Original Article

Two Years of Treatment With Dehydroepiandrosterone Does Not Improve Insulin Secretion, Insulin Action, or Postprandial Glucose Turnover in Elderly Men or Women

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To determine if dehydroepiandrosterone (DHEA) replacement improves insulin secretion, insulin action, and/or postprandial glucose metabolism, 112 elderly subjects with relative DHEA deficiency ingested a labeled mixed meal and underwent a frequently sampled intravenous glucose tolerance test before and after 2 years of either DHEA or placebo. Despite restoring DHEA sulphate concentrations to values observed in young men and women, the changes over time in fasting and postprandial glucose concentrations, meal appearance, glucose disposal, and endogenous glucose production were identical to those observed after 2 years of placebo. The change over time in postmeal and intravenous glucose tolerance test insulin and C-peptide concentrations did not differ in men treated with DHEA or placebo. In contrast, postmeal and intravenous glucose tolerance test change over time in insulin and C-peptide concentrations were greater ($P < 0.05$) in women after DHEA than after placebo. However, since DHEA tended to decrease insulin action, the change over time in disposition indexes did not differ between DHEA- and placebo-treated women, indicating that the slight increase in insulin secretion was a compensatory response to a slight decrease in insulin action. We conclude that 2 years of replacement of DHEA in elderly men and women does not improve insulin secretion, insulin action, or the pattern of postprandial glucose metabolism. *Diabetes* 56:753–766, 2007

Plasma dehydroepiandrosterone (DHEA) concentrations and glucose tolerance both decrease with age (1–6). In addition, plasma DHEA concentrations have been reported to be inversely correlated with BMI, visceral fat, plasma insulin concentrations, and insulin action (1,7–10). Furthermore, treatment with DHEA increases glucose uptake in vitro and improves glucose tolerance in mice, decreases body fat in *fa/fa* rats, prevents diabetes in *ob/ob* mice, and enhances glucose-induced insulin secretion in Wistar rats (11–17). These observations have led to speculation that the age-related fall in DHEA concentrations either causes or exacerbates glucose intolerance and likely has contributed to the widespread empirical use of DHEA as a putative "anti-aging" drug.

Studies in humans examining the effects of DHEA on carbohydrate metabolism have been less convincing. Whereas DHEA replacement improves insulin action in individuals with absolute DHEA deficiency (18), it has been reported to improve (19–21), have no effect (22–25), or decrease (26) insulin action in subjects with intact adrenals. However, all of the above have studied a relatively small number of patients (i.e., less than 15 patients per group) for a relatively short period of time (i.e., ≤12 months). In addition, to our knowledge, no study has concurrently assessed the effect of DHEA replacement on insulin secretion and action, leaving open the question as to whether a change in one of the parameters observed in some studies is a primary effect of DHEA or merely represents a compensatory response to a change in the other.

We have recently reported that 24 months of DHEA replacement in physiological doses had no beneficial effects on quality of life, body composition, or physical performance in either elderly men or women (29). We also observed that DHEA replacement did not alter net insulin action measure with the unlabeled meal minimal model. The current studies extend those observations by concurrently assessing insulin action (measured using both the labeled and unlabeled "oral" and "intravenous" glucose minimal models) after meal ingestion and intravenous glucose injection and insulin secretion (measured using C-peptide–based models). Disposition indexes were calculated to determine if DHEA-induced changes in insulin secretion (if observed) were appropriate for the prevailing level of insulin action. Glucose turnover was measured using a triple-tracer approach to determine if DHEA altered postprandial glucose disposal, suppression of endogenous glucose production, and/or the rate of appearance of the ingested carbohydrate.

We report that 2 years of DHEA replacement in elderly DHEA-deficient men and women does not improve glucose tolerance, alter postprandial glucose turnover, increase insulin action, or enhance insulin secretion. These

TABLE 1
Subject characteristics

	Elderly men (placebo)	Elderly men (DHEA)	Elderly women (placebo)	Elderly women (DHEA)
n	29	28	29	27
Age (years)	67.1 ± 0.6	68.4 ± 0.6	70.4 ± 0.8	68.4 ± 0.6
BMI (kg/m^2)	27.4 ± 0.3	27.1 ± 0.5	27.9 ± 0.5	26.4 ± 0.5
Percent body fat	29.1 ± 0.6	26.0 ± 1.3	42.4 ± 0.8	42.3 ± 1.3
Lean body mass (kg)	62.2 ± 0.6	59.7 ± 0.9	39.7 ± 0.5	38.9 ± 0.6
$V_{O_{2max}}$ (ml · kg^{-1} · min^{-1})	40.4 ± 0.6	41.7 ± 1.2	37.6 ± 0.6	39.6 ± 1.1
DHEA-S (ng/ml)	0.67 (0.5–0.2)	0.63 (0.4–1.0)	0.32 (0.3–0.4)	0.38 (0.3–0.5)
Bioavailable estradiol (pg/ml)	9.2 (7.0–11.8)	8.3 (7.0–10.8)	2.8 (1.6–5.2)	2.6 (1.6–6.2)
Bioavailable testosterone (ng/dl)	52.8 (46.4–63.7)	62.3 (52.4–69.0)	NA	NA

Data are medians $\pm$ SE or medians (upper and lower interquartile range).

data argue strongly against a role of DHEA deficiency in the pathogenesis of the age-associated decline in glucose tolerance.

RESEARCH DESIGN AND METHODS

Experimental design. The study was conducted as a randomized placebo-controlled double-blind trial for 2 years. The study design and methods have previously been described in detail (27–29). In brief, men whose bioavailable testosterone (non-sex hormone–binding globulin bound) concentration was <103 ng/dl and DHEA sulphate (DHEA-S) concentration was <1.57 μg/dl and women who were not on hormonal replacement therapy and whose DHEA-S concentration was <0.95 μg/dl were eligible for study. These cutoffs represent the 15th percentile of levels for normal young men and women, respectively (5). All volunteers were in good general health, and subject characteristics are given in Table 1. The baseline data examining the effects of age and sex on insulin secretion, insulin action, and glucose metabolism in elderly and young men and women have previously been published (27,28). The effects of DHEA replacement on quality of life, body composition, physical performance, and net insulin action measured with the unlabeled meal minimal model also have been recently reported (29). To be able to directly compare concordance or discordance (if observed), data from all subjects for whom there were data available for both the meal and intravenous glucose tolerance test before and after 24 months of treatment are presented here. Specifically, the present article includes data from 27 of the 30 elderly women randomized to 50 mg per day DHEA, 29 of the 30 elderly women randomized to placebo, 28 of the 30 elderly men randomized to 75 mg per day DHEA, and 29 or 32 elderly men randomized to placebo. Of the men receiving DHEA or placebo, one each were lost to follow-up, whereas of the women on DHEA, three were lost to follow-up. Samples from the other subjects were not available because of technical problems encountered during the conduct of the studies. A third group of men also was given a testosterone patch as part of a separate but related experiment previously described (29).

All subjects consumed a weight maintenance diet (55% carbohydrate, 15% protein, and 30% fat) provided by the General Clinical Research Center kitchen for 3 days preceding study. Subjects were admitted at 1600 h on the afternoon before study and were given a standard 10 kcal/kg meal (55% carbohydrate, 15% protein, and 30% fat), which was consumed between 1700 and 1730 h. No additional food was eaten until the next morning. On one occasion, a mixed meal (10 kcal/kg, 45% carbohydrate, 15% protein, 40% fat) consisting of scrambled eggs, Canadian bacon, and [1-^{13}C]glucose Jell-O (containing 1.2 g per kg body wt of dextrose) was consumed within 15 min (27,28). An infusion of [6-^{3}H]glucose (1.2 μCi/ml; New England Nuclear, Boston, MA) was started at time 0, and the rate varied to mimic the anticipated rate of appearance of the [1-^{13}C]glucose contained within the meal (30). At the same time, the rate of infusion of [6,6-^{2}H$_2$]glucose was altered so as to approximate the anticipated pattern of fall in endogenous glucose production, thereby minimizing the change in plasma glucose enrichment (30). On another occasion, 0.33 g/kg glucose containing [6,6-^{2}H$_2$]glucose was injected at time 0 and 0.02 units/kg insulin at time 20 min (27). Arterialized venous blood was the collected at frequent intervals as previously described (27,28).

Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at −20°C until assay. Plasma glucose concentrations were measured using a glucose oxidase method (Yellow Spring Instruments, Yellow Springs, OH). Plasma insulin concentrations were measured using a chemilumines-cence assay with reagents obtained from Beckman (Access Assay; Beckman, Chaska, MN). Plasma C-peptide concentrations were measured by radioim-munoassay (Linco Research, St. Louis, MO). Interassay coefficient of variation

was 6.5% for the insulin assay and 10% for the C-peptide assay. Body composition was measured using dual-energy X-ray absorptiometry (DPX Scanner; Lunar Corporation, Madison, WI). Visceral fat was measured by a single-slice computerized tomographic scan at the level of L2/L3 (31). Peak oxygen uptake (V$_{O_{2max}}$) was measured using a standard treadmill stress test (32). Knee extensor strength was measured by having each subject lift a progressively higher weight using a bilateral leg press machine (Cybex, Medway, MA) until the one-repetition maximum was reached. Consecutive attempts were separated by 1 min of rest (33). Subjects were familiarized with the equipment and test procedures before data collection.

Calculations. The "oral" and "intravenous" glucose minimal models (34–36) were used to interpret plasma glucose and insulin concentrations measured after meal ingestion or glucose injection. The "oral" and "intravenous" models assume that insulin action on glucose production and disposal emanates from a compartment remote from plasma, which is usually identified with the interstitium. The most important parameters of the model are net insulin sensitivity (S_i), which measures the ability of insulin to stimulate glucose disposal and inhibit glucose production, and net glucose effectiveness (GE), which measures the ability of glucose per se to stimulate glucose disposal and inhibit glucose production. Similarly, the labeled "oral" and labeled "intrave-nous" glucose minimal models (37–39) were used to assess the selective effect of insulin (i.e., S_i^*) and glucose (GE*) on glucose disposal.

The "oral" and "intravenous" C-peptide minimal models (34,40,41), incor-porating age-associated changes in C-peptide kinetics, as measured by Van Cauter et al. (42), were used to interpret plasma glucose and C-peptide concentrations measured during the tests. The models assume that insulin secretion is made up of two components. The "oral" model assumes a dynamic component (Phi$_{dynamic}$) that defines the response to the rate of increase in glucose concentration and a static component (Phi$_{static}$) that evaluates the response to an increment in glucose above basal. Similarly, the "intravenous" model assumes a rapid component (Phi$_1$), which presumably represents release of previously docked insulin granules and is commonly referred to as first-phase insulin secretion, and a slower component (Phi$_2$), which represents the response to a given increment in glucose and is commonly referred to as second-phase insulin secretion. The overall β-cell response to glucose (Phi$_{total}$) is a composite of Phi$_{dynamic}$ and Phi$_{static}$ for the "oral" model and a composite of Phi$_1$ and Phi$_2$ for the "intravenous" model. The calculations also assume that neither DHEA nor placebo alters C-peptide clearance.

As previously suggested (43,44), the appropriateness of insulin secretion for the prevailing level of insulin resistance can be determined by calculating disposition indexes. "Oral" model disposition indexes (DI$_{dynamic}$, DI$_{static}$, and DI$_{total}$) were calculated by multiplying Phi$_{dynamic}$, Phi$_{static}$, and Phi$_{total}$, respec-tively, by net insulin action (S_i) determined with the "oral" model. Similarly, "intravenous" model disposition (DI$_1$, DI$_2$, and DI$_{total}$) are calculated by multiplying Phi$_1$, Phi$_2$, and Phi$_{total}$, respectively, by net insulin action (S_i) determined with the "intravenous" model. First-pass hepatic insulin extraction in the basal state and during the meal was determined by calculating insulin secretion using plasma C-peptide concentrations and the C-peptide minimal model and by calculating posthepatic delivery using plasma insulin concen-trations and the insulin minimal model (45).

The systemic rates of meal appearance, endogenous glucose production, and glucose disappearance were calculated using Radziuk's two-compartment model (46) by using the triple-tracer approach (30,47). In brief, rate of meal appearance, which measures the systemic rate of appearance of the ingested glucose that is not initially extracted by the splanchnic bed as it passes from gut to the hepatic vein, was calculated by multiplying the rate of appearance of [1-^{13}C]glucose (obtained from the infusion rate of [6-^{3}H]glucose and the clamped plasma ratio of [6-^{3}H]glucose and [1-^{13}C]glucose) by the meal

enrichment (i.e., the ratio of total glucose to tracer in the meal). Endogenous glucose production was calculated from the infusion rate of $[6,6\text{-}^2H_2]$glucose and the clamped plasma ratio of $[6,6\text{-}^2H_2]$glucose to endogenous glucose concentration. Glucose disappearance was calculated by subtracting the change in glucose mass from the overall rate of glucose appearance (i.e., meal appearance plus endogenous glucose production). As previously discussed in detail (30,47), this approach is virtually model independent, yielding essentially the same results when interpreted using steady-state or non–steady-state assumptions and either a one-compartment or two-compartment model.

Values from -30 to 0 min were averaged and considered as basal. Area above basal was calculated using the trapezoidal rule. Parameters of all models were estimated by using the SAAMII software (48). Measurement errors have been assumed to be independent and Gaussian, with zero mean and variance for glucose and tracer glucose as described by Dalla Man et al. (38) and for C-peptide as described by Toffolo et al. (45).

Statistical analysis. Data are presented as means $\pm$ SE as well as median and upper and lower interquartile ranges. Area above basal was calculated using the trapezoidal rule. The change from baseline (i.e., 24-month value minus baseline value) was compared in the DHEA and placebo groups using the Student's t test. A P value of <0.05 was considered to be statistically significant. Power calculations based on data from previous studies indicated that the mean and SD for glucose tolerance was 457 ± 58 mmol/l and that for the disposition index was 533 ± 67 10^{-4} dl $\cdot$ kg^{-1} $\cdot$ min^{-1} (2) per picomole per liter. To detect a 20% difference with power of 0.9 would require $n = 10$ in each group to reliably test both parameters. To avoid testing the same hypothesis multiple times, integrated responses and slopes were tested.

RESULTS

Plasma DHEA-S, estrogen and testosterone concentrations, and body composition. The effects of DHEA replacement on plasma hormone concentrations and body composition have been described in detail elsewhere (29). In brief, plasma DHEA-S concentrations were no different in elderly men and women treated for 2 years with either placebo or DHEA (0.67 vs. 0.63 ng/ml men and 0.32 vs. 0.38 ng/ml women; NS). DHEA replacement resulted in a delta increase ($P < 0.001$) in plasma estrogen concentrations in both the elderly men (19.8 pg/ml) and the elderly women (20.9 pg/ml). Two years of DHEA replacement did not alter BMI, visceral fat, percent body fat, or fat-free mass in the elderly men or elderly women. DHEA replacement also did not alter peak V_{O_2}, leg isometric knee extension, double leg press, or chest press (Table 1).

Plasma glucose, insulin, and C-peptide concentrations observed after meal ingestion. Fasting glucose concentrations in the elderly men did not differ before treatment in the DHEA and placebo groups (5.3 ± 0.1 vs. 5.3 ± 0.1 mmol/l). Four elderly men in the DHEA group and seven in the placebo group had impaired fasting glucose (100–125 mg/dl), and one subject in the placebo group had a fasting glucose of 135 mg/dl that was confirmed on a second occasion. Similarly, fasting glucose concentrations in the elderly women did not differ before treatment in the DHEA and placebo groups (5.0 ± 0.1 vs. 5.2 ± 0.1 mmol/l). Two elderly women in the DHEA group and four in the placebo group had impaired fasting glucose.

The change (i.e., 24 months minus baseline) from baseline of fasting concentration and the postprandial increments (i.e., area above basal) of glucose, insulin, and C-peptide concentrations in the elderly men did not differ after 2 years of treatment with DHEA or placebo (Fig. 1). The change from baseline of fasting and the postprandial increment of glucose also did not differ in elderly women after 2 years of treatment with DHEA or placebo (Fig. 2). Furthermore, the change from baseline in fasting insulin and C-peptide concentrations also did not differ between the DHEA and placebo groups of elderly women (Fig. 2). On the other hand, the postprandial increment in insulin

concentrations after 2 years of treatment with DHEA was slightly (but not significantly) greater than that observed at baseline (73.8 ± 7.8 vs. 61.3 ± 7.8 nmol/l per 6 h), whereas the postprandial increment in insulin after 2 years of treatment with placebo was slightly (but not significantly) lower than that observed at baseline (63.8 ± 7.0 vs. 65.6 ± 6.5 nmol/l per 6 h). This resulted in a significantly greater ($P < 0.05$) change from baseline of the postprandial increment in insulin after treatment with DHEA than after treatment with placebo.

A similar pattern was observed with C-peptide. The postprandial increment in C-peptide concentrations after 2 years of treatment with DHEA was slightly (but not significantly) greater than that observed at baseline (819 ± 75 vs. 733 ± 51 nmol/l per 6 h), whereas the postprandial increment in C-peptide after 2 years of treatment with placebo was slightly (but not significantly) lower than that observed at baseline (652 ± 34 vs. 662 ± 37 nmol/l per 6 h). This resulted in a significantly greater ($P < 0.05$) change from baseline in the postprandial increment after treatment of the elderly women with DHEA than after treatment with placebo.

Plasma glucagon, growth hormone, and cortisol concentrations observed after meal ingestion. The change (i.e., 24 months minus baseline) from baseline of fasting concentration and the postprandial increments (i.e., area above basal) of glucagon, growth hormone, and cortisol concentrations in the elderly men did not differ after 2 years of treatment with DHEA or placebo (Fig. 3). In addition, the change from baseline of fasting and the postprandial increment of glucagon, growth hormone, and cortisol concentrations also did not differ in the elderly women after 2 years of treatment with DHEA or placebo (Fig. 4).

Meal rate of appearance, endogenous glucose production, and glucose disappearance observed after meal ingestion. The changes from baseline in fasting rates of endogenous glucose production and glucose disappearance did not differ after 2 years of treatment with DHEA from those observed after 2 years of treatment with placebo in either the elderly men or women (Figs. 5 and 6). The change from baseline in the postprandial decrement in endogenous glucose production and the postprandial increment in glucose disappearance also did not differ after 2 years of treatment with DHEA or placebo.

Plasma glucose, insulin, and C-peptide concentrations observed after intravenous injection of glucose. The change from baseline of fasting and post-intravenous glucose increments in glucose, insulin, and C-peptide concentrations did not differ in the elderly men (Fig. 5) or elderly women (Fig. 6) after 2 years of treatment with DHEA or placebo (Figs. 7 and 8).

Insulin action, glucose effectiveness, insulin secretion, and disposition indexes observed after meal ingestion or intravenous glucose injection. The change from baseline in net insulin action (S_i) measured with either the unlabeled "oral" or unlabeled "intravenous" glucose minimal models did not differ in the elderly men or women after 2 years of treatment with DHEA or placebo (Table 2).

The change from baseline in the ability of insulin to stimulate glucose uptake (S_i^*) measured with either the labeled "oral" or labeled "intravenous" glucose "minimal" models also did not differ in the elderly men or women after 2 years of treatment with DHEA or placebo.

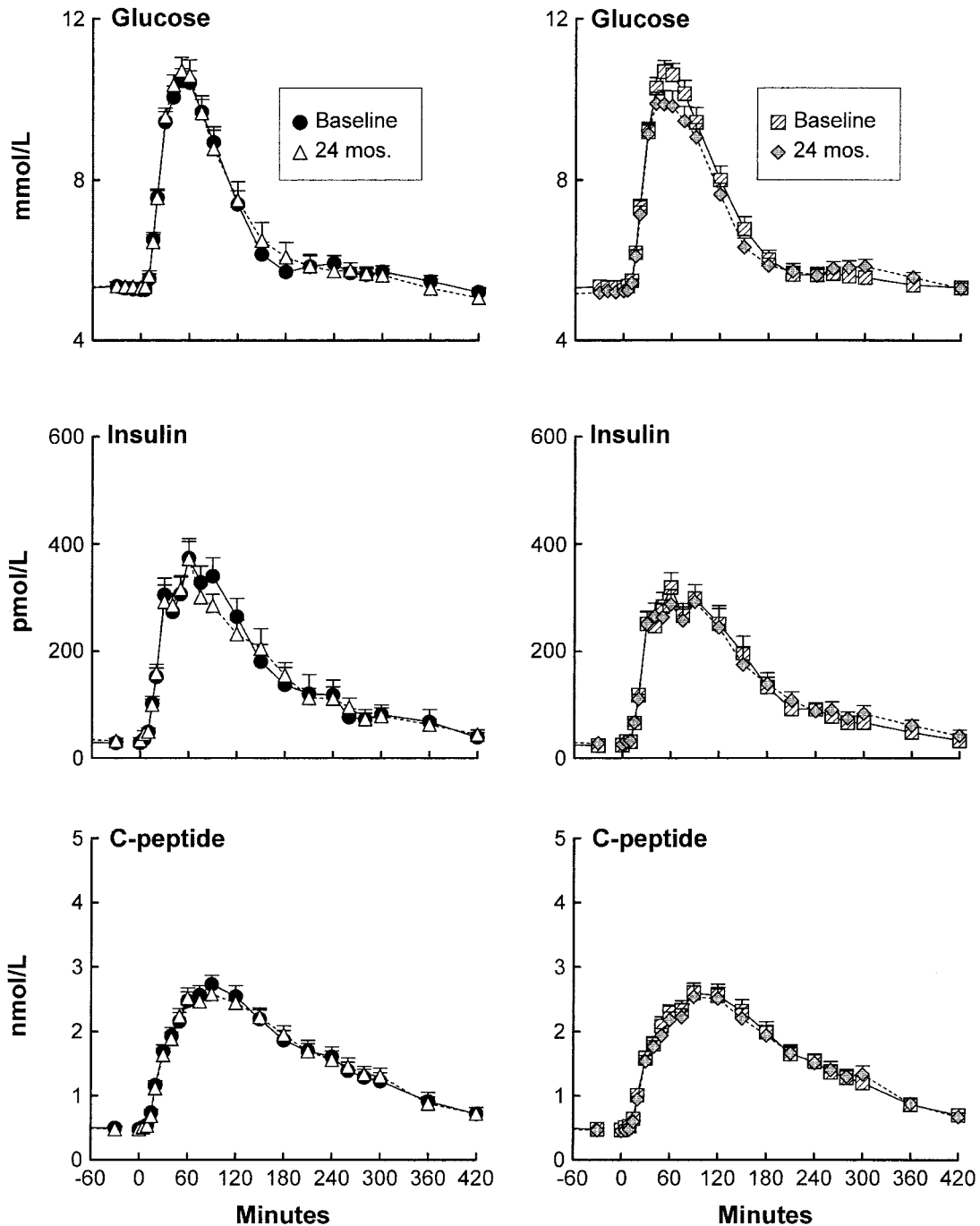The change from baseline in net GE and the ability of

FIG. 1. Plasma glucose, insulin, and C-peptide concentrations observed in elderly men after meal ingestion before (baseline) and after 2 years of treatment with either placebo or DHEA.

glucose to stimulate its own uptake (GE*) measured with either the unlabeled and labeled "intravenous" glucose models did not differ in the elderly men or women after 2 years of treatment with DHEA or placebo.

The change from baseline in "oral" indexes of insulin secretion including $Phi_{dynamic}$, Phi_{static}, and Phi_{total} did not differ in the elderly men or women after 2 years of treatment with DHEA from those observed after 2 years of treatment with placebo. The change from baseline in insulin secretion indexes calculated with the "intravenous" minimal model during the intravenous glucose tolerance test in the elderly men also did not differ in the DHEA and placebo groups. On the other hand, the change from

baseline in Phi_2 and Phi_{total} (but not Phi_1) calculated with the "intravenous" minimal model was greater ($P < 0.05$) in the elderly women after 2 years of treatment with DHEA than after 2 years of treatment with placebo. However, since insulin action tended to decrease in the elderly women treated with DHEA, the change from baseline in the disposition indexes measured during the intravenous glucose tolerance test did not differ in the DHEA and placebo groups, indicating that the small increase in Phi_{static} and Phi_{total} in the elderly women on DHEA was an appropriate compensatory response for the small decrease in insulin action. Similarly, the change in disposition indexes from baseline calculated with the "oral" minimal

TABLE 2
Meal and intravenous glucose tolerance test indexes of insulin action and secretion

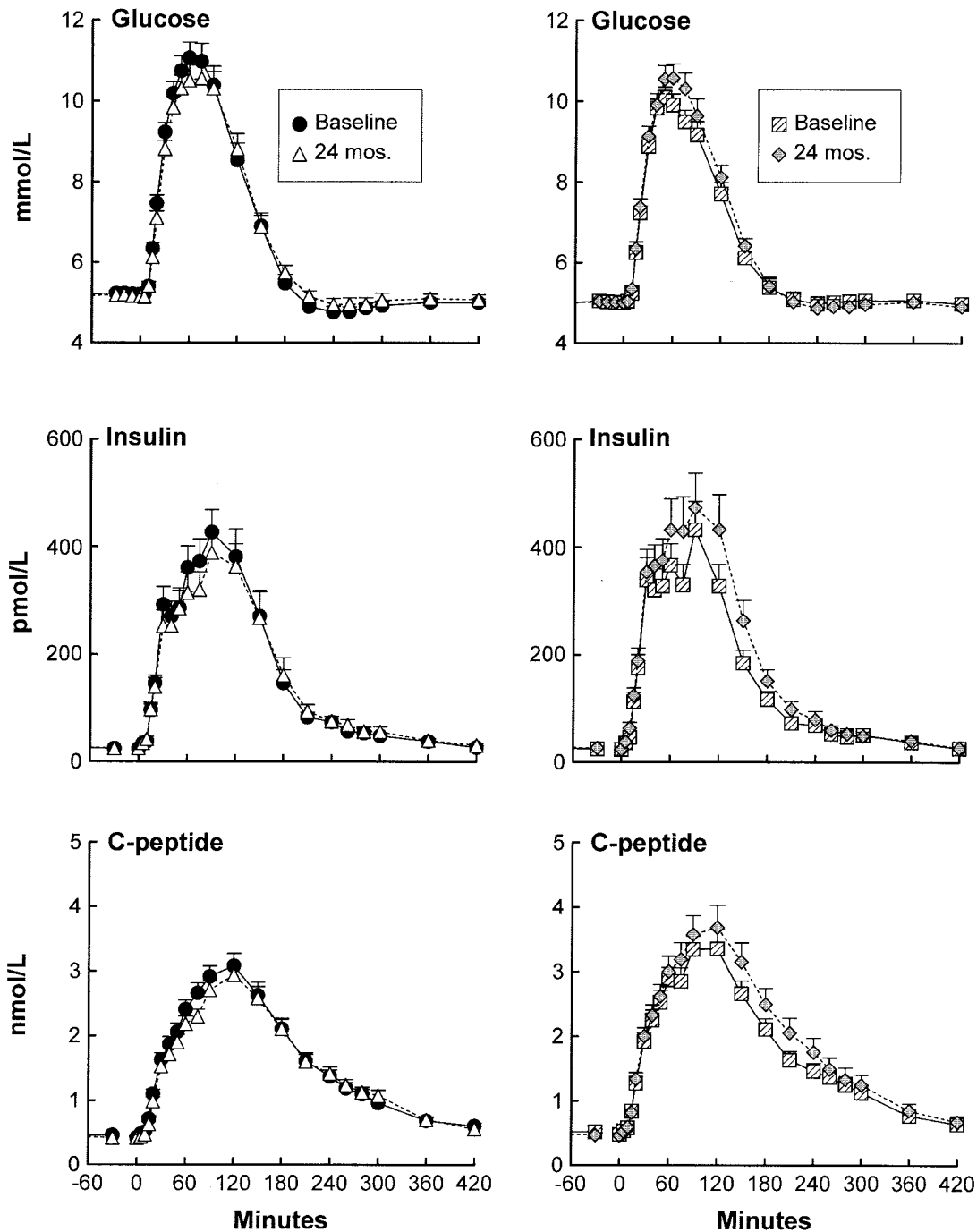|	S_I (10^{-4} dl/kg/min per μU/ml)	Phi$_{dynamic}$ (10^{-9})	Phi$_{static}$ (10^{-9} min^{-1})	Phi$_{total}$ (10^{-9} min^{-1})	DI (10^{-14} dl/kg per min^2 per pmol/l)	GE (dl/kg per min)	S_I^* (10^{-4} dl/kg/min per μU/ml)	GE* (dl/kg per min)	Hepatic extraction of insulin
Meal indexes									
Women									
Placebo									
Pre-	12.5 ± 1.6	444.7 ± 53.2	32.7 ± 1.9	36.7 ± 2.2	712.2 ± 85.9	0.03 ± 0.0	7.6 ± 1.2	0.02 ± 0.0	0.43 ± 0.03
Post-	12.7 ± 1.6	442.2 ± 49.1	31.4 ± 2.0	35.7 ± 2.7	735.8 ± 130.1	0.04 ± 0.0	8.3 ± 1.2	0.02 ± 0.0	0.39 ± 0.03
Delta	0.2 ± 1.0	−2.5 ± 41.1	−1.3 ± 2.2	−1.0 ± 2.8	23.6 ± 90.3	0.01 ± 0.0	0.7 ± 0.9	0.00 ± 0.0	0.04 ± 0.03
DHEA-S									
Pre-	12.8 ± 1.9	553.5 ± 61.5	38.1 ± 3.2	42.8 ± 3.6	778.9 ± 87.8	0.04 ± 0.0	8.7 ± 1.6	0.02 ± 0.0	0.45 ± 0.02
Post-	10.9 ± 1.6	557.0 ± 62.4	38.7 ± 2.9	43.4 ± 3.0	697.9 ± 70.8	0.04 ± 0.0	7.0 ± 1.2	0.02 ± 0.0	0.39 ± 0.03
Delta	−1.9 ± 1.2	3.5 ± 64.9	0.6 ± 1.7	0.6 ± 1.9	−81.0 ± 58.9	0.00 ± 0.0	−1.7 ± 1.0	0.02 ± 0.0	0.06 ± 0.03
Men									
Placebo									
Pre-	11.7 ± 1.8	419.2 ± 28.6	34.1 ± 1.9	37.7 ± 2.1	685.0 ± 87.1	0.04 ± 0.0	9.6 ± 1.3	0.02 ± 0.0	0.39 ± 0.04
Post-	11.9 ± 1.4	411.9 ± 44.5	33.1 ± 2.2	36.6 ± 2.5	672.6 ± 67.0	0.04 ± 0.0	9.0 ± 1.3	0.02 ± 0.0	0.37 ± 0.03
Delta	0.2 ± 1.5	−7.3 ± 34.4	−1.0 ± 1.5	−1.1 ± 1.6	−12.4 ± 78.6	0.00 ± 0.0	−0.6 ± 1.0	0.00 ± 0.0	0.02 ± 0.03
DHEA-S									
Pre-	13.5 ± 1.4	476.3 ± 51.0	29.7 ± 1.8	33.1 ± 2.0	685.3 ± 62.7	0.04 ± 0.0	10.0 ± 1.3	0.02 ± 0.0	0.44 ± 0.03
Post-	13.6 ± 1.6	448.0 ± 44.4	30.3 ± 2.1	33.5 ± 2.3	677.0 ± 63.1	0.04 ± 0.0	10.9 ± 1.7	0.02 ± 0.0	0.44 ± 0.02
Delta	0.1 ± 1.4	−28.3 ± 43.3	0.6 ± 1.6	0.3 ± 1.7	−8.3 ± 70.1	0.00 ± 0.0	0.9 ± 1.5	0.02 ± 0.0	0.00 ± 0.00
IVGTT indexes									
Women									
Placebo									
Pre-	6.6 ± 0.9	104.8 ± 9.1	8.9 ± 0.5	14.3 ± 0.9	148.8 ± 18.8	0.03 ± 0.0	6.0 ± 0.9	0.02 ± 0.0	0.62 ± 0.02
Post-	5.7 ± 0.7	106.3 ± 10.0	8.3 ± 0.6	13.2 ± 0.8	122.3 ± 15.5	0.03 ± 0.0	7.7 ± 1.1	0.02 ± 0.0	0.54 ± 0.02
Delta	−0.9 ± 0.6	1.5 ± 9.5	−0.6 ± 0.4	−1.1 ± 0.6	−26.5 ± 15.4	0.00 ± 0.0	1.7 ± 1.2	0.00 ± 0.0	0.08 ± 0.02
DHEA-S									
Pre-	6.4 ± 0.9	143.0 ± 12.1	10.0 ± 0.8	16.9 ± 1.2	166.3 ± 24.1	0.03 ± 0.0	6.0 ± 0.8	0.02 ± 0.0	0.60 ± 0.02
Post-	5.4 ± 1.0	160.2 ± 25.6	11.0 ± 0.8	17.8 ± 1.4	140.8 ± 18.4	0.03 ± 0.0	7.2 ± 1.8	0.02 ± 0.0	0.60 ± 0.02
Delta	−1.0 ± 0.6	17.2 ± 21.8	1.0 ± 0.8	0.9 ± 1.3	−25.5 ± 22.2	0.00 ± 0.0	1.2 ± 1.5	0.00 ± 0.0	0.00 ± 0.02
Men									
Placebo									
Pre-	5.8 ± 0.8	130.3 ± 15.1	10.3 ± 0.8	16.6 ± 1.3	153.3 ± 19.8	0.03 ± 0.0	6.7 ± 1.1	0.02 ± 0.0	0.53 ± 0.03
Post-	5.5 ± 0.8	135.5 ± 11.7	10.3 ± 0.7	17.2 ± 1.2	150.1 ± 20.2	0.03 ± 0.0	7.6 ± 1.2	0.02 ± 0.0	0.49 ± 0.02
Delta	−0.3 ± 0.4	5.2 ± 9.5	0.0 ± 0.6	0.6 ± 0.8	−3.2 ± 10.6	0.00 ± 0.0	0.9 ± 0.5	0.00 ± 0.0	0.04 ± 0.02
DHEA-S									
Pre-	6.7 ± 0.9	137.8 ± 14.6	10.7 ± 1.1	19.6 ± 1.7	190.3 ± 25.7	0.03 ± 0.0	7.4 ± 1.1	0.02 ± 0.0	0.59 ± 0.03
Post-	5.7 ± 0.9	132.0 ± 10.7	11.0 ± 0.8	17.8 ± 1.1	143.0 ± 19.7	0.03 ± 0.0	7.5 ± 0.9	0.02 ± 0.0	0.54 ± 0.02
Delta	−1.0 ± 0.6	−5.8 ± 10.4	0.3 ± 0.7	−1.8 ± 1.3	−47.3 ± 22.4	0.00 ± 0.0	0.1 ± 0.6	0.00 ± 0.0	0.05 ± 0.02

FIG. 2. Plasma glucose, insulin, and C-peptide concentrations observed in elderly women after meal ingestion before (baseline) and after 2 years of treatment with either placebo or DHEA.

model also did not differ in the elderly women treated with DHEA from those observed in the elderly women treated with placebo.

Hepatic insulin extraction. The change from baseline in hepatic insulin extraction after meal ingestion did not differ in elderly women or men after 2 years of treatment with either placebo or DHEA (Table 2).

DISCUSSION

Billions of dollars are spent each year by people who take DHEA supplements in hope of preventing many of the biological consequences of aging. Speculation that a de-

cline in DHEA-S concentrations causes and/or exacerbates age-associated deterioration in glucose tolerance likely has contributed to DHEA's popularity. However, the present data argue strongly against the use of DHEA for this purpose, since they establish that treatment of elderly men and women with DHEA for 2 years does not improve carbohydrate tolerance. DHEA replacement did not alter either fasting or postprandial glucose concentrations or fasting or postprandial glucose turnover. Furthermore, DHEA replacement did not improve insulin action nor did it enhance insulin secretion. While DHEA replacement resulted in slightly higher insulin and C-peptide concen-
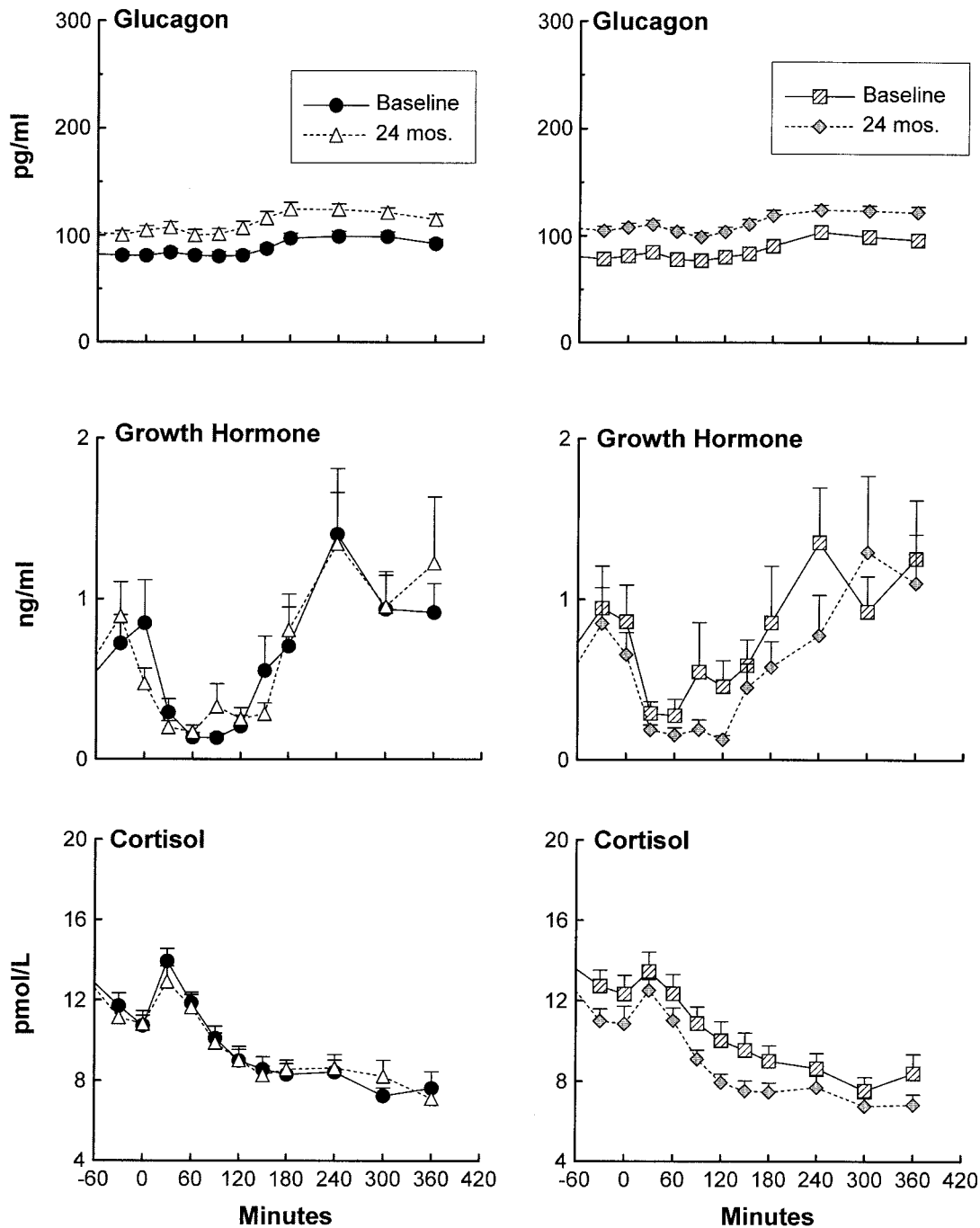
FIG. 3. Plasma glucagon, growth hormone, and cortisol concentrations observed in elderly men after meal ingestion before (baseline) and after 2 years of treatment with either placebo or DHEA.

trations after meal ingestion and a slight increase in second-phase insulin secretion after intravenous injection of glucose in elderly women, in both instances, disposition indexes remained unchanged, indicating that these subtle increases in insulin secretion were a compensatory response to an equally subtle decrease in insulin action. Taken together, these data provide no evidence that DHEA deficiency contributes to the carbohydrate intolerance of aging.

Glucose concentration increases when glucose appearance exceeds glucose disappearance. In the fasting state, glucose appearance is primarily due to release of glucose by the liver (49). However, the situation changes after

eating, when glucose appearance equals the sum of the rate of appearance of the glucose contained in the meal plus the rate of release of glucose by the liver (49). At least in theory, treatment with DHEA could alter postprandial glucose turnover in the absence of a change in glucose concentration if DHEA had offsetting effects on glucose appearance and disappearance. The present data provide no evidence of such an effect. The pattern of change in meal appearance, endogenous glucose production, and glucose disappearance were virtually identical after 2 years of treatment with DHEA or placebo. Pre- and postprandial glucose concentrations also were superimposable in the DHEA and placebo groups. When compared
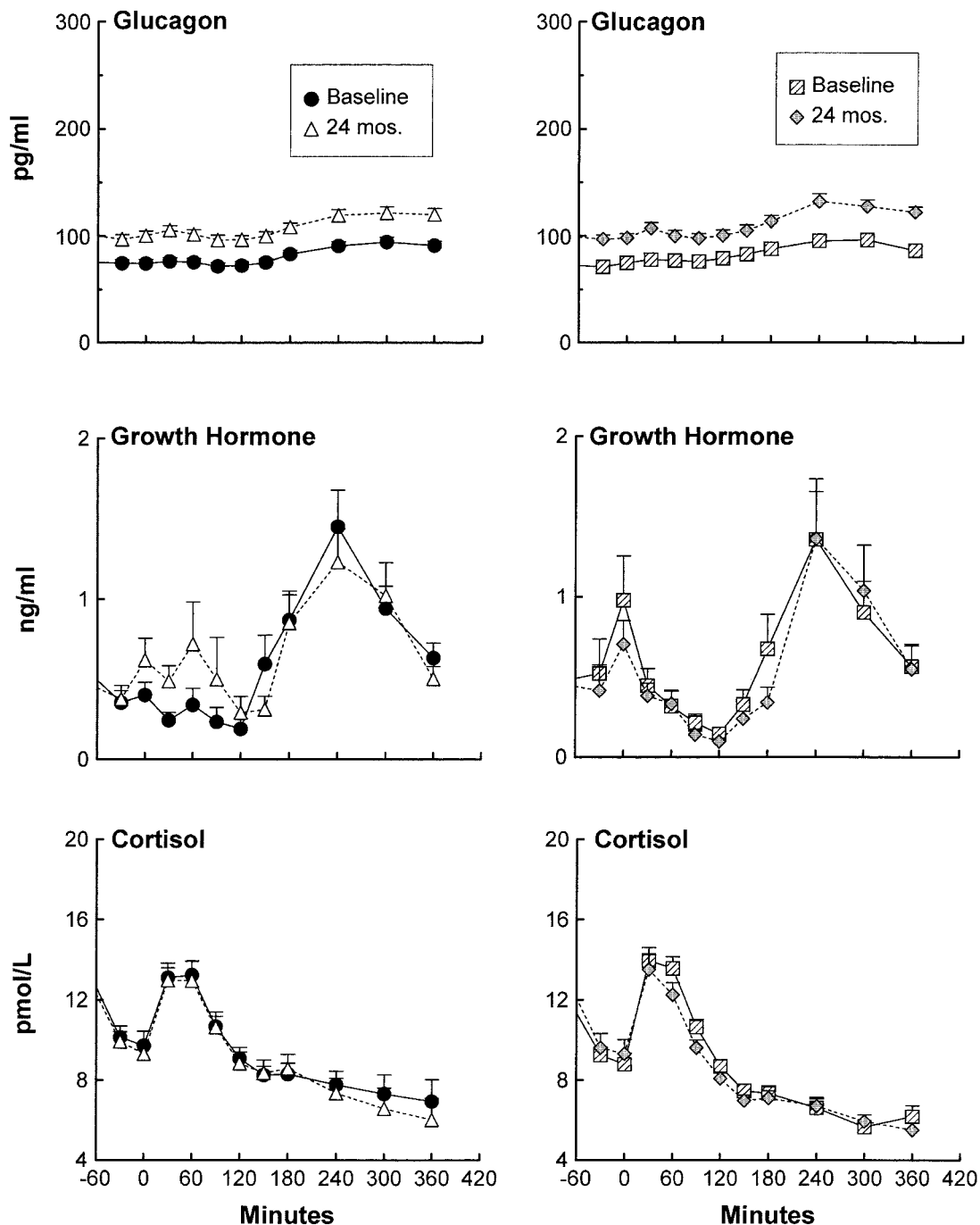
FIG. 4. Plasma glucagon, growth hormone, and cortisol concentrations observed in elderly women after meal ingestion before (baseline) and after 2 years of treatment with either placebo or DHEA.

with young individuals of the same sex, the cause of postprandial hyperglycemia differs in elderly men and women, with lower rates of disposal being the primary cause in the former and higher rates of meal appearance in the latter (28). Of note, there was no suggestion that treatment with DHEA had any effect on either of these parameters. Therefore, these data provide no evidence that treatment with DHEA improves either glucose tolerance or alters the pattern of postprandial glucose turnover in elderly men or women.

In vitro and animal experiments suggest that DHEA can improve insulin action (11–17). On the other hand, studies in humans have been less convincing, perhaps because

many used indirect methods to assess insulin action (19,26) or involved young or middle-aged subjects who did not have documented DHEA deficiency (19,20,22,23,25, 26,50). Of note, the studies of Lasco et al. (21), Mortola and Yen (26), and Villareal and Holloszy (19) perhaps most closely resemble the present experiments. Lasco et al. (21) insulin action measured with a euglycemic clamp was higher in 10 postmenopausal women (age ~58 years) treated with 25 mg DHEA for 12 months than in 10 age-matched women treated with placebo. On the other hand, Mortola and Yen (26) reported that treatment of six middle-aged to elderly women (ages 46–61 years) with 1,600 mg DHEA for 28 days resulted in no change in
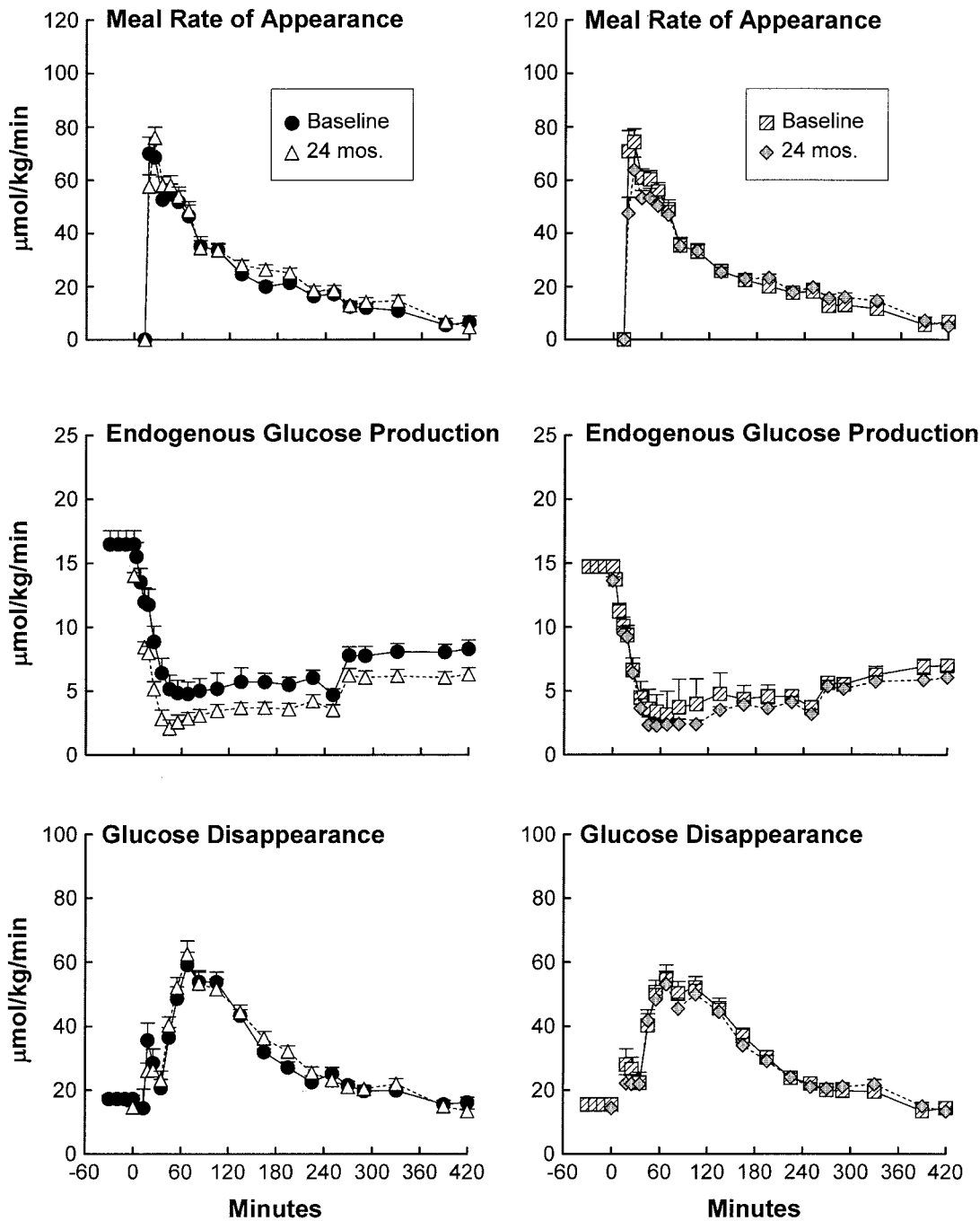
FIG. 5. Meal appearance, endogenous glucose production, and glucose disappearance observed in elderly men after meal ingestion before (baseline) and after 2 years of treatment with either placebo or DHEA.

glucose concentration but higher insulin concentrations measured during an oral glucose tolerance test, implying a decrease in insulin action. In contrast, Villareal and Holloszy (19) reported that whereas treatment of 14 elderly women (age 71 years) and 14 elderly men (age 72 years) with 50 mg/day DHEA for 1 year did not alter glucose concentrations measured during an oral glucose tolerance test, it resulted in lower insulin concentrations than those observed in matched women and men treated with placebo, implying either an increase in insulin action or an increase in hepatic insulin extraction.

The present experiments sought to clarify these conflicting findings. In an effort to do so, we directly measured

insulin action in elderly men and women after 2 years of treatment with either DHEA or placebo. As previously reported (29), net insulin action measured with the "oral" minimal model did not differ in the DHEA and placebo groups. The present data extend this observation by demonstrating that net insulin action measured in the same individuals with the "intravenous" minimal model also did not differ and that the ability of insulin to stimulate glucose disposal measured with the labeled "oral" and labeled "intravenous" minimal models also did not differ in the DHEA and placebo groups. The lack of an effect of DHEA on insulin action is compelling, since a large number of subjects were studied using two different
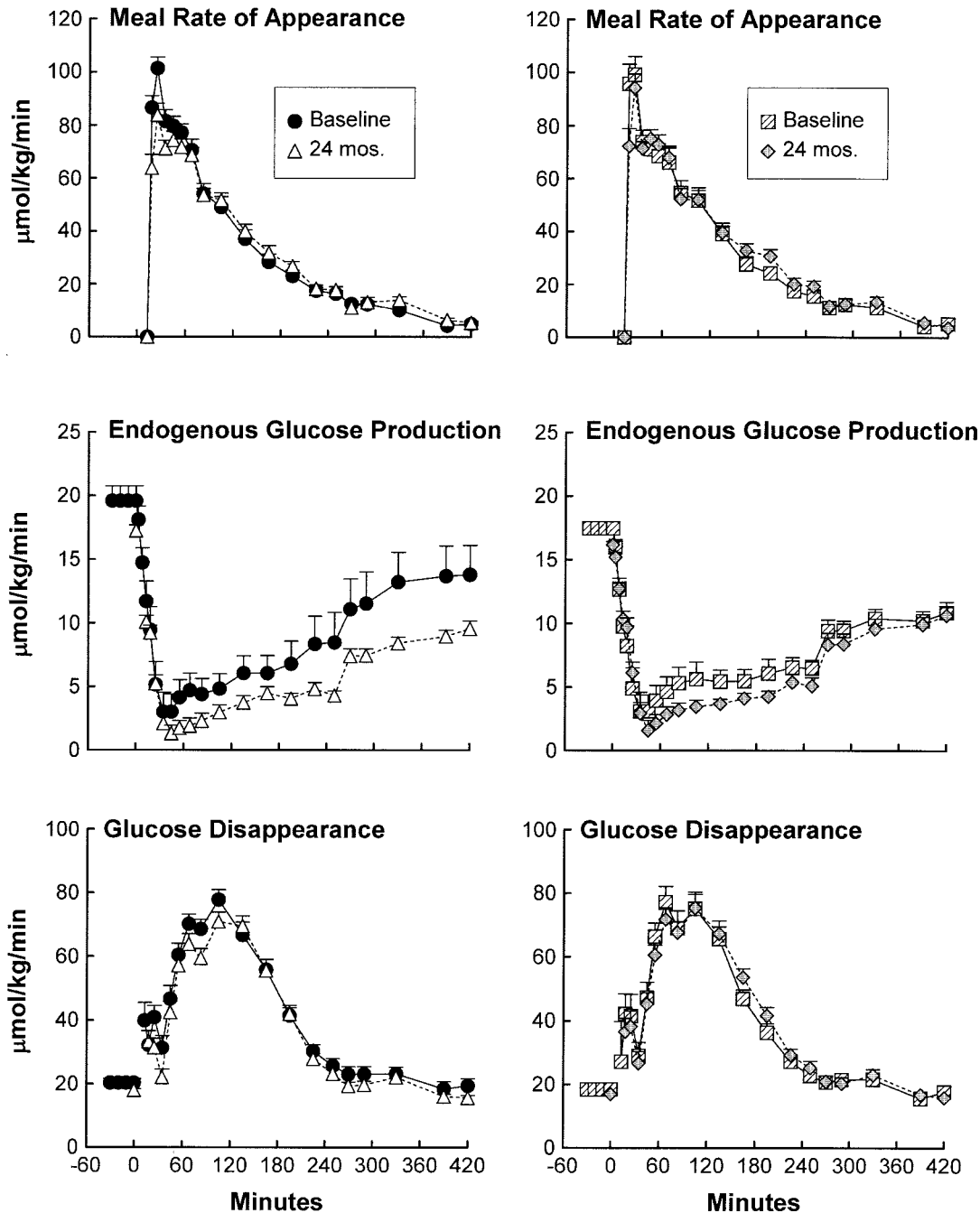
FIG. 6. Meal appearance, endogenous glucose production, and glucose disappearance observed in elderly women after meal ingestion before (baseline) and after 2 years of treatment with either placebo or DHEA.

well-validated methods of measuring insulin action (34–39). In addition, the labeled and unlabeled models provide independent assessments of insulin action. Thus, whereas previous studies suggest that short-term DHEA replacement may worsen, have no effect on, or improve insulin action in some elderly subjects (19–26), the present data indicate that such effects are either minimal or transient, since 2 years of DHEA replacement had no detectible effect on insulin action in either elderly men or women.

We are unaware of any studies that have directly measured the effects of DHEA replacement on insulin secretion in humans. However, studies in rats and in isolated islets have shown that DHEA can enhance glucose-induced insulin secretion (11,12,16). The present studies

used C-peptide–based models to assess glucose-induced insulin secretion both in response to intravenous glucose and after ingestion of a mixed meal when incretins and other nutrients are present. Using these approaches, there was no evidence that DHEA replacement enhanced insulin secretion in elderly men. The situation was somewhat more complex in the elderly women. Plasma C-peptide concentrations after meal ingestion after 2 years of DHEA replacement were slightly higher than baseline values measured before randomization, whereas they were slightly lower than baseline after 2 years of treatment with placebo. This resulted in a significantly greater change from baseline in the DHEA group. On the other hand, DHEA had no effect on indexes of insulin secretion
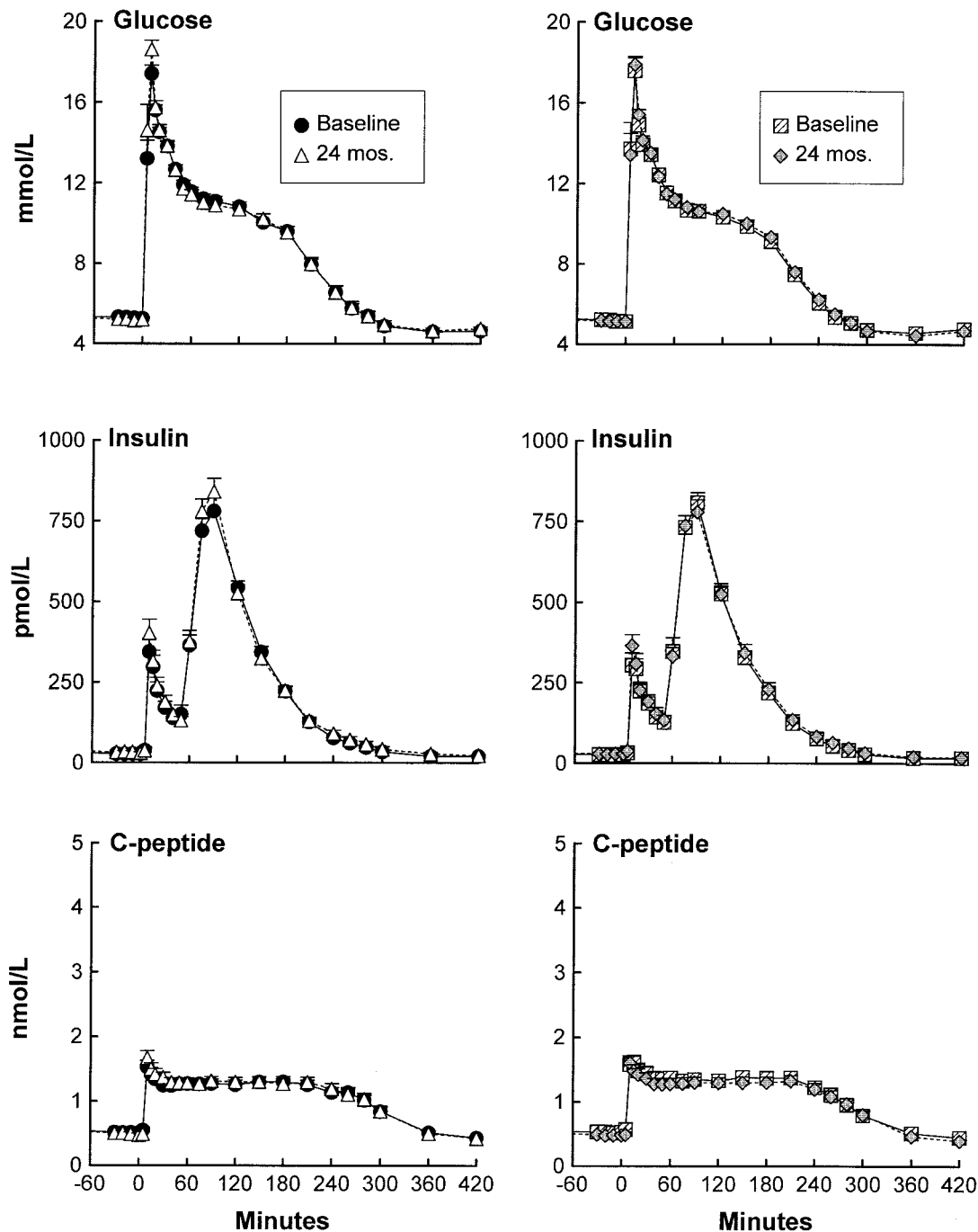
FIG. 7. Plasma glucose, insulin, and C-peptide concentrations in observed elderly men after intravenous glucose injection at time 0 and insulin injection at time 20 min before (baseline) and after 2 years of treatment with either placebo or DHEA.

measured with the "oral" C-peptide model. Conversely, both the change from baseline and actual plasma glucose and C-peptide concentrations were virtually identical in the DHEA and placebo groups after intravenous glucose injection. However, the "intravenous" C-peptide model suggested a small but significant increase in second-phase insulin secretion (i.e., Phi_2) after DHEA treatment. Perhaps most importantly, when the appropriateness of insulin secretion for the prevailing level of insulin action was assessed by calculating disposition indexes, there was no hint of an effect of DHEA replacement on insulin secretion after either meal ingestion or intravenous glucose injection. Furthermore, treatment with DHEA had no effect on

hepatic insulin extraction. Thus, rather than directly enhancing β-cell function, the slight but nonsignificant decrease in insulin action that occurred during DHEA replacement in the elderly women appears to have been offset by an appropriate compensatory increase in insulin secretion. Thus, there was no evidence of an independent effect of DHEA on insulin secretion in either the elderly men or women.

The present studies suffer from certain limitations. The elderly men and women had to be in good health to be eligible for the study. In addition, very few of the elderly subjects were overtly obese. Therefore, we cannot exclude the possibility that DHEA replacement may be of value in
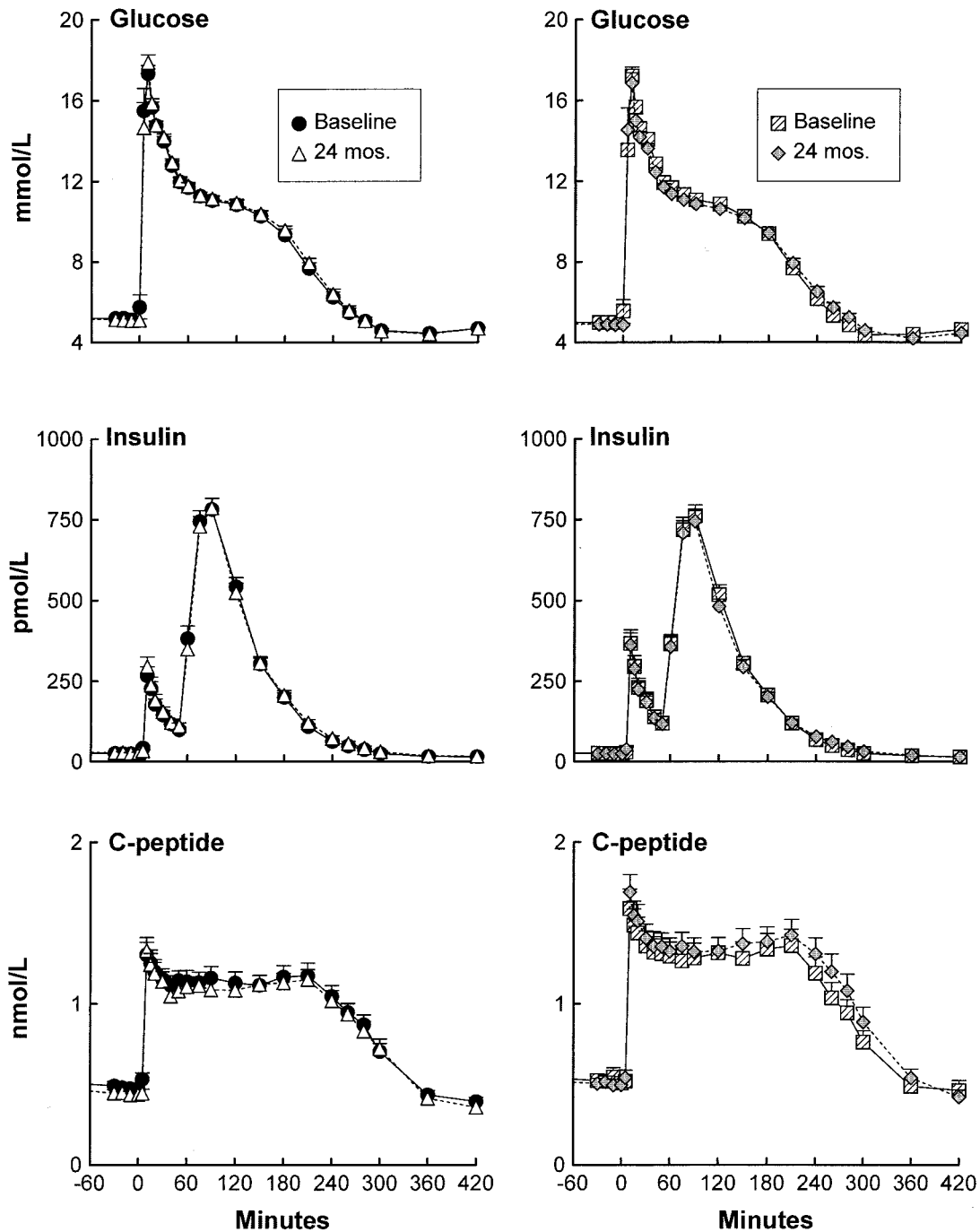
FIG. 8. Plasma glucose, insulin, and C-peptide concentrations observed in elderly women after intravenous glucose injection at time 0 and insulin injection at time 20 min before (baseline) and after 2 years of treatment with either placebo or DHEA.

elderly subjects who either have other diseases or are obese. The elderly subjects had relative (i.e., plasma DHEA concentrations less than the 15th percentile of those observed in healthy young subjects) rather than absolute DHEA deficiency. More marked effects of DHEA replacement on carbohydrate metabolism are likely to be observed in elderly individuals with absolute DHEA deficiency (e.g., postmenopausal women who have had an adrenalectomy). The subjects were studied before and after 2 years of DHEA replacement. Therefore, it is possible that DHEA exerted a short-term effect on carbohydrate metabolism that waned over time. If so, this presumably would limit the long-term clinical utility of DHEA replace-

ment. Similarly, we cannot rule out the possibility that an effect of DHEA replacement would have been observed if we had given it for longer than 2 years. We doubt this is the case, since in vitro effects of DHEA are detectible within hours (13,14,17) and when observed in vivo in animals occur after days to weeks of treatment rather than years (11,12,15,16). Although the numbers were small, there was no evidence that subjects with baseline fasting glucose concentrations <100 mg/dl responded any differently than individuals whose fasting glucose concentration was >100 mg/dl. Finally, as part of a parallel study, the elderly men also had low testosterone concentrations (29). It is therefore possible that DHEA replacement may be more effec-

tive in elderly men with isolated DHEA deficiency. However, since DHEA and bioavailable testosterone concentrations both decrease with age, isolated DHEA deficiency is likely to be the exception rather than the rule.

In summary, 2 years of treatment of elderly DHEA-deficient men and women with DHEA in amounts sufficient to restore plasma concentrations to those present in healthy young individuals has no effect on insulin secretion, insulin action, hepatic insulin extraction, postprandial glucose concentrations, or postprandial glucose turnover. These data strongly argue against a role of DHEA deficiency in the pathogenesis of age-related deterioration in glucose tolerance. They also provide further evidence that DHEA has little or no value as an anti-aging drug in elderly subjects and therefore should not be used for this purpose.

REFERENCES

1. Denti L, Pasolini G, Sanfelici L, Ablondi F, Freddi M, Benedetti R, Valenti G: Effects of aging on dehydroepiandrosterone sulfate in relation to fasting insulin levels and body composition assessed by bioimpedance analysis. *Metabolism* 46:826–832, 1997
2. Field AE, Colditz GA, Willett WC, Longcope C, McKinlay JB: The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. *J Clin Endocrinol Metab* 79:1310–1316, 1994
3. Orentreich N, Brind JL, Rizer RL, Vogelman JH: Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J Clin Endocrinol Metab* 59:551–555, 1984
4. Orentreich N, Brind JL, Vogelman JH, Andres R, Baldwin H: Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. *J Clin Endocrinol Metab* 75:1002–1004, 1992
5. Barrett-Connor E, Khaw K, Yen SSC: A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl J Med* 315:1519–1524, 1986
6. Chen M, Bergman RN, Pacini G, Porte D Jr: Pathogenesis of age-related glucose intolerance in man: insulin resistance and decreased β-cell function. *J Clin Endocrinol Metab* 60:13–20, 1985
7. Haffner SM, Valdez RA, Mykkänen L, Stern MP, Katz MS: Decreased testosterone and dehydroepiandrosterone sulfate concentrations are associated with increased insulin and glucose concentrations in nondiabetic men. *Metabolism* 43:599–603, 1994
8. Piédrola G, Novo E, Serrano-Gotarredona J, de Teresa ML, Escobar-Jiménez F, Garcia-Robles R: Relationship between insulin sensitivity and dehydroepiandrosterone sulfate in patients with ischemic heart disease. *Horm Metab Res* 29:566–571, 1997
9. Herranz L, Megia A, Grande C, Gonzalez-Gancedo P, Pallardo F: Dehydroepiandrosterone sulphate, body fat distribution and insulin in obese men. *Int J Obes* 19:57–60, 1995
10. De Pergola G, Triggiani V, Giorgino F, Cospite MR, Garruti G, Cignaraelli M, Guastamacchia E, Giorgino R: The free testosterone to dehydroepiandrosterone sulphate molar ratio as a marker of visceral fat accumulation in premenopausal obese women. *Int J Obes* 18:659–664, 1994
11. Gansler TS, Mueller S, Cleary MP: Chronic administration of dehydroepiandrosterone reduces pancreatic β-cell hyperplasia and hyperinsulinemia in genetically obese Zucker rats. *Proc Soc Exp Biol Med* 180:155–162, 1985
12. Cleary MP, Zabel T, Sartin JL: Effects of short-term dehydroepiandrosterone treatment on serum and pancreatic insulin in Zucker rats. *J Nutr* 118:382–387, 1988
13. Nakashima N, Haji M, Umeda F, Nawata H: Effect of dehydroepiandrosterone on glucose uptake in cultured rat myoblasts. *Horm Metab Res* 27:491–494, 1995
14. Nakashima N, Haji M, Sakai Y, Ono Y, Umeda F, Nawata H: Effect of dehydroepiandrosterone on glucose uptake in cultured human fibroblasts. *Metabolism* 44:543–548, 1995
15. Ishizuka T, Kajita K, Kiura A, Ishizawa M, Kanoh Y, Itaya S, Kimura M, Muto N, Mune T, Morita H, Yasuda K: DHEA improves glucose uptake via activations of protein kinase C and phosphatidylinositol 3-kinase. *Am J Physiol Endocrinol Metab* 276:E196–E204, 1999
16. Dillon JS, Yaney GC, Zhou Y, Voilley N, Bowen S, Chipkin S, Bliss CR, Schuit FC, Prentki M, Waxman DJ, Corkey BE: Dehydroepiandrosterone sulfate and β-cell function: enhanced glucose-induced secretion and altered gene expression in rodent pancreatic β-cells. *Diabetes* 49:2012–2020, 2000
17. Perrini S, Natalicchio A, Laviola L, Belsanti G, Montrone C, Cignarelli A, Minielli V, Grano M, De Pergola G, Giorgino R, Giorgino F: Dehydroepiandrosterone stimulates glucose uptake in human and murine adipocytes by inducing GLUT1 and GLUT4 translocation to the plasma membrane. *Diabetes* 53:41–52, 2004
18. Dhatariya K, Bigelow ML, Nair KS: Effect of dehydroepiandrosterone replacement on insulin sensitivity and lipids in hypoadrenal women. *Diabetes* 54:765–769, 2005
19. Villareal DT, Holloszy JO: Effect of DHEA on abdominal fat and insulin action in elderly women and men: a randomized controlled trial. *JAMA* 292:2243–2248, 2004
20. Kawano H, Yasue H, Kitagawa A, Hirai N, Toshida T, Soejima H, Miyamoto S, Nakano M, Ogawa H: Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. *J Clin Endocrinol Metab* 88:3190–3195, 2003
21. Lasco A, Frisina N, Morabito N, Gaudio A, Morini E, Trifiletti A, Basile G, Nicita-Mauro V, Cucinotta D: Metabolic effects of dehydroepiandrosterone replacement therapy in postmenopausal women. *Eur J Endocrinol* 145:457–461, 2001
22. Vogiatzi MG, Boick MA, Vlachopapadopoulou E, El-Rashid R, New MI: Dehydroepiandrosterone in morbidly obese adolescents: effects on weight, body composition, lipids, and insulin resistance. *Metabolism* 45:1011–1015, 1996
23. Usiskin KS, Butterworth S, Clore JN, Arad Y, Ginsberg HN, Blackard WG, Nestler JN: Lack of effect of dehydroepiandrosterone in obese men. *Int J Obes* 14:457–463, 1990
24. Callies F, Fassnacht M, van Vlijmen JC, Koehler I, Huebler D, Seibel MJ, Arlt W, Allolio B: Dehydroepiandrosterone replacement in women with adrenal insufficiency: effects on body composition, serum leptin, bone turnover, and exercise capacity. *J Clin Endocrinol Metab* 86:1968–1972, 2001
25. Nestler JE, Barlascini CO, Clore JN, Blackard WG: Dehydroepiandrosterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity in normal men. *J Clin Endocrinol Metab* 66:57–61, 1988
26. Mortola JF, Yen SSC: The effects of oral dehydroepiandrosterone on endocrine-metabolic parameters in postmenopausal women. *J Clin Endocrinol Metab* 71:696–704, 1990
27. Basu R, Breda E, Oberg AL, Powell CC, Dalla Man C, Basu A, Vittone JL, Klee GG, Arora P, Jensen MD, Toffolo G, Cobelli C, Rizza RA: Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action and clearance. *Diabetes* 52:1738–1748, 2003
28. Basu R, Dalla Man C, Campioni M, Basu A, Klee G, Toffolo G, Cobelli C, Rizza RA: Effects of age and sex on postprandial glucose metabolism: differences in glucose turnover, insulin secretion, insulin action, and hepatic insulin extraction. *Diabetes* 55:2001–2014, 2006
29. Nair KS, Rizza RA, O'Brien P, Dhatariya K, Short KR, Nehra A, Vittone JL, Klee GG, Basu A, Basu R, Cobelli C, Toffolo G, Dalla Man C, Tindall DJ, Melton LJ III, Smith GE, Khosla S, Jensen MD: DHEA in elderly women and DHEA or testosterone in elderly men. *N Engl J Med* 355:1647–1659, 2006
30. Basu R, Di Camillo B, Toffolo G, Basu A, Shah P, Vella A, Rizza R, Cobelli

C: Use of a novel triple-tracer approach to assess postprandial glucose metabolism. *Am J Physiol Endocrinol Metab* 284:E55–E69, 2003

31. Jensen MD, Kanaley JA, Reed JE, Sheedy PF: Measurement of abdominal and visceral fat with computed tomography and dual-energy x-ray absorptiometry. *Am J Clin Nutr* 61:274–278, 1995

32. Proctor DN, Sinning WE, Walro JM, Sieck GC, Lemon PW: Oxidative capacity of human muscle fiber types: effects of age and training status. *J Appl Physiol* 78:2033–2038, 1995

33. Taafe DR, Marcus R: Dynamic muscle strength alterations to detraining and retraining in elderly men. *Clin Physiol* 17:311–324, 1997

34. Moore MC, Shulman GI, Giaccari A, Pagliassotti MJ, Cline G, Neal D, Rossetti L, Cherrington AD: Effect of hepatic nerves on disposition of an intraduodenal glucose load. *Am J Physiol* 265:E487–E496, 1993

35. Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, Cobelli C: Minimal model estimation of glucose absorption and insulin sensitivity from oral test: validation with a tracer method. *Am J Physiol Endocrinol Metab* 287:E637–E643, 2004

36. Bergman RN, Ider YZ, Bowden CR, Cobelli C: Quantitative estimation of insulin sensitivity. *Am J Physiol* 236:E667–E677, 1979

37. Cobelli C, Vicini P, Toffolo G, Caumo A: The hot IVGTT minimal models: simultaneous assessment of glucose disposal indices and hepatic glucose release. In *MinMod 94*. Lovejoy J, Bergman RN, Eds. Baton Rouge, LA, Louisiana Press, 1996, p. 202–239

38. Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, Cobelli C: Measurement of selective effect of insulin on glucose disposal from labeled glucose oral test minimal model. *Am J Physiol Endocrinol Metab* 289:E909–E914, 2005

39. Dalla Man C, Yarasheski KE, Caumo A, Robertson H, Toffolo G, Polonsky KS, Cobelli C: Insulin sensitivity by oral glucose minimal models: validation against clamp. *Am J Physiol Endocrinol Metab* 289:E954–E959, 2005

40. Toffolo G, De Grandi F, Cobelli C: Estimation of β-cell sensitivity from intravenous glucose tolerance tests C-peptide data. *Diabetes* 44:845–854, 1995

41. Toffolo G, Cefalu WT, Cobelli C: β-Cell function during insulin-modified intravenous glucose tolerance test successfully assessed by the C-peptide minimal model. *Metabolism* 48:1162–1166, 1999

42. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377, 1992

43. Bergman RN, Phillips LS, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man. *J Clin Invest* 68:1456–1467, 1981

44. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JP, Palmer JP, Port DJ: Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 42:1663–1672, 1993

45. Toffolo G, Campioni M, Rizza R, Cobelli C: A minimal model of insulin secretion and kinetics to assess hepatic insulin extraction. *Am J Physiol Endocrinol Metab* 290:E169–E176, 2006

46. Radziuk J, Norwich KH, Vranic M: Experimental validation of measurements of glucose turnover in nonsteady state. *Am J Physiol* 234:E84–E93, 1978

47. Toffolo G, Basu R, Dalla Man C, Rizza RA, Cobelli C: Assessment of postprandial glucose metabolism: conventional dual versus triple tracer method. *Am J Physiol Endocrinol Metab* 291:E800–E806, 2006

48. Barret PHR, Bell BM, Cobelli C, Golde H, Schumitzky A, Vicini P, Foster D: SAAM II: simulation, analysis and modeling software for tracer and pharmacokinetic studies. *Metabolism* 47:484–492, 1998

49. Dinneen S, Gerich J, Rizza R: Carbohydrate metabolism in non-insulin-dependent diabetes mellitus. *N Engl J Med* 327:707–713, 1992

50. Jedrzejuk D, Medras M, Milewicz A, Demissie M: Dehydroepiandrosterone replacement in healthy men with age-related decline of DHEA-S: effects on fat distribution, insulin sensitivity and lipid metabolism. *Aging Male* 6:151–156, 2003