-Echevarría *et al.* 2007), while others found positive ones (Nugent *et al.* 2001, Dimopoulos *et al.* 2006). The relation between *n-6* PUFA and insulin sensitivity could depend on the localization of the FAs, so that high levels in serum would be related to insulin resistance, while in AT or skeletal muscle they would help to improve sensitivity. If this was the case, the reduction in serum *n-6* PUFA in DHEA-treated rats observed in our work might be related to the lower insulin concentrations found in these animals. By

Table 5 Selected fatty acids in interscapular brown adipose tissue from experimental groups

	Control (n=10)	DHEA (n=11)	P
Fatty acids (%)			
12:0	0.04 ± 0.007	0.03 ± 0.017	NS
14:0	0.95 ± 0.048	0.85 ± 0.048	NS
16:0	25.28 ± 0.254	24.82 ± 0.379	NS
18:0	5.59 ± 0.439	6.66 ± 0.234	0.050
16:1 <i>n-9</i>	0.53 ± 0.028	0.63 ± 0.024	0.019
16:1 <i>n-7</i>	1.53 ± 0.170	0.80 ± 0.077	0.002
18:1 <i>n-9</i>	46.81 ± 0.410	47.45 ± 0.234	NS
18:1 <i>n-7</i>	7.30 ± 0.274	6.84 ± 0.532	NS
18:2 <i>n-6</i>	8.88 ± 0.134	7.67 ± 0.130	<0.001
18:3 <i>n-6</i>	0.07 ± 0.003	0.12 ± 0.007	<0.001
20:3 <i>n-6</i>	0.10 ± 0.008	0.20 ± 0.014	<0.001
20:4 <i>n-6</i>	1.19 ± 0.121	1.55 ± 0.171	NS
18:3 <i>n-3</i>	0.10 ± 0.038	0.16 ± 0.049	NS
18:4 <i>n-3</i>	0.04 ± 0.005	0.14 ± 0.011	<0.001
20:5 <i>n-3</i>	0.03 ± 0.003	0.06 ± 0.010	0.022
22:6 <i>n-3</i>	0.06 ± 0.006	0.08 ± 0.008	NS
18:1 <i>trans</i>	0.06 ± 0.007	0.07 ± 0.012	NS
18:2 <i>cis, trans</i>	0.09 ± 0.005	0.10 ± 0.004	0.035
18:2 <i>trans, cis</i>	0.07 ± 0.005	0.15 ± 0.007	<0.001
SFA	32.41 ± 0.282	33.06 ± 0.354	NS
MUFA	56.55 ± 0.460	56.21 ± 0.408	NS
N-6 PUFA	10.57 ± 0.253	9.95 ± 0.228	NS
N-3 PUFA	0.25 ± 0.046	0.47 ± 0.057	0.009
N-6/n-3 PUFA	54.4 ± 8.0	23.9 ± 2.54	0.004
TRANS FA	0.22 ± 0.009	0.32 ± 0.015	<0.001

Data are presented as mean ± s.e.m. Comparisons were performed by the Student's *t*-test. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data in bold represents statistical significance, *P* < 0.05

contrast to *n*-6 FAs, our results show increased proportions of *n*-3 PUFA in brown AT in rats receiving DHEA. It has been proposed that *n*-3 PUFA in brown AT are related to UCP-1 levels (Takahashi & Ide 2000), and DHEA has been shown to increase brown fat UCP-1 levels (Ryu *et al.* 2003); therefore, we hypothesize that DHEA treatment could enhance brown fat thermogenesis, at least in part, through increments in *n*-3 PUFA contents.

Of note, the changes in PUFA proportions led to significant reductions in *n*-6/*n*-3 PUFA ratios in s.c. and brown ATs—and the same trend was followed by the other adipose regions analyzed—of DHEA-treated rats. It has been proposed that high *n*-6/*n*-3 PUFA ratios are associated with greater body fat accumulation, cardiovascular risk, and inflammatory processes (Ghafoorunissa *et al.* 2005, Ailhaud *et al.* 2006, Riediger *et al.* 2008); therefore, the lower *n*-6/*n*-3 ratios observed in the present work could be a part of the protective actions attributed to DHEA.

It is interesting to highlight that the effects of DHEA on AT seem to be depot-specific, since the changes observed varied among the different tissues analyzed. It appeared that the s.c. white AT and the interscapular brown AT were more affected by the treatment, while the changes in the visceral depots were less pronounced. These results could be related, among other possible causes, to the fact that this experiment was conducted in female rats, since previous work performed in humans showed a gender-specific action of DHEA on AT, its effects being more evident in visceral fat among men and in s.c. fat among women (Hernández-Morante *et al.* 2008). Further studies should be carried out in order to confirm this hypothesis.

Finally, in our experiment, DHEA administration also modified significantly the estimates of desaturase activities. In serum of DHEA-treated rats, the marker of SCD activity on C18 (ratio 18:1/18:0) was increased, while SCD on C16 (ratio 16:1/16:0) was reduced. These indices are commonly used as estimates of hepatic desaturase activity, based on the fact that in the fasting state most serum FAs are derived from the liver (Sjögren *et al.* 2008). Some authors have suggested that higher SCD activity leading to increased synthesis of palmitoleic acid might be involved in metabolic alterations associated with obesity (Gil-Campos *et al.* 2008, Paillard *et al.* 2008). Therefore, these inverse changes observed in serum SCD markers that imply decreased palmitoleic and increased oleic synthesis could partly mediate the beneficial effects of DHEA. In AT, in contrast,

Figure 1 Desaturase activities estimated by product/substrate ratios of different fatty acids. Values in the DHEA group (light bars) are expressed as percentages of average values from the control group (dark bars). Serum ratios reflect indirectly hepatic desaturase activities. (A) Estimates of delta-9 desaturase activity on C16; (B) Estimates of delta-9 desaturase activity on C18; (C and D) Estimates of delta-6 desaturase activity. oWAT, periovarian adipose tissue; mWAT, mesenteric adipose tissue; scWAT, s.c. adipose tissue; iBAT, interscapular adipose tissue. Significant differences at *P<0.05; **P<0.01; ***P<0.001

the two markers of SCD activity were lower in the DHEA group than in the control one, the difference being significant in the s.c. and brown depots. It is worth highlighting that our results show opposite trends in serum and AT 18:1/18:0 in response to DHEA treatment. These changes are in agreement with the findings by Imai *et al.* and Gómez *et al.* The former observed that DHEA induces SCD in liver to increase 18:1 synthesis (Imai *et al.* 2001), which would explain the higher 18:1/18:0 ratio found in serum in our work, while the latter found that DHEA inhibits SCD expression in adipocytes *in vitro* (Gómez *et al.* 2002), leading to the lower 18:1/18:0 ratio in AT, mainly in the s.c. and brown depots. In addition, we found a general increment in the markers of delta-6-desaturase activity in the DHEA group. To our knowledge, no previous data are available on the effects of DHEA on delta-6-desaturase activity. Considering that SCD activity in AT has been associated with insulin resistance (Dobrzyn & Ntambi 2005, Sjögren *et al.* 2008), and that delta-6-desaturase has been reported to be involved in insulin-stimulated glucose uptake (Das 2005), this DHEA-related reduction in SCD markers in AT together with the increased delta-6-desaturase estimates observed in our work could reflect another mechanism by which DHEA improves insulin sensitivity.

It must be kept in mind that other major tissues, such as liver and muscle, play an important role in insulin resistance and they also seem to be targets for DHEA; therefore, they should be taken into account when dealing with systemic insulin sensitivity. Nevertheless, the contribution of AT should not be underestimated, considering that it is one of the main sites for insulin action and the principal source for circulating FAs in the body.

In summary, we have demonstrated for the first time that pharmacological administration of DHEA modifies significantly the FA profiles in serum and different AT depots in rats, and that changes vary according to the tissue studied. The hormone affected mainly *n*-9 MUFA, palmitoleic acid, *n*-6 PUFA, and *n*-3 PUFA, and also the markers of desaturase activities. Although the relationships among DHEA, FAs and insulin sensitivity are still poorly understood, we hypothesize that changes in FAs due to DHEA treatment may be related to DHEA's insulin-lowering effect, and speculate that the lower insulin levels might be accompanied by improved insulin sensitivity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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