Dehydroepiandrosterone Alters Zucker Rat Soleus and Cardiac Muscle Lipid Profiles

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High levels of serum free fatty acids (FFA) and lower proportions of polyunsaturated (PU) FAs, specifically arachidonic acid (AA), are common in obesity, insulin resistance (IR), and type 2 diabetes mellitus. Dehydrepiandrosterone (DHEA) decreases body fat content, dietary fat consumption, and insulin levels in obese Zucker rats (ZR), a genetic model of human youth onset obesity and type 2 diabetes. This study was conducted to investigate DHEA's effects on lean and obese ZR serum FFA levels and total lipid (TL) FA profiles in heart and soleus muscle. We postulated that DHEA alters serum FFA levels and tissue TL FA profiles of obese ZR so that they resemble the levels and profiles of lean ZR. If so, DHEA may directly or indirectly alter tissue lipids, FFA flux, and perhaps lower IR in obese ZR. Lean and obese male ZR were divided into six groups with 10 animals in each: obese *ad libitum* control, obese pair-fed, obese DHEA, lean *ad libitum* control, lean pair-fed, and lean DHEA. All animals had *ad libitum* access to a diet whose calories were 50% fat, 30% carbohydrate, and 20% protein. Only the diets of the DHEA treatment groups were supplemented with 0.6% DHEA. Pair-fed groups were given the average number of calories per day consumed by their corresponding DHEA group, and *ad libitum* groups had 24-h access to the DHEA-free diet. Serum FFA levels and heart and soleus TL FA profiles were measured. Serum FFA levels were higher in obese (~1 mmol/L) compared to lean (~0.6 mmol/L) ZR, regardless of group. In hearts, monounsaturated (MU) FA were greater and PU FA were proportionally lower in obese compared to the lean rats. In soleus, saturated and MU FA were greater and PU FA were proportionally lower in the obese compared to the lean rats. DHEA groups displayed significantly increased proportions of TL AA and decreased oleic acid in both muscle types. Mechanisms by which DHEA alters TL FA profiles are a reflection of changes occurring within specific lipid fractions such as FFA, phospholipid, and triglyceride. This study provides initial insights into DHEA's lipid altering effects.

[Exp Biol Med Vol. 226(8):782–789, 2001]

This study was funded by a Grant from the American Heart Association.

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Received June 21, 2000.
Accepted April 19, 2001.

0037-9727/01/2268-0782$15.00

Key words: fatty acids; insulin resistance; Zucker Rats; DHEA; PPAR

We have shown that dehydroepiandrosterone (DHEA), the most abundant human adrenal steroid (1), decreases the rate of weight gain in the *fa/fa* obese Zucker rat (ZR) (2). Others demonstrated that DHEA decreases fat intake and body weight in rats (3–6), decreases serum triglyceride (TG) levels in hyperlipidemic rats (7), inhibits coronary atherosclerosis in rabbits (8), and decreases insulin resistance (IR) in diabetic mice (9, 10). Some clinical studies report that serum DHEA levels are significantly lower in type 2 diabetic patients when compared to nondiabetic controls (11, 12).

Abnormalities observed in the obese ZR include hyperinsulinemia as a reflection of IR (4), hyperlipidemia (5), and beginning at mid-life, hyperglycemia (13). In this study we demonstrate that the obese ZR has twice the level of fasting serum free fatty acids (FFA) as their lean counterparts. Similar to the obese ZR, human obesity and type 2 diabetes is accompanied by elevated fasting serum FFA levels (14, 15). High FFA levels may be a significant contributor to IR and compensatory hyperinsulinemia (16). In a prospective study of over 4000 Caucasians, it has been shown that a 0.12 m*M* increase in fasting plasma FFA level correlated with a 30% increase in the risk of developing type 2 diabetes over a 2-year follow-up period (17).

Alterations in FA flux may be an important metabolic consequence central to the pathophysiology of the obese ZR (18, 19). However, control mechanisms governing FA flux have not been determined. Moreover, such mechanisms may be difficult to establish. After all, FAs are critical energy substrates, building blocks for cell membrane components, precursors of cell mediators such as gene expression (20). Such diverse roles suggest complex and specific regulatory factors governing FA flux.

Because this lipid flux may be central to the development of IR, tissue FA profiles may play a more important role than FFA levels. After all, skeletal muscle IR is the primary defect of patients with type 2 diabetes (21). The

pathogenesis of IR in skeletal muscle has been investigated intensively during the past 15 years; however, the pathophysiologic mechanism(s) remain to be determined. One major problem has been, and still is, inadequate understanding of the FA regulation of IR at the tissue level.

We are not the only investigators to report FA differences in lean and obese ZRs (22). It has been demonstrated that these changes are largely due to differences in the membrane phospholipid fraction (23). One study reports that tissue phospholipids of obese rats include lower proportions of arachidonic acid (AA) than lean rats (24). Other groups show that decreased proportions of AA in muscle tissue correlates with decreased insulin sensitivity and complications of type 2 diabetes (25, 26). Moreover, other investigators state that these AA profile differences imply an abnormality either in the ability of AA formation from linoleic acid at the delta 6 desaturase step, or an abnormality in the catabolism/distribution of AA (27).

Because DHEA alters FFA mobilization, and because increased levels of serum FFA and increased muscle FA hydrolysis often lead to IR and diabetes mellitus (28), perhaps a DHEA-related decreases in IR may be due to specific DHEA-mediated alterations in tissue lipid FA profiles. For example, Imai *et al.* (29) show that DHEA increases hepatic oleic acid in rats. In this study we hypothesize that DHEA would lower serum FFA levels in conjunction with altering FA profiles in muscle tissue. Specifically, this study investigates DHEA effects on serum FFA levels and on TL FA profiles of skeletal and cardiac muscle in lean and obese ZRs. In this study we do not claim that DHEA alters IR, and IR is not measured. Therefore, any discussion that associates DHEA treatment and IR reduction is speculative.

Materials and Methods

Animals. Obese (n = 30) and lean (n = 29) male ZR 16 to 24 weeks old were obtained from our colony in the Department of Physiology of the Louisiana State University Medical Center (New Orleans, LA). All animals were maintained on a 12:12-hr light:dark cycle (lights out at 0600 hr) in a room whose temperature was maintained at 22° + 1°C. Prior to the study, all animals were fed Purina Rodent Laboratory Chow #5001. The physiologic fuel value, as reported by the manufacturer, is 3.30 kcal/g. The proportions of energy as carbohydrate, protein, and fat are 63.7%, 30.4%, and 5.9%, respectively. Both food and water were available *ad libitum.* Animals were housed individually in wire mesh cages for each experiment.

Specialized Diet. A specialized macronutrient (MN) diet, as described in previous communications (2, 6), was combined such that the proportions of energy as carbohydrate, protein, and fat were 30%, 20%, and 50%, respectively. Our previous studies demonstrate that when given a three-bowl choice among carbohydrate, protein, and fat, both lean and obese ZR consume these MN in the proportions used in this study's design. Throughout this study, each animal was presented one dish containing the modified MN diet in a single bowl. The FA composition of the diet is presented in Table 3. The dishes were attached to the cages via a spring to prevent spillage. Additionally, each dish had a modified metal cover and disk to prevent the animal from kicking food out of the dish. Body weights and food intakes were determined between 0800 and 0900 hr. As indicated, pair feeding was conducted daily at 0900 hr. Any spilled diet was collected on the paper placed under each cage. The diet's consistency was "paste-like" and spillage was minimal. The diet of the groups designated "DHEA" was supplemented with 0.6% (wt/wt) DHEA.

Study Design and Procedures. On Day 1, 59 male ZRs 16 to 24 weeks old were divided into lean and obese, then weight/age matched, and subsequently divided into six groups (1–6). Group 1 contained 10 obese ZRs and was designated male obese DHEA. Group 2 contained 10 obese ZRs and was designated male obese pair-fed (PF). Group 3 contained 10 lean ZRs and was designated male lean DHEA. Group 4 contained nine lean ZRs and was designated male lean PF. The other two groups were 10 obese and 10 lean rats that were designated *ad libitum* control, and were given 24-hr *ad libitum* access to the DHEA-free diet throughout the experiment. The diet of each treatment group was supplemented with 0.6% DHEA. In previous publications, we have established DHEA dose-response curves and have demonstrated that the most significant antiobesity DHEA effects occur at the 0.6% level (3, 6).

All animals were given an adjustment period during which they consumed the DHEA-free diet for 7 days (from Days 1 to 7). For the subsequent 7 days (Days 8 through 14), Groups 2 and 4 were PF to the caloric intake of their corresponding lean or obese DHEA group. Each animal in the PF groups was given the average number of calories consumed by their corresponding DHEA group from the previous day. An introduction of 0.6% DHEA in the diet of ZR in our colony corresponds to a 50% reduction in caloric intake during the first 24 hr. Therefore, on the first day of pair feeding, PF groups were given one-half the amount they had consumed the previous day. At the end of the experiment, all animals were fasted for 14 hr, sacrificed, serum analyzed for FFA levels, and soleus and hearts were analyzed for TL FA profiles.

Animals consumed the diet as described in each group and were sacrificed via rapid decapitation after a 14-hr fast. Trunk blood from each was collected over ice in nonheparinized glass tubes (10 × 75 mm) and centrifuged at 2500 rpm for 20 min at −4°C in an IEC Centra-7R refrigerated centrifuge. Four 350-µl aliquots of serum were stored in a biologic freezer (−80°C), and were later assayed for insulin, glucose, triglyceride (TG), and FFA or nonesterified FA.

Serum FFA, insulin, DHEA-S, glucose, TG, and total cholesterol were determined as described elsewhere (30–34).

Epididymal, perirenal, and retroperitoneal fat depots

were removed and their weights were recorded. Left soleus and whole hearts were removed, weighed, frozen in liquid nitrogen, and stored at −80°C until TL FA profile determination.

Soleus and heart TL FA profiles were determined by gas-liquid chromatography. Each muscle was homogenized with a Virtus Electric Homogenizer "45" with nanograde (Mallinckrodt) chloroform-methanol solution (2:1, v/v). TL was extracted by the method of Folch *et al.* (35). The washed lipid extract was evaporated to dryness under a vacuum and was redissolved in 10 ml of chloroform. The TL extract was transmethylated with 6% sulfuric acid in anhydrous methanol in a Teflon-lined screw-cap tube at 75°C for 15 hr. The FA methyl esters were extracted with hexane and were stored under nitrogen prior to injection directly onto the column of a gas chromatograph.

The gas chromatograph was equipped with a flame ionization detector and an automatic digital integrator. The 30-m fused-silica capillary column had an internal diameter of 0.32 mm and was coated with a 0.2-μm film of highly polar cyanosilicone liquid phase (SP 2330, Supelco Inc., Bellefonte, PA). The column temperature was held at 170°C for 4 min and was then programmed to increase at a rate of 5°C/min to 220°C. The injection port and the detector temperatures were held at 250°C. Helium was the carrier gas at a flow rate of 25 ml/min; the inlet pressure was ~517 × 103 Pa.

A standard mixture of FA methyl esters (Applied Science Labs, State College, PA) was run daily for standardization and proper identification of the FA. The individual FA were calculated and expressed as a proportion (mass percentage) of the TL FA present in the sample.

Statistical Analysis. Variations in experimental measurements were examined by one-way analysis of variance (ANOVA) on a Power Macintosh 7600/120 Superanova program Abacus Concepts. Significance for $P < 0.05$ was measured using Fisher's projected least square difference, giving the exact P value for each comparison.

For all tables presented, rows not sharing the same letter are significantly different ($P < 0.05$). Values in columns are not statistically compared. Values are presented as mean + SEM.

Results

Table I contains percentage of body weight (BW) changes on Days 8 and 14 compared with Day 1, and caloric intake (CI) on Days 5, 8, 11, and 14. On Day 8, the change in BW was not different within phenotypes; however, between phenotypes, obese groups had a more rapid weight gain than lean groups. On Day 14 the change in BW of both PF groups was not different from each respective Day 1 BW. However, during the 7 days of treatment, lean and obese DHEA groups lost 5.4% and 9% of their Day 1 BW, respectively. Both *ad libitum* groups gained significantly more weight and consumed significantly more calories than their corresponding DHEA group.

On Day 5, CI was not different within phenotypes; however, obese groups consumed significantly more calories than the lean groups. During Days 8, 11, and 14, both DHEA groups consumed significantly fewer calories per day compared with the pre-treatment period (Days 1 to 7). This significant difference is not indicated in the table. The CI of the lean DHEA group steadily increased during the treatment period; this was not observed in the obese DHEA group whose CI were not different from Day 1 levels throughout the experiment. The obese DHEA group demonstrated a greater proportional decrease in calories consumed compared with the lean DHEA group.

Table II contains weights (g) of hearts, left soleus, retroperitoneal (RP) adipose tissue (AT), perirenal (PR) AT, and epididymal (EPID) AT for each group. Neither hearts nor soleus weights were affected by phenotype or were altered by pair-feeding or DHEA treatment. All AT depots weighed more in obese groups compared with lean ZR groups. The weights of RP AT and EPID AT were significantly diminished after 7 days of DHEA treatment in both lean and obese ZR. PR AT weight was not altered by DHEA treatment.

Table III contains the TL FA profiles for whole hearts of each group. For comparison purposes, the TL profile of the 50% fat diet is included. Total proportions (%) are recorded for FAs that are 12 through 22 carbons in length and then are summed for omega-6, saturated, MU, and PU FA. Table IV is designed in the same fashion (without the diet's

Table I. Percent Body Weight Change (% BW from Day 1 on Days 8 and 14 and Caloric Intakes in Kilocalories on Days 5, 8, and 14 Are Presented

% BW Δ	OAC	OPF	ODHEA	LAC	LPF	LDHEA
Day 8 vs 1	2.6 ± 1.4^a	2.5 ± 1.2^a	3.0 ± 1.2^a	1.2 ± 0.7^b	1.3 ± 0.6^b	1.0 ± 1.1^b
Day 14 vs 1	**3.2 ± 1.6^a**	**−0.8 ± 1.2^b**	**−9.0 ± 0.8^c**	**1.7 ± 1.1^b**	**1.4 ± 1.4^b**	**−5.4 ± 1.2^d**
Caloric intakes (kcals)						
Day 5	82.1 ± 3.6^a	85.9 ± 4.6^a	84.8 ± 3.5^a	62.1 ± 2.5^b	60.3 ± 3.7^b	61.2 ± 2.7^b
Day 8	**79.2 ± 2.1^a**	See ODHEA	**36.5 ± 2.2^b**	**58.7 ± 2.1^c**	See LDHEA	**41.1 ± 1.5^d**
Day 11	**80.0 ± 2.5^a**	See ODHEA	**38.3 ± 2.0^b**	**59.2 ± 3.0^c**	See LDHEA	**48.1 ± 1.0^d**
Day 14	**78.4 ± 3.1^a**	See ODHEA	**36.6 ± 1.5^b**	57.0 ± 3.2^c	See LDHEA	55.2 ± 2.1^c

Note. Values are presented as means, + SEM. Rows not sharing the same letter are significantly different ($P < 0.05$). OAC, obese *ad libitum* control; OPF, obese pair-fed; ODHEA, obese DHEA; LAC, lean *ad libitum* control; LPF, lean pair-fed, LDHEA, lean DHEA. DHEA effects are typed in **bold.**

Table II. Weight (grams) of Hearts, Soleus Muscles, Retroperitoneal (RP) Adipose Tissues (AT), Perirenal (PR) AT, and Epididymal (EPID) AT Are Recorded as Means + SEM

Tissue wt (g)	OAC	OPF	ODHEA	LAC	LPF	LDHEA
Heart	1.04 ± 0.07[a]	1.07 ± 0.09[a]	1.03 ± 0.10[a]	1.04 ± 0.10[a]	1.00 ± 0.10[a]	1.12 ± 0.11[a]
Soleus	0.14 ± 0.04[a]	0.13 ± 0.02[a]	0.14 ± 0.03[a]	0.15 ± 0.03[a]	0.15 ± 0.03[a]	0.16 ± 0.03[a]
RP AT	**7.68 ± 0.67[a]**	**7.42 ± 0.51[a]**	**5.70 ± 0.40[b]**	**1.77 ± 0.14[c]**	**1.62 ± 0.14[c]**	**1.09 ± 0.17[d]**
PR AT	2.95 ± 0.35[a]	2.89 ± 0.21[a]	2.57 ± 0.30[a]	0.65 ± 0.08[b]	0.62 ± 0.06[b]	0.62 ± 0.07[b]
EPID AT	**12.2 ± 1.07[a]**	**11.39 ± 0.8[a]**	**8.10 ± 0.66[b]**	**3.74 ± 0.55[c]**	**3.54 ± 0.39[c]**	**2.74 ± 0.42[d]**

Note. Rows not sharing the same letter are significantly different ($P < 0.05$). OAC, obese *ad libitum* control; OPF, obese pair-fed; ODHEA, obese DHEA; LAC, lean *ad libitum* control; LPF, lean pair-fed; LDHEA, lean DHEA. DHEA effects are typed in **bold.**

Table III. Heart TL FA Profiles Are Recorded as Means ± SEM

FA	DIET	OAC	OPF	ODHEA	LAC	LPF	LDHEA
12:0	0.0	0.1[a]	0.1[a]	0.1[a]	0.1[a]	0.1[a]	0.1[a]
14:0	0.3	0.4 ± 0.2[a]	0.4 ± 0.1[a]	0.4[a]	0.4 ± 0.1[a]	0.4[a]	0.4 ± 0.1[a]
14:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:0	13.5	15.6 ± 1.2[a]	14.4 ± 0.9[a]	15.6 ± 1.2[a]	10.5 ± 0.8[b]	10.3 ± 0.9[b]	9.4 ± 1.1[b]
16:1	0.2	1.6 ± 0.3[a]	1.9 ± 0.3[a]	1.6 ± 0.4[a]	0.6 ± 0.1[b]	0.4 ± 0.1[b]	0.4 ± 0.1[b]
18:0	11	14.9 ± 1.1[a]	13.8 ± 0.8[a]	14.8 ± 0.9[a]	17.7 ± 0.8[b]	17.9 ± 0.5[b]	18.9 ± 0.5[b]
18:1	40.2	**20.3 ± 2.1[a]**	**21.6 ± 2.9[a]**	**16.9 ± 0.8[b]**	**14.6 ± 0.8[b,c]**	**14.5 ± 0.8[b,c]**	**10.4 ± 0.5[d]**
18:2	29.3	18.9 ± 0.9[a]	18.9 ± 1.0[a]	18.1 ± 1.1[a]	19.1 ± 1.7[a]	19.1 ± 0.9[a]	18.0 ± 1.9[a]
20:0	0.3	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]
18:3	2.0	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]
20:1	0.2	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]
20:2	0.5	0.4 ± 0.1[a]	0.3 ± 0.1[a]	0.5 ± 0.2[a]	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.4 ± 0.1[a]
20:3	0.0	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.4 ± 0.1[a]
22:0	0.4	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]
20:4	0.0	**11.7 ± 2.0[a]**	**10.0 ± 1.7[a]**	**15.7 ± 0.7[b]**	**16.3 ± 1.0[b]**	**17.1 ± 2.0[b]**	**23.3 ± 0.9[c]**
20:5	0.1	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]
24:0	0.2	0.4 ± 0.1[a]	0.5 ± 0.1[a]	0.4 ± 0.1[a]	0.4[a]	0.4[a]	0.4[a]
24:1	0.0	0.3[a]	0.3[a]	0.3[a]	0.6[b]	0.6 ± 0.1[b]	0.8 ± 0.2[b]
22:5	0.0	1.9 ± 0.3[a]	1.7 ± 0.2[a]	1.9 ± 0.3[a]	1.7 ± 0.3[a]	1.6 ± 0.3[a]	1.8 ± 0.3[a]
22:6	0.0	9.6 ± 0.4[a]	8.7 ± 1.4[a]	9.5 ± 0.8[a]	9.9 ± 1.3[a]	10.6 ± 1.1[a]	10.2 ± 1.4[a]
ω-6	29.8	**31.4 ± 1.1[a]**	**29.6 ± 1.1[a]**	**34.7 ± 0.7[b]**	**36.1 ± 1.9[b]**	**36.9 ± 2.1[b]**	**42.1 ± 2.7[c]**
SFA	25.7	31.8 ± 0.9[a]	30.8 ± 1.1[a]	31.4 ± 1.4[a]	30.9 ± 1.0[a]	30.0 ± 1.2[a]	28.9 ± 1.4[a]
MUFA	40.6	**22.9 ± 2.8[a]**	**24.6 ± 3.1[a]**	**18.9 ± 1.2[b]**	**15.6 ± 1.1[c]**	**14.7 ± 1.4[c]**	**11.6 ± 1.0[d]**
PUFA	31.9	**41.1 ± 2.5[a]**	**39.9 ± 3.1[a]**	**45.9 ± 1.1[b]**	**48.7 ± 1.4[b]**	**50.5 ± 1.9[b]**	**55.6 ± 0.9[c]**

Notes. The TL profile of the diet is recorded in the first column. Rows not sharing the same letter are significantly different ($P \leq 0.05$). OAC, obese *ad libitum* control; OPF, obese pair-fed; ODHEA, obese DHEA; LAC, lean *ad libitum* control; LPF, lean pair-fed; LDHEA, lean DHEA. DHEA effects are typed in **bold.**

FA profile), presenting the profiles for soleus muscles of each group.

With respect to phenotypes, the percentage of saturated FA in the soleus was greater in the obese compared with the lean groups. This increase is the result of increases in the proportions of myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0) in the obese soleus. Significantly higher proportions of palmitic and stearic acid in obese hearts was not great enough to significantly increase the percentage of saturated FA.

The percentage of MUFA in heart and soleus was significantly greater in obese compared with lean ZR. This difference was due to proportional increases in palmitoleic acid (16:1), oleic acid (18:1), and nervonic acid (24:1) in the heart profiles and to palmitoleic acid for the soleus. The percentage of PUFA in hearts and soleus was significantly lower in the obese compared with the lean control groups. This decrease was due to proportionally lower heart arachidonic acid (20:4) and to soleus linoleic acid (18:2), AA, and docosahexaenoic acid (22:6) in obese compared with lean ZR. The heart and soleus muscle omega-6 FAs are proportionally higher in obese compared with lean rats.

The soleus percentage of saturated FA, MUFA, and PUFA was not altered after 7 days of pair feeding or DHEA treatment in either lean or obese ZR groups. However, AA proportions were significantly increased in both DHEA groups. The heart percentage of MUFA was significantly lower, and the percentage of PU FA was significantly higher than controls in both DHEA-treated lean and obese rats. The percentage of MU FA was significantly greater in heart for both obese and lean DHEA groups. This increase was due to the proportional elevations in oleic acid. There was a significant increase in the percentage of PU FA in heart for both lean and obese DHEA groups when compared with their corresponding controls. This percentage of PU FA increase was solely due to increased proportions of AA. The

Table IV. Soleus TL FA Profiles Are Recorded as Means ± SEM

FA	OAC	OPF	ODHEA	LAC	LPF	LDHEA
12:0	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.2 ± 0.1[a]	0.3 ± 0.1[a]	0.2 ± 0.1[a]
14:0	1.5 ± 0.2[a]	1.4 ± 0.2[a]	1.5 ± 0.2[a]	0.6 ± 0.1[b]	0.5 ± 0.1[b]	0.5 ± 0.1[b]
14:1	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]
16:0	22.4 ± 1.5[a]	22.0 ± 2.1[a]	21.8 ± 2.0[a]	14.3 ± 1.2[b]	12.5 ± 1.4[b]	12.6 ± 1.4[b]
16:1	6.8 ± 0.6[a]	6.6 ± 0.7[a]	6.5 ± 1.1[a]	1.6 ± 0.4[b]	1.4 ± 0.3[b]	1.3 ± 0.3[b]
18:0	9.2 ± 1.7[a]	8.5 ± 1.0[a]	8.7 ± 0.9[a]	13.5 ± 1.2[b]	13.6 ± 0.6[b]	14.1 ± 0.6[b]
18:1	26.4 ± 1.3[a]	24.7 ± 1.6[a]	24.7 ± 1.7[a]	23.6 ± 2.1[a]	24.1 ± 1.6[a]	24.0 ± 1.6[a]
18:2	18.1 ± 0.9[a]	17.9 ± 1.1[a]	17.3 ± 1.1[a]	24.6 ± 1.6[b]	23.7 ± 0.9[b]	23.5 ± 1.0[b]
20:0	0.4[a]	0.4[a]	0.4[a]	0.4[a]	0.4[a]	0.4[a]
18:3	0.7 ± 0.1[a]	0.7 ± 0.2[a]	0.6 ± 0.1[a]	0.8 ± 0.2[a]	0.7 ± 0.2[a]	0.7 ± 0.2[a]
20:1	0.4[a]	0.4[a]	0.4[a]	0.4[a]	0.4[a]	0.4[a]
20:2	0.7 ± 0.1[a]	0.8 ± 0.2[a]	0.9 ± 0.3[a]	0.9 ± 0.2[a]	0.8 ± 0.2[a]	0.8 ± 0.3[a]
20:3	0.5 ± 0.1[a]	0.6 ± 0.1[a]	0.6 ± 0.2[a]	0.6 ± 0.2[a]	0.7 ± 0.2[a]	0.7 ± 0.2[a]
22:0	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]
20:4	**3.3 ± 0.4[a]**	**3.6 ± 0.3[a]**	**6.1 ± 0.6[b]**	**6.9 ± 1.0[b]**	**7.3 ± 1.4[b]**	**8.9 ± 1.0[c]**
20:5	0.5 ± 0.1[a]	0.6 ± 0.2[a]	0.7 ± 0.3[a]	0.7 ± 0.3[a]	0.5 ± 0.2[a]	0.5 ± 0.2[a]
24:0	0.4 ± 0.2[a]	0.5 ± 0.2[a]	0.5 ± 0.2[a]	0.6 ± 0.3[a]	0.5 ± 0.2[a]	0.4 ± 0.1[a]
24:1	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]
22:5	1.1 ± 0.2[a]	1.2 ± 0.2[a]	1.3 ± 0.2[a]	1.0 ± 0.2[a]	1.3 ± 0.2[a]	1.2 ± 0.2[a]
22:6	3.5 ± 0.9[a]	3.8 ± 0.7[a]	4.2 ± 0.8[a]	5.8 ± 0.6[b]	6.0 ± 0.5[b]	6.1 ± 0.5[b]
ω-6	22.6 ± 1.1[a]	22.9 ± 1.3[a]	24.9 ± 1.4[a]	33.0 ± 1.7[b]	32.5 ± 1.6[b]	33.9 ± 1.4[b]
SFA	33.5 ± 0.9[a]	32.8 ± 0.8[a]	33.6 ± 0.9[a]	28.1 ± 1..0[b]	27.8 ± 1.0[b]	27.9 ± 1.1[b]
MUFA	34.8 ± 1.4[a]	34.1 ± 1.5[a]	32.9 ± 1.9[a]	28.8 ± 1.2[b]	26.4 ± 1.2[b]	26.3 ± 1.5[b]
PUFA	28.6 ± 3.1[a]	28.9 ± 2.1[a]	31.1 ± 2.8[a]	41.3 ± 1.6[b]	41.2 ± 1.8[b]	41.7 ± 1.8[b]

Notes. The TL profile of the diet is recorded in the first column. Rows not sharing the same letter are significantly different ($P \leq 0.05$). OAC, obese *ad libitum* control; OPF, obese pair-fed; ODHEA, obese DHEA; LAC, lean *ad libitum* control; LPF, lean pair-fed; LDHEA, lean DHEA. DHEA effects are typed in **bold.**

hearts, but not soleus muscles, of DHEA groups have significantly greater proportions of omega-6 FAs.

Heart TL content was approximately 30 mg of lipid/g of tissue wet weight. This value was not different among groups or between phenotypes (data not shown).

Serum insulin (nanograms per milliliter), DHEA-sulfate (nanograms per milliliter), glucose (milligrams per decaliter), TG (milligrams per decaliter), cholesterol (milligrams per decaliter), and FFA (m*M*) levels in PF and DHEA groups are recorded in Table V. In general agreement with previous studies, TG, cholesterol, and FFA were higher in obese compared with lean rats. For all serum parameters except for DHEA-sulfate, obese PF levels were significantly greater than lean PF. Insulin levels were significantly decreased in the obese DHEA, but not the lean DHEA group. DHEA-sulfate levels were significantly increased in both DHEA groups. No other serum parameters were altered in obese DHEA or lean DHEA groups. PF groups were not different from the *ad libitum* controls.

Discussion

While serum FFA levels in obese were almost two times higher than in lean ZR, there were no effects after 7 days of DHEA treatment. However, there were tissue-specific FA profile differences between lean and obese ZR. For example, the proportion of linoleic acid was significantly greater in the soleus of the lean compared with the obese ZR. This specific FA difference was not observed in heart muscle. Both lean and obese DHEA groups had significantly greater proportions of AA and lower proportions of oleic acid in both muscle types when compared with both of their controls.

High serum FFA levels often accompany IR states such as obesity and type 2 diabetes (15, 36). Several investigators

Table V. Serum Levels for the Indicated Parameters Are Recorded as Means ± SEM

Serum parameters	OAC	OPF	ODHEA	LAC	LPF	LDHEA
Insulin (ng/mL)	6.2 ± 0.8[a]	**5.9 ± 0.4[a]**	**4.8 ± 0.2[b]**	1.4 ± 0.2[c]	1.6 ± 0.2[c]	1.4 ± 0.2[c]
DHEA-S (ng/mL)	6.0 ± 3.1[a]	**6.4 ± 4.5[a]**	**51.8 ± 12.8[b]**	4.1 ± 2.0[a,c]	**4.5 ± 2.5[a,c]**	**31.2 ± 5.8[d]**
Glucose (mmol/L)	9.7 ± 0.7[a]	9.1 ± 0.5[a]	8.4 ± 0.6[a,b]	7.7 ± 0.5[b]	7.4 ± 0.4[b]	7.7 ± 0.4[b]
TG (mmol/L)	2.4 ± 0.2[a]	2.2 ± 0.2[a]	2.1 ± 0.3[a]	0.7 ± 0.1[b]	0.7 ± 0.1[b]	0.7 ± 0.1[b]
Cholesterol (mmol/L)	2.5 ± 0.1[a]	2.6 ± 0.2[a]	2.5 ± 0.1[a]	1.8 ± 0.1[b]	1.8 ± 0.2[b]	1.9 ± 0.1[b]
FFA (mmol/L)	1.121 ± 0.15[a]	1.115 ± 0.12[a]	1.071 ± 0.10[a]	0.620 ± 0.10[b]	0.624 ± 0.09[b]	0.582 ± 0.08[b]

Notes. Rows not sharing the same letter are significantly different ($P \leq 0.05$). OAC, obese *ad libitum* control; OPF, obese pair-fed; ODHEA, obese DHEA; LAC, lean *ad libitum* control; LPF, lean pair-fed; LDHEA, lean DHEA. DHEA effects are typed in **bold.**

have demonstrated that experimentally increasing FFA levels in healthy humans, via triglyceride/heparin infusions, can acutely induce IR (37–44). Additionally, high FFA levels eventually lead to a gradual decreased glucose-stimulated insulin secretion, which may manifest at the onset of hyperglycemia.

A human study demonstrated that FFA levels of ~0.8 m*M* corresponded to an increase in glucose-6-phosphate concentrations in muscle biopsies (45). This may suggest an inhibitory effect of FFA on glycogen synthase activity at this level. However, lower FFA levels (~0.5 m*M*) did not correspond to a difference in intramuscular glucose-6 phosphate concentrations. Interestingly, the FFA levels in our lean rats are slightly lower, and in our obese rats, are slightly higher than 0.8 m*M*. Perhaps slight elevations in serum FFA levels over long time periods lead to IR by inhibition of GLUT-4. Such inhibition may be followed by a reduction of muscle glycogen synthesis and glucose oxidation. However, we believe that altered lipid FA profiles, rather than FFA levels, has a greater influence on IR development at the tissue level.

Specifically, in this study, the proportion of AA in hearts and soleus was significantly greater in both DHEA-treated lean and obese ZR. Oleic acid was the only other FA whose profile was altered in the DHEA group. A study conducted in humans demonstrated that higher levels of LC PU FA, such as AA, in skeletal muscle has been connected with improved peripheral insulin sensitivity (25).

Proportional elevations in serum omega-6 FA correlate with the onset of type 2 diabetes mellitus (46). Because ω-6 FA class switching and inter-conversions are not possible, we propose that DHEA may alter ω-6 utilization such that AA proportionally increases in muscle tissue. In turn, ω-6 FA in serum may be decreasing. This may reflect a mechanism by which DHEA could reduce IR. Other studies investigating lipid-lowering drugs show that PU FAs decreased and MU FAs increased in obese ZR plasma and liver (47). Another study showed no change in plasma PL AA proportions in NZB/WF1 mice treated with DHEA (48). These studies, however, did not investigate muscle lipid profiles.

Investigators who have measured skeletal muscle and adipose lipoprotein lipase support the view that muscle, and not adipose tissue, is the primary site of FA clearance (49). Several investigators have proposed that myocardial AA, a modulator of cellular signaling pathways, plays an important role as a cardioprotective agent during and after ischemia (50–53). Therefore, the mechanisms by which AA acts as a second messenger may explain its cardioprotective effect in myocardial ischemia.

Analysis of muscle FA profiles from nondiabetic patients with coronary artery disease show that insulin sensitivity positively correlates with the proportion of LC PU FA, specifically AA (25–27). From these and other studies (54), lower proportions of muscle PL AA have been implicated in the impairment of insulin sensitivity and have been shown to promote complications of type 2 diabetes. Specifically, tissue insulin deficiency leads to a relative decrease in AA (28). DHEA-related increases in AA noted in our study may reflect the mechanism by which DHEA could reduce IR.

The primary defect in patients with diabetes mellitus is in skeletal muscle (21), and this defect may be directly related to the proportions of LC PU FA uptake by muscle tissue. While there are few studies that support a DHEA role in reducing IR (55, 56), our paper is the first to demonstrate that DHEA treatment alters PU FA profiles in muscle tissue. We believe that these specific DHEA-related lipid alterations reflect key regulatory changes that may explain DHEA's ability to decrease IR. Perhaps, as suggested by Phinney *et al.* (27), there is a role for abnormal omega 6 FA metabolism in the etiology of ZR obesity. Other enzymes may also play a role in the DHEA-related changes. Specifically, it has been demonstrated that DHEA-treated rats have increased levels of malic enzyme, long-chain fatty-acyl coenzyme A hydrolyase, and catalase (57). While such alterations are attributable to hepatic lipid profile changes, these studies did not measure muscle tissue lipid profiles.

In a pilot study, similar to the results reported by Cleary *et al.* (22), we show that subcutaneous adipose FA profiles are different between lean and obese ZRs. However, 7 days of DHEA treatment did not have an effect. This outcome is likely for several reasons. First, adipose tissue contains few FAs greater than 18 carbons long. Second, the rate of adipose FA turnover is not as rapid as in other tissue. Therefore, 7 days of DHEA treatment may not be long enough to demonstrate a change. Such changes occurring within 7 days of DHEA treatment seem to be better observed in muscle tissue than in adipose tissue. Other investigators, however, suggest that such adipose-specific changes may occur in the PL fractions (23, 58).

While such DHEA-related changes demonstrated in our study correspond with improved states of IR, several questions remain unanswered. Because serum FFA levels, but not serum profiles, were measured, proportional DHEA-related changes in specific serum FA were not determined. Future studies should determine the profiles of specific serum and muscle tissue lipid fractions, such as the phospholipid, FFA, triglyceride, and/or cholesteryl ester. The identification of the location where such DHEA-related alterations are occurring could elucidate a possible mechanism for the TL profile changes we observed. The determination of such mechanisms may prove to be a significant step in assessing a DHEA-related effect on IR. Because of the specific DHEA-related lipid alterations, we believe that the importance and implications of this study are a tremendous beginning step in attributing a DHEA effect on IR.

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