CLINICAL STUDY

Effect of DHEA-sulfate on adiponectin gene expression in adipose tissue from different fat depots in morbidly obese humans

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Abstract

Objective: A growing body of studies has demonstrated the inverse relationship between DHEA-sulfate (DHEA-S) and the pathological alterations associated with the metabolic syndrome. However, the mechanism by which DHEA-S treatment operates has not been elucidated completely. Adiponectin, an adipose-specific protein, is thought to have anti-diabetic and anti-atherosclerotic properties. Because fat depots differ in the impact of their relationship with the undesirable consequences of obesity, the aim of the present study was to investigate the effect of DHEA-S on adiponectin expression in both s.c. and visceral tissues in a morbidly obese population.

Design and methods: We studied the *in vitro* expression of the adiponectin gene from paired biopsies of human visceral and s.c. adipose tissue, obtained from men and women (body mass index = 48.68 ± 7.43 kg/m²). Adipocytes were incubated for 24 h with or without DHEA-S. Adiponectin mRNA was measured by reverse transcription-quantitative PCR.

Results: In this population, DHEA-S plasma values were 141 ± 105 µg/100 ml. Serum adiponectin values were under normal ranges. In basal conditions, s.c. tissue expressed higher amounts (58%) of adiponectin mRNA than visceral tissue ($P = 0.027$). Adiponectin expression was differentially regulated in the two depots by DHEA-S. There was a significant increase in adiponectin expression specifically in the visceral tissue ($P = 0.020$), but no significant effect of DHEA-S on the s.c. tissue ($P = 0.738$).

Conclusions: In the present study, for the first time in humans, we have shown that DHEA-S treatment is a strong upregulator of adiponectin gene expression in omental adipocytes, suggesting that the positive effects observed by DHEA-S treatment in humans suffering from metabolic syndrome could be exerted through overexpression of adiponectin in the visceral depot.

European Journal of Endocrinology 155 593–600

Introduction

The adrenal hormone DHEA and its sulfate derivative, DHEA-S, are secreted almost exclusively by the cortex of the adrenal gland. Although DHEA-S has few intrinsic androgenic actions, it is the major circulating steroid hormone in humans. DHEA can be converted by peripheral tissues, such as the gonads, skin, and adipose tissue (AT) into potent androgenic and estrogenic hormones, such as androstenedione, testosterone, estrone, and 17β-estradiol (1, 2).

A large number of studies have examined the inverse relationship between overweight, central obesity, and plasma levels of DHEA and DHEA-S (3, 4). Furthermore, several clinical trials have demonstrated that DHEA treatment reduces body fat (5) and increases lean body mass (5, 6). A high concentration of DHEA in cultures of preadipocytes has been reported to reduce the rate of

differentiation of 3T3-L1 preadipocytes into mature, lipid-filled adipocytes by as much as 50% (7, 8), and also decrease preadipocyte proliferation (9). However, the mechanism by which DHEA or DHEA-S treatment affect adiposity has not yet been determined.

Insulin resistance is often associated with increased body weight and obesity, with intra-abdominal obesity being more detrimental than peripheral obesity (10–12). It has been suggested that DHEA may be related to insulin resistance and hyperinsulinemia, and that this relationship could be a consequence of obesity. It has also been shown that reduced DHEA may directly contribute to insulin resistance by increasing insulin binding to its own receptor (13).

Various adipocyte-secreted proteins have been described that are altered in obesity and affect insulin sensitivity, and might, therefore, provide a link between these two pathological states. Among these

adipocytokines, the product of the adipose most-abundant gene transcript 1 (apM1), adiponectin, appears to play an important role in carbohydrate and lipid metabolism (14).

Adiponectin is a potent insulin enhancer in mouse models of obesity, lipoatrophy and diabetes, and hypoadiponectinemia has been linked to insulin resistance in humans. Although adiponectin is secreted only from AT, its levels are paradoxically lower in obese than in lean humans (15). Body fat distribution has also been associated with plasma adiponectin levels and AT expression, this association being inverse with central obesity (16). This protein appears to be a major modulator of insulin action by enhancing insulin-mediated suppression of hepatic glucose production (17). Moreover, plasma adiponectin levels in diabetic subjects with coronary artery disease (CAD) are lower than in diabetic patients without CAD, suggesting that adiponectin may have anti-atherogenic properties (18). The association of low adiponectin levels with obesity, insulin resistance, CAD, and dyslipidemia indicates that this protein may be an important marker of the metabolic syndrome.

Thus, the aim of the present study was to determine the effect of DHEA-S treatment on adiponectin gene expression in human primary AT cultures from morbidly obese patients and to analyze a possible differential effect in two abdominal (s.c. and visceral) fat depots.

Subjects and methods

Subjects

Visceral and s.c. abdominal AT biopsies were obtained from a total of 25 Caucasians subjects (10 men and 15 women; mean age 42.8 ± 10.2 years) with morbid obesity (body mass index (BMI) ≥ 40 kg/m²), from the General Surgery Service of 'Virgen de la Arrixaca' University Hospital, undergoing laparoscopic gastric bypass surgery due to obesity. After an overnight fast, the AT biopsies were taken as paired samples from the two AT depots at the beginning of the surgical procedure. Visceral AT was taken from the omental depot. The abdominal s.c. AT was taken 5 cm lateral from umbilicus. All biopsies were transported in sterile containers within 30 min of removal and were used in subsequent cultures.

The protocols were approved by the Ethics Committee of the 'Virgen de la Arrixaca' University Hospital, and the subjects gave written informed consent before the biopsies were obtained.

Anthropometric measurements

The evaluation of obesity was carried out according to the criteria proposed by the Spanish Society for the Study of Obesity (19). Weight was determined in

subjects wearing light clothes and bare-footed, using a digital electronic weighing scale. Height was determined using a Harpenden digital stadiometer (range 0.70–2.05 m), with the subject upright and the head in the Frankfurt plane. From these data, BMI was calculated.

Total body fat (%) was measured by bioimpedance with a TANITA Model TBF-300 (TANITA Corporation of America, Arlington Heights, IL, USA). Body fat distribution was assessed using waist circumference at the level of midway between the lower rib margin and the iliac crest, and hip, the widest circumference over the great trochanters. The waist-to-hip ratio was calculated from these measurements.

Serum hormone determinations

Blood samples were collected the day before surgery following an overnight fast. Serum was separated after centrifugation and stored at -70°C until analyzed.

Insulin and sex hormone-binding globulin (SHBG) were determined by IRMA with reagents from Biosource (Fleurus, Belgium), and Orion Diagnostica (Espoo, Finland) respectively. The sensitivity of the method was 1 $\mu\text{U/ml}$ for insulin and 0.5 nmol/l for SHBG. The intra-assay coefficient of variation (CV) was 4.5% at a serum insulin concentration of 6.6 $\mu\text{U/ml}$ and 2.1% at 53 $\mu\text{U/ml}$; and 5.3% at a serum SHBG concentration of 17.7 nmol/l and 5.6% at 156.8 nmol/l.

17 β -Estradiol and testosterone were determined by ELISA/competition with biotinylated/estreptavidine technology with reagents purchased from Boehringer Mannheim Immunodiagnosics (Meylan, France). Androstenedione and DHEA-S were determined by RIA with reagents purchased from Immunotech (Marseille, France), and Diagnostic Systems Laboratories, Inc. (Webster, TX, USA) respectively. The assays had sensitivities of 0.1 ng/ml for androstenedione and 1.7 $\mu\text{g}/100$ ml for DHEA-S. The intra-assay CVs were 8.9% at a mean androstenedione concentration of 0.58 ng/ml and 4.1% at a mean value of 6.68 ng/ml; 9.4% at a mean DHEA-S concentration of 20.3 $\mu\text{g}/100$ ml and 6.3% at 593.3 $\mu\text{g}/100$ ml. The normal ranges according to the hospital standards were as follows: insulin, 5 ± 25 $\mu\text{U/ml}$; 17 β -estradiol, 10 ± 39 pg/ml in males and 10 ± 147 pg/ml in females in the follicular phase; 110 ± 338 during menses; 27 ± 247 in the luteal phase; and 0.01 ± 46 in the postmenopausal state; androstenedione, 0.61 ± 3.71 ng/ml in males and 0.46 ± 3.39 ng/ml in females; testosterone, 2 ± 8.5 pg/ml in males and 0.01 ± 0.86 pg/ml in females; DHEA-S, 281 ± 606 $\mu\text{g}/100$ ml in males from 17 to 50 years and 195 ± 507 $\mu\text{g}/100$ ml in females; SHBG, 10 ± 50 nmol/l in males and 30 ± 90 nmol/l in females.

Plasma adiponectin concentrations were measured by ELISA being plasma samples diluted 500-fold with a buffer (Mediagnost, Reutlingen, Germany). The sensitivity of the method was 0.6 ng/ml. The intra- and inter-assay CVs were less than 4.7 and 6.7% respectively.

Serum glucose concentration was measured in duplicate by the glucose oxidase method. Fasting insulin resistance index (homeostasis model assessment (HOMA)) was calculated with the accepted formula (20):

$$\text{HOMA} = \frac{\text{fasting glucose (mmol/l)} \times \text{fasting insulin (mIU/l)}}{22.5}.$$

Human primary adipose tissue cultures

After laparoscopy procedure, in each patient, paired AT samples were obtained from both fat depots (s.c. and visceral; 4–5 g). The AT was carefully dissected out from skin and vessels and cut into small pieces (average weight 10 mg), and all subsequent procedures were carried out under laminar airflow and under sterile conditions. All cultures were performed in duplicate. Explants were then placed at 37 °C for 24 h in a humidified atmosphere containing 7% CO₂ in 100 mm diameter dishes. AT (800–1000 mg) was placed in 5 ml Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum. After 24 h, the medium was replaced in every well. DHEA-S (1 µM) was added in s.c. and visceral AT cultures, in order to determine possible regional differences in the effect of this hormone on adiponectin expression. Incubation was continued for 24 h.

Adipocyte isolation and fat-cell data determination

Adipocytes were isolated by the method of Rodbell (21) with modifications. The s.c. and omental fat tissues were digested in DMEM containing 0.5 mg/ml type-II collagenase (Sigma) and 1% BSA (Sigma) for 20–30 min at 37 °C under constant shaking. The reaction was stopped by dilution in PBS and filtered on a silk screen in order to retain undigested explants; isolated adipocytes were separated from the stromal-vascular fraction by floatation. The floating packed cells were washed twice with PBS. From the isolated adipocytes, an aliquot was photographed under microscope using a digital camera connected to an image analyzer (MIP-Microm, Barcelona, Spain). Mean fat-cell diameter was determined by measuring the diameter of at least 200 cells.

The total fat-cell number was calculated by dividing the weight of total body fat by the average of the mean fat-cell weights of the two AT regions studied: s.c. and visceral (3).

RNA extraction from isolated adipocytes

After 24 h incubation, AT explants were digested as described earlier. Total RNA was extracted from isolated adipocytes using RNeasy Kit (Qiagen) according to the instructions of the company, except that the fat cake

was removed by centrifugation before loading the purifying columns.

RNA was quantified by measuring absorbancy at 260 and 280 nm. The integrity of the RNA was checked by visual inspection of the two rRNAs 18S and 28S on a 1% agarose gel.

Real-time PCR measurement of adiponectin mRNA

Reverse transcription (RT) was performed using random hexamers as primers and Thermoscript reverse transcriptase (Invitrogen) with 1 µg total RNA for each sample.

Quantitative real-time PCR was performed using an ABI PRISM 7900 HT Sequence Detection System as described by the provider (Applied Biosystems, Foster City, CA, USA). PCR Master MIX (Perkin-Elmer, Norwalk, CT, USA) containing Hot Start Taq DNA polymerase. Taqman probes for human adiponectin and 18S rRNA as internal control were also supplied by Applied Biosystems (Assay-by-Design). All samples were determined as duplicates, and for a negative control the same setup was used except for the addition of RT. No PCR product was detected under these latter conditions. In brief, adiponectin mRNA and 18S RNA were amplified in separated wells at 95 °C for 10 min and thereafter repeating cycles comprised 95 °C for 30 s and 60 °C for 60 s for annealing and extension steps respectively. During the extension step, an increase in fluorescence was measured in real-time.

Relative quantities of transcript were calculated using the $2^{-\Delta\Delta C_t}$ formula, where C_t is defined as the cycle number at which fluorescence is statistically significantly above background; ΔC_t is the difference in C_t of the gene of adiponectin and C_t of 18S; and $\Delta\Delta C_t$ is the difference in ΔC_t of unknown sample and ΔC_t of the calibrator/control sample (22). The results are expressed in arbitrary units with one unit being the mean mRNA levels determined in the control group. Amplification of specific transcripts was further confirmed by subjecting the amplification products to agarose gel electrophoresis.

Statistical analysis

Clinical and anthropometric data are presented as mean $\pm$ S.E.M. The ranges of these results are also shown. Data for gene expression, expressed in arbitrary units, are also presented as mean $\pm$ S.E.M.

Comparisons between men and women were analyzed by means of Student's *t*-test. To analyze the DHEA-S effect on adiponectin expression in s.c. and visceral depots, obtained from the same individual, we compared AT cultures without treatment, which was denominated 'control group', with the DHEA-S treated cultures, and we have expressed the adiponectin gene expression changes obtained by

the DHEA-S effect by n -fold changes with respect to the control. The population was normally distributed; therefore, Student's paired t -test was used for comparing data from the samples derived from the two adipose depots in each individual subject, and to compare data from control (untreated) and DHEA-S-treated samples. All statistical analyses were carried out using SPSS for windows (release 12.0; SPSS, Inc., Chicago, IL, USA).

Results

General characteristics

Table 1 contains several obesity parameters of the population studied ($n=25$) and the characteristics of men ($n=10$) and women ($n=15$). BMI was higher than 40 kg/m^2 , indicating that the patients studied suffered from morbid obesity. There were no significant differences in age or BMI between genders. The number of adipocytes was higher than 5×10^{10} , and with a size greater than $108 \mu\text{m}$ diameter; therefore, the population studied showed hypertrophy and hyperplasia of adipocytes (23). The s.c. adipocyte diameter was significantly higher than visceral ($P=0.012$) in the total population. Visceral diameter was significantly higher in men than in women ($P=0.020$; Table 1).

Hormonal status and several glucose metabolism parameters of this population were also studied (Table 2). Significant differences were found between men and women. As expected, men presented higher DHEA-S and free testosterone values than their female counterparts ($P=0.025$ and 0.003 respectively). However, plasma adiponectin values were higher in women ($P=0.019$).

As was expected, HOMA index of this morbidly obese population was elevated, in fact, HOMA was twice the normal range (3.8) (20). In the light of this result, we can define this population as insulin resistant.

Basal adiponectin expression in samples from the two adipocyte depots

Paired AT biopsies from the s.c. and the visceral depot were obtained from the laparoscopy obesity surgery. Adiponectin expression in the s.c. and visceral AT was measured after 24-h culture. Using group mean data, in basal conditions, the s.c. tissue expressed significantly higher amounts ($P=0.027$) of adiponectin mRNA than visceral tissue (Fig. 1). In the s.c. tissue, adiponectin expression was 58% higher than in the omental.

When we compared the paired samples, this difference was still observed ($P=0.048$). Data showed no significant differences between the genders in basal AT adiponectin gene expression in either s.c. AT ($P=0.655$) or in the visceral depot ($P=0.199$). Therefore, data were analyzed in the total population.

Adiponectin expression response to DHEA-S from grouped samples from the two different depots

Adiponectin expression was differentially regulated in the two depots by DHEA-S. Thus, at 24-h culture, there was a significant increase ($P=0.020$) in adiponectin expression, specifically in the omental tissue, compared with the basal conditions (Fig. 2). In contrast, no significant effect of DHEA-S on the s.c. tissue was found ($P=0.738$).

When the study was performed in the paired samples from each individual, the difference observed in the omental tissue compared with the basal condition still remained significant ($P=0.022$; paired t -test). In this way, the s.c. adipocytes showed no significant differences ($P=0.529$). Data show no significant differences between the genders in the adiponectin expression response to DHEA-S. Therefore, data were analyzed in the total population.

Table 1 General characteristics and fat-cell data of the total population studied.

Clinical characteristics	Total population ($n=25$)	Men ($n=10$)	Women ($n=15$)	P
Age (years)	43 ± 2 (28–66)	41 ± 3 (31–59)	44 ± 3 (28–66)	0.475
Weight (kg)	133.6 ± 5.1 (95.9–202.3)	156.2 ± 6.4 (133.8–202.3)	118.6 ± 4.1 (95.9–160.0)	<0.001
Height (cm)	165 ± 2 (150–190)	173 ± 3 (161–190)	159 ± 2 (150–173)	0.001
BMI (kg/m^2)	48.7 ± 1.5 (37.0–70.0)	51.9 ± 2.7 (37.3–70.0)	46.5 ± 1.5 (37.0–59.4)	0.073
WC (cm)	140.9 ± 4.1 (104.5–179.6)	155.8 ± 4.4 (141.7–179.6)	131.0 ± 4.7 (104.5–167.3)	0.001
WHR	0.99 ± 0.02 (0.82–1.36)	1.07 ± 0.04 (0.97–1.36)	0.94 ± 0.02 (0.82–1.09)	0.002
%Fat	46.6 ± 1.5 (24.5–56.7)	40.9 ± 2.6 (24.5–53.5)	50.3 ± 1.0 (43.5–56.7)	0.005
Fat-cell number ($\times 10^9$)	9.29 ± 0.59 (4.08–14.91)	8.12 ± 0.93 (4.08–11.78)	10.14 ± 0.69 (6.12–14.91)	0.094
Visceral adipocyte size (μm)	109 ± 2 (84–120)	114 ± 2 (106–120)	104 ± 3 (84–120)	0.020
Subcutaneous adipocyte size (μm)	116 ± 2 (90–135)	120 ± 3 (109–135)	112 ± 3 (90–127)	0.085

Data are presented as mean $\pm$ S.E.M. (range). P values were calculated using Student's t -test to compare men versus women. Significant differences ($P < 0.05$) are shown in bold. BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio.

Table 2 Hormonal status in the studied population.

	Total population (n=25)	Men (n=10)	Women (n=15)	P
DHEA-S (µg/100 ml)	141 ± 22 (10–427)	196 ± 42 (10–497)	99 ± 15 (27–165)	0.025
17β-Estradiol (pg/ml)	46 ± 10 (10–96)	23 ± 6 (13–33)	52 ± 13 (10–96)	0.080
Free testosterone (pg/ml)	6.7 ± 1.9 (0.2–31.9)	13.9 ± 3.1 (3.2–31.9)	1.2 ± 0.3 (0.2–3.7)	0.003
SHBG (nmol/l)	37 ± 6 (11–133)	26 ± 4 (11–49)	46 ± 9 (22–133)	0.112
Androstenedione (ng/ml)	1.4 ± 0.1 (0.3–3.0)	1.5 ± 0.2 (0.8–2.3)	1.4 ± 0.2 (0.3–3.0)	0.744
Adiponectin (µg/ml)	19.54 ± 2.13 (3.73–48.46)	14.10 ± 2.51 (3.73–30.24)	23.69 ± 2.87 (9.89–48–46)	0.019
Insulin (µU/ml)	27.3 ± 4.1 (5.7–80.2)	23.7 ± 5.3 (6.2–50.0)	29.8 ± 6.1 (5.7–80.2)	0.487
Glucose (mg/dl)	122.35 ± 17.72 (76.00–293.00)	119.60 ± 30.23 (84.00–293.00)	123.50 ± 21.48 (76.00–280.00)	0.919
HOMA-IR	7.00 ± 1.07 (2.60–15.82)	6.35 ± 2.54 (3.42–15.82)	7.32 ± 0.97 (2.60–9.99)	0.664
C-peptide (ng/ml)	4.4 ± 0.6 (1.4–14.2)	5.0 ± 1.4 (3.4–14.2)	4.1 ± 0.4 (1.4–6.6)	0.467

Data are presented as mean ± S.E.M. (range). P values were calculated using Student's *t*-test to compare men versus women. Significant differences (*P* < 0.05) are shown in bold. DHEA-S, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; HOMA-IR, homeostasis-model assessment-insulin resistance.

Discussion

In the recent years, major advances include the recognition that AT is a highly secretory organ that releases a variety of factors, which can affect both insulin action and other functions (24). Also, it is well established that body fat distribution can affect some diseases related to adipose mass. The regional variations in adipocyte lipolytic responses and the differential expression of certain genes and secretory products have provided a framework from which to explore the differential impact of s.c. versus visceral AT on clinical risk (25, 26).

We have compared the *in vitro* adiponectin gene expression from adipocytes derived from two fat depots, s.c. and visceral in morbidly obese patients. Our results showed a statistically significant difference between the two compartments with a higher expression of adiponectin in s.c. human adipose culture versus

visceral tissue (Fig. 1). These data are in disagreement with a study of Yang *et al.* (27) performed in obese women, who showed no depot difference in adiponectin mRNA levels. On the other hand, a study performed by Lihn *et al.* (28) showed significant differences in adiponectin gene expression only in lean women, but not in obese ones; whereas Fisher *et al.* (29) demonstrated a significantly lower adiponectin gene expression and protein content in omental AT compared

Figure 1 Adiponectin mRNA expression in the subcutaneous (black bar) and the omental (white bar) tissues in the basal state in the total population. Adiponectin mRNA level was measured with the real-time PCR and normalized to 18S using the $\Delta\Delta C_t$ method of relative quantification. Data are reported as mean ± S.E.M., and results are presented as percent of visceral group (visceral tissue value = 1). **P* < 0.05 (Student's *t*-test).

Figure 2 Differences in adiponectin gene expression between control and DHEA-sulfate-treated samples in (A) visceral and (B) subcutaneous adipose tissues. Adiponectin mRNA level was measured with the real-time PCR and normalized to 18S using the $\Delta\Delta C_t$ method of relative quantitation. Data are reported as mean ± S.E.M., and results are presented as percent of control group (control value = 1). **P* < 0.05 (Student's *t*-test). NS, no significant difference.

with s.c. abdominal tissue. These apparent contradictory data could be due to differences in the general characteristics of the populations studied, i.e. BMI, age, body fat distribution, and hormonal status. Most of these studies in gene expression are carried out on AT obtained from dermolipectomies in very heterogeneous groups or in populations that are not well characterized. The present study was performed in a morbidly obese population, with a very high fat-cell size and number compared with the reviewed literature (23).

The hormonal status was also studied. Data showed higher plasma levels of insulin, androstendione, and SHBG, compared with the normal ranges according to the hospital standards, and lower DHEA-S plasma concentrations. These results confirm other studies performed in obese populations with similar serum hormone alterations (3). In concordance with previous results (15), in our population, women showed higher plasma adiponectin levels than men. In addition, circulating adiponectin values in these morbidly obese subjects were below data from normal weight populations reported in the reviewed literature, especially for men (30). These data could be influencing the insulin-resistant state of this morbidly obese population. Indeed, the relationship between obesity and insulin resistance has been widely demonstrated (31). In this regard, in this morbidly obese population, both HOMA index and insulin values exceeded the normal range, which indicates that these morbidly obese patients could be classified as insulin-resistant subjects (20).

The main goal of the present study was to investigate the effect of a short-term DHEA-S incubation on human primary AT culture to elucidate the effect of this hormone on adiponectin gene expression. It has been shown previously that a decline in the circulating concentration of DHEA-S (the most abundant circulating steroid in humans) is linked to a number of disorders, including obesity and associated co-morbidities (insulin resistance, type-2 diabetes, and atherosclerosis) (3, 32, 33).

In this assay, we have observed that DHEA-S upregulates AT adiponectin gene expression in morbidly obese patients more than twofold (Fig. 2). This is the first study that demonstrates in humans the effect of DHEA-S in adiponectin gene expression in AT culture. A previous study by Karbowska *et al.* (34), who found a similar increase of adiponectin expression caused to DHEA treatment, was performed on rats.

The effect of DHEA-S on adiponectin expression was restricted to visceral AT in these morbidly obese patients. These results could be of interest in the treatment of morbid obesity taking into account the fact that visceral AT plays a major role in the pathogenesis of the metabolic syndrome (35).

The molecular aspects of DHEA or DHEA-S are still unclear (36). It has been postulated that DHEA-S could be related to adiponectin expression by its conversion to a more potent androgen, testosterone. However, this

hormone has recently been shown to be a negative regulator of adiponectin production in humans (37). We have demonstrated a positive regulation of DHEA-S on adiponectin gene expression. Based on this evidence, it seems unlikely that DHEA-S exerted its effects via activation of androgen receptors.

Previous studies have revealed that DHEA-S acts as a peroxisome proliferator-activated receptor (PPAR) ligand (38). Indeed, PPAR γ and DHEA have been related in the study of Karbowska *et al.* (34). On the other hand, Iwaki *et al.* (39) have identified a functional PPAR-responsive element in the promoter region of the gene-encoding adiponectin. Therefore, adiponectin expression and secretion are increased by activators of PPAR γ (40). In this context, PPAR γ could act as an important link between DHEA-S and adiponectin, and the adiponectin expression enhancement found in the human AT primary culture in the present study could be mediated by this transcription factor.

However, the analysis of the specific mechanism of action of DHEA-S, and the parallel influence on other genes, is limited in the present study because of the quantity of fat. The introduction of the laparoscopy techniques in the surgery process of morbid obesity has limited the accessibility to AT, specifically to the visceral depot.

Previous studies have shown that DHEA supplementation has beneficial effects on human diabetes and atherosclerosis (6), reporting a significant increase in insulin sensitivity in response to DHEA treatment. Also, it has been shown that adiponectin has anti-diabetic and anti-atherogenic properties (41). Thus, in the light of our results, we hypothesized that the beneficial effect of DHEA-S on atherosclerosis and diabetes observed in humans might be caused indirectly through an activation of adiponectin gene expression in visceral AT.

In conclusion, for the first time in humans, we have shown that DHEA-S treatment is a strong upregulator of adiponectin gene expression in primary AT culture, especially in the omental depot, suggesting that the observed positive effects of DHEA-S treatment in humans attending to the metabolic syndrome could be exerted indirectly by overexpression of adiponectin in the visceral fat depot.

Acknowledgements

The authors wish to thank P Gomez-Abellan, C Piernas and J A Jiménez-Bastida for their contributions and support, especially in the AT culture development. We also thank the staff from the General Surgery Service, 'Virgen de la Arrixaca' University Hospital, Murcia (Spain), especially to Dr Lujan, for their skilled technical assistance. We acknowledge the *Institut Louis Bugnard, Unite Recherches sur les Obesites INSERM U586*, Toulouse, France for the 1-year period in which Dr Garaulet improved gene expression techniques.

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Received 24 April 2006

Accepted 17 July 2006