DHEA (dehydroepiandrosterone) replacement is not part of the current standard of care in hypoadrenal subjects. Animal studies have shown that DHEA administration prevents diabetes. To determine the physiological effect of DHEA replacement on insulin sensitivity in adrenal-deficient women, we performed a single-center, randomized, double-blind, placebo-controlled, crossover study in 28 hypoadrenal women (mean age 50.2 ± 2.87 years) who received a single 50-mg dose of DHEA daily or placebo. After 12 weeks, insulin sensitivity was assessed using a hyperinsulinemic-euglycemic clamp. DHEA replacement significantly increased DHEA-S (sulfated ester of DHEA), bioavailable testosterone, and androstenedione and reduced sex hormone-binding globulin levels. Fasting plasma insulin and glucagon were lower with DHEA (42 ± 4.94 vs. 53 ± 6.58 pmol/l [P = 0.005] and 178 ± 11.32 vs. 195.04 ± 15 pmol/l [P = 0.02], respectively). The average amount of glucose needed to maintain similar blood glucose levels while infusing the same insulin dosages was higher during DHEA administration (358 ± 24.7 vs. 320 ± 24.6 mg/min; P < 0.05), whereas endogenous glucose production was similar. DHEA also reduced total cholesterol (P < 0.005), triglycerides (P < 0.011), LDL cholesterol (P < 0.05), and HDL cholesterol (P < 0.005). In conclusion, replacement therapy with 50 mg of DHEA for 12 weeks significantly increased insulin sensitivity in hypoadrenal women, thereby suggesting that DHEA replacement could have a potential impact in preventing type 2 diabetes. Diabetes 54:765–769, 2005
estrogen status had been steady for >6 months and women on other forms of hormone replacement therapy (e.g., thyroid) whose dosage had remained the same for >6 months were eligible.

Exclusion criteria included BMI >35 kg/m², a fasting blood glucose >120 mg/dl, or a history of sex hormone–dependent malignancy. Women with a history of cerebrovascular disease, cardiovascular disease (other than hypertension), live or renal disease were also excluded. Women who were pregnant or breast-feeding or postmenopausal women who had been on hormone replacement therapy for <6 months were not eligible to participate.

After the initial screening, 33 subjects were randomized into the study. In all, five subjects did not complete the study; one withdrew after 10 weeks in the first arm of the study as she suffered from diarrhea, which stopped when the drug (DHEA) was withdrawn; two finished the first arm of the study, but declined to complete the second half (both on placebo); one withdrew at 11 weeks, citing work pressure (on DHEA); and one unblinded herself by having blood levels taken (on DHEA). Thus, complete data are presented on 28 subjects.

This was a single-center, randomized, placebo-controlled, double-blind, crossover study. Study participants self-administered 50 mg of micronized pharmaceutical-grade DHEA (Spectrum Chemicals and Laboratory Products, Gardena, CA) or an identically encapsulated placebo (Clinical Encapsulation Services, Newington, NY) as a single daily dose for 12 weeks. There was a 2-week washout period between treatment phases. At screening, subjects underwent a physical examination that including a graded submaximal treadmill exercise test with continuous electrocardiogram monitoring to exclude ischemic heart disease. Blood was drawn for fasting glucose, complete blood count, and renal and liver function tests. All subjects were standardized onto divided doses of hydrocortisone as their glucocorticoid replacement therapy (mean dosage 24.4 ± 7.0 mg).

Randomization was done by the Division of Biostatistics at Mayo Clinic. Subjects began the first 12-week phase of the study at least 3 weeks after hydrocortisone standardization. At the end of the 12-week period, subjects were admitted overnight to the General Clinical Research Center at Mayo Clinic. After an overnight fast, blood samples were collected for measurements of glucose, DHEA-S, cortisol, androstenedione, IGF-I, IGFBP-1, and -3, lipid profile, and bioavailable testosterone. Body composition was measured by dual X-ray absorptiometry.

Subjects were fasted from 10:00 p.m. the night before the clamp until completion of the study the next day. At 4:00 a.m., a 3-mg/kg fat-free body mass (FFM) bolus of [6,6-2H]glucose (CIL, Boston, MA) was given intravenously as a priming dose into an indwelling forearm catheter. Next, a 3-h infusion of [6,6-2H]glucose at 3 mg/kg FFM · h⁻¹ was given to measure fasting endogenous glucose production (EGP). At 7:00 a.m., the deuterated glucose infusion was stopped and an intravenous insulin infusion was begun at 1.5 mU/kg FFM · min⁻¹ for 7 h. A retrograde intravenous cannula was placed in the distal forearm. This hand was placed in a hotbox, as previously described, to exclude ischemic heart disease. Blood was drawn for fasting glucose, complete blood count, and renal and liver function tests. All subjects were standardized onto divided doses of hydrocortisone as their glucocorticoid replacement therapy (mean dosage 24.4 ± 7.0 mg).

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After the clamp period, there was a 2-week washout period, followed by another 12-week trial period on the alternative drug. At the end of the second trial period, subjects were studied in the same manner as after the first trial period.

Hormone measurements. Plasma concentrations of DHEA-S were measured by a competitive chemiluminescent immunoassay on the Immulite automated immunoassay system (Diagnostic Products, Los Angeles, CA). Bioavailable testosterone was measured with a differential precipitation of sex hormone–binding globulin (SHBG) by ammonium sulfate after equilibration of the serum sample with tracer amounts of tritiated testosterone. Androstenedione was measured by a two-site immunoradiometric assay (IRMA) (Diagnostic Systems, Webster, TX). SHBG was measured with a solid-phase, two-site chemiluminescent immunometric assay on the Immulite automated immunoassay system (Diagnostic Products).

Insulin and cortisol concentrations were measured using a two-site immunoenzymatic assay performed on the Access automated immunoassay system (Beckman, Chaska, MN). Glucagon concentrations were measured by a direct, double-antibody radioimmunoassay (RIA) (Linco Research, St. Charles, MO). IGFBP-1 and -3 were measured by a two-site IRMA (Diagnostic Systems). Estradiol levels were measured by the Estradiol double-antibody RIA (Diagnostic Products).

Glucose measurements. Plasma glucose was measured using an enzymatic technique. Plasma [2H]glucose enrichment was measured as previously described using a gas chromatograph/mass spectrometer (19). EGP was measured using tracer dilution technique using steady-state plasma [2H]glucose meter ratios (19).

Data are means ± SE.

Lipids. Total cholesterol was measured on the Hitachi 912 chemistry analyzer using a Technicon cholesterol reagent (Bayer, Tarrytown, NY). HDL cholesterol was measured on the Hitachi 912 chemistry analyzer using direct HDLC plus reagent (Roche Diagnostics, Indianapolis, IN). Triglycerides were measured on the Hitachi 912 chemistry analyzer using a Technicon triglyceride reagent (Bayer).

Statistical analysis. A paired t test was performed to determine whether there were any differences between various outcome measures after DHEA treatment and placebo in all subjects. We also performed two-way repeated-measures ANOVAs with the factors treatment sequence (S), indicating which treatment was given first, the particular treatment (T: DHEA or placebo), and the interaction of these two main effects, thereby indicating whether the effects of DHEA varied depending on whether the drug preceded or followed placebo (S × T). Data are given as means ± SE.

RESULTS

DHEA effect on body composition. DHEA had no significant effect on body weight, fat mass, or FFM (data not given). Body weights were 72.5 ± 2.31 kg at the end of the placebo period and 72.3 ± 2.5 kg at the end of the DHEA period (P = 0.72).

Effects associated with treatment sequence. No significant effects associated with the sequence in which treatments were administered were noted, except for aspartate transerase (AST) and alkaline phosphatase (AP), so the results of the other end points are reported using the paired analysis. For both AST and AP, a statistically significant interaction was observed, indicating that the nature of the observed effects depended on which treatment was given first. In both instances, it appears that the observed effects of DHEA were less when it preceded placebo; when statistical tests were performed for DHEA effect separately for each sequence, the effect was significant when DHEA was given second or nonsignificant when it was given first.

Hormone levels. DHEA administration significantly increased DHEA-S, total and bioavailable testosterone, and androstenedione levels, whereas it reduced SHBG levels (Table 1). DHEA also significantly reduced fasting plasma insulin and glucagon levels, whereas it tended to increase IGF-I and estradiol levels (P = 0.058 and 0.051, respectively).

Plasma lipids, liver enzymes, and hematological data. DHEA significantly decreased total, LDL, and HDL cholesterol and triglyceride levels (Table 2). DHEA did not affect hematocrit, hemoglobin, or liver enzymes.

Glucose levels, glucose kinetics, and glucose clamp results. Fasting glucose levels showed a trend to be lower
During DHEA than during placebo administration (4.67 ± 0.54 vs. 4.83 ± 0.56 mmol/l), but the difference was not significant. EGP in the postabsorptive state during DHEA and placebo administration was similar (19.93 ± 0.56 vs. 19.94 ± 0.49 μmol · kg FFM⁻¹ · min⁻¹; P = 0.996).

During the glucose clamp (Fig. 1), the circulating insulin and glucose levels were similar. In contrast, plasma glucagon levels fell during insulin infusion (P < 0.0001) and during the three time points when the levels were lower (P < 0.0001) in the subjects during DHEA replacement than during placebo replacement. The average amount of dextrose infused from 180 to 420 min to maintain similar glucose levels was higher during DHEA compared with placebo administration (358 ± 24.7 vs. 320 ± 24.6 mg/min; P < 0.05). The total volume of 40% dextrose infused to maintain similar glucose levels was higher during the DHEA period compared with the placebo period (305 ± 22.5 vs. 276 ± 22.3 ml; P < 0.05).

**DISCUSSION**

The important finding of the current study is the enhanced insulin sensitivity observed in hypoadrenal subjects receiving replacement doses of DHEA. The fasting insulin levels were also lower during DHEA replacement than with placebo, despite no significant change in plasma glucose. The rate of EGP did not change with DHEA replacement, despite a lower fasting glucagon, demonstrating that DHEA had no effect on EGP. In contrast, although plasma insulin levels were identical during glucose clamp in both studies, the amount of glucose required to maintain similar glucose levels was higher after DHEA replacement. These results suggest that DHEA replacement results in enhanced insulin-induced glucose disposal, thus reflecting enhanced insulin sensitivity. We did not measure EGP during insulin infusion and, therefore, we cannot categorically exclude the possibility that the different rate of glucose infusion was due entirely to glucose disposal. However, based on previous reports, the amount of insulin we infused should have completely suppressed EGP (20).

The duration of DHEA administration and the differences in methodologies used to assess insulin sensitivity may explain the differences between the current study and many previous studies. The current study used the classic euglycemic glucose clamp (18), which is a sensitive approach to measuring insulin sensitivity. All subjects were admitted to the General Clinical Research Center on the day before the study and were kept on a similar diet and similar activity levels. In addition, we also standardized the replacement to a single glucocorticoid in all study subjects. Through these means, the measurement of insulin sensitivity was likely to have been more sensitive than in many other studies.

Previous studies administering 50–200 mg DHEA in similar groups of subjects have not shown significant changes in glucose tolerance (13–16). A few studies looking at the effects of DHEA in healthy elderly subjects have shown improvements using indirect measures of insulin sensitivity (21). Therefore, the results of this study suggest that DHEA may be a useful therapy for the treatment of insulin resistance in hypoadrenal subjects.

**TABLE 2**

Lipids and hematological and liver enzyme levels

<table>
<thead>
<tr>
<th></th>
<th>After 12 weeks of placebo</th>
<th>After 12 weeks of DHEA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.6 ± 0.17</td>
<td>4.2 ± 0.16</td>
<td>0.007</td>
</tr>
<tr>
<td>Calculated LDL cholesterol (mmol/l)</td>
<td>2.76 ± 0.15</td>
<td>2.56 ± 0.13</td>
<td>0.048</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.08 ± 0.06</td>
<td>0.97 ± 0.06</td>
<td>0.004</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.7 ± 0.15</td>
<td>1.5 ± 0.13</td>
<td>0.011</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.1 ± 0.15</td>
<td>13.5 ± 0.20</td>
<td>0.472</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>38.0 ± 0.48</td>
<td>38.5 ± 0.57</td>
<td>0.235</td>
</tr>
<tr>
<td>Aspartate transaminase (units/l)</td>
<td>24.7 ± 1.89</td>
<td>23.0 ± 1.48</td>
<td>0.253</td>
</tr>
<tr>
<td>Alanine transaminase (units/l)</td>
<td>23.3 ± 4.48</td>
<td>21.6 ± 2.20</td>
<td>0.672</td>
</tr>
<tr>
<td>Alkaline phosphatase (units/l)</td>
<td>145 ± 13.8</td>
<td>131 ± 10.0</td>
<td>0.375</td>
</tr>
</tbody>
</table>

Data are means ± SE.
sensitivity (12,21). These elderly people, unlike the subjects in the current study, were not completely DHEA deficient. Diamond et al. (21), using a 10% topical formulation of DHEA for 12 months, found an 11% reduction in fasting glucose associated with a 17% reduction in fasting insulin; however, the 75-g oral glucose tolerance testing results were unchanged between treatment groups.

There is additional evidence suggesting that DHEA has a role in reducing age-related increases in insulin levels, insulin resistance, and blood glucose (22). Jakubowicz et al. (12) studied 22 healthy men (mean age 57 years) using 100 mg DHEA per day. In this group, serum insulin decreased from 35.3 to 25.8 mU/ml, whereas serum glucose declined from 93.4 to 88.9 mg/ml, suggesting an improvement in insulin sensitivity. Serum insulin and glucose did not change significantly in the placebo group. However, no formal tests of insulin sensitivity were done. Kawano et al. (23) reported an improvement in insulin sensitivity measured by a fasting glucose measurement in 24 hypercholesterolemic but otherwise healthy men age 54 ± 1 years after 12 weeks on 25 mg of oral DHEA. There was no change in fasting insulin levels compared with placebo.

In contrast to the above studies, observational studies have demonstrated that low DHEA-S levels are associated with hyperglycemia and insulin resistance (24,25). Animal studies have demonstrated a significant effect of DHEA in preventing diabetes (8), but these are pharmacological effects. In contrast to the animal data, results from studies in healthy human volunteers (8) have been inconclusive. The human studies have shown that DHEA may improve insulin sensitivity (11,12,21,23), has no effect on it (9,10, 26–28), or actually worsens it (29). The current study, based on a crossover design in the same subjects using the hyperinsulinemic glucose clamp, clearly demonstrated that DHEA replacement enhances insulin action. The current findings have to be interpreted in the context of DHEA replacement in women who have no adrenal gland. It is not clear from the current study whether patients receiving DHEA in pharmacological dosages or elderly patients with reduced DHEA levels receiving DHEA replacement respond in a similar manner. In general, women have low testosterone levels, so that in adrenalectomized women, we anticipated seeing more of an effect of DHEA replacement than in men. It remains to be determined if a similar effect can be observed in adrenalectomized men as well. Elderly men and women have lower DHEA levels than younger people (30). However, elevating DHEA from the levels of elderly people to that of young people may have a differential effect in men and women.

Further issues arise when comparing the absolute DHEA deficiency in hypoadrenal subjects and the relative DHEA deficiency in healthy elderly patients. These differences increase the difficulty of inferring results from one group to another. However, the premise of several studies looking at DHEA replacement in both groups is that both DHEA-deficient conditions are interchangeable. This link between the relative DHEA deficiency seen with normal aging and the absolute state seen in hypoadrenal subjects is compounded by the similar findings of studies, such as those looking at different measures of general well-being, libido, and mood (9,10,13,31). The results of our study, therefore, may be important in other groups of relatively hypoadrenal subjects, such as those in intensive care, because it is in this group that adrenal insufficiency is common (32) and in whom insulin therapy has been shown to improve outcomes (33).

We measured other hormone levels that can impact insulin action such as glucagon, cortisol, and IGF-I. Although plasma glucagon levels in the postabsorptive state decreased with DHEA administration, we did not observe any decrease in EGP. In contrast, IGF-I levels tended to increase, which may have contributed to the increased insulin sensitivity (34) that occurred after DHEA administration.

Another observation of great interest is the decrease in triglyceride and total, LDL, and HDL cholesterol levels after DHEA administration. Reductions in total and HDL cholesterol levels have been reported by some authors (28,31,35), but not others (11,26). Although the mechanisms by which this occurs are not fully understood, these reductions are thought to be mediated by the effects of androgens on increasing hepatic lipase activity, thus impairing hepatic cholesterol formation (36). This may be a reflection on administering DHEA orally, thus increasing hepatic levels. Although systemic levels of testosterone remain relatively low, the levels of the androgenic metabolites of DHEA undergoing a hepatic first-pass effect may be sufficient to cause these changes. This may explain the relative lack of effect on serum lipids when DHEA was given transdermally or sublingually (21,27). Overall, the total−to−HDL cholesterol ratio remained unchanged between the two arms of the study (4.75 ± 0.33 vs. 4.64 ± 0.34, DHEA vs. placebo; P = 0.25). The mechanism for the reduction in triglycerides remains unclear, as the increase in activity in peroxisome proliferator-activated receptor-γ activity reported with DHEA administration would not account for the magnitude of change seen (37).

In summary, the current study clearly demonstrated that 12 weeks of supplemental DHEA results in an increase in insulin sensitivity. This conclusion is based on the increased glucose infusion rate required after DHEA administration to maintain similar glucose levels during identical insulin administration after both placebo and DHEA in hypoadrenal women. Based on the current results, serious consideration for DHEA replacement in deficient states is warranted, but further work is needed before the use of DHEA can be routinely recommended in hypoadrenal subjects. Alterations in triglyceride and LDL levels may also benefit in preventing cardiovascular death.

ACKNOWLEDGMENTS

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Cushing Disease Support and Research Foundation (http://www.cushing.org), and Internet self-help groups for hypoadrenal subjects (www.healinglight.com and http://groups.yahoo.com/group/Addisons_Disease).

REFERENCES