Insulin Resistance and Advancing Age: What Role for Dehydroepiandrosterone Sulfate?

Giuseppe Paolisso, Stefania Ammendola, Mario Rotondi, Antonio Gambardella, Maria Rosaria Rizzo, Gherardo Mazziotti, Maria Rosaria Tagliamonte, Carlo Carella, and Michele Varricchio

The relationship between insulin resistance and aging is still debated. This study aims to investigate the role that age-related differences in plasma dehydroepiandrosterone sulfate (DHEAS) concentration may have on insulin action. For this reason, 75 subjects (42 men and 33 women) with a wide age range (21 to 106 years) were studied. In all subjects, plasma DHEAS and total testosterone concentrations were measured and a euglycemic clamp was used, but substrate oxidation was not determined in centenarians (n = 15). Plasma DHEAS correlated with age ($r = -.77$, $P < .001$) and whole-body glucose disposal (WBGD) ($r = .57$, $P < .001$). After controlling for age, sex, body fat, and waist to hip ratio (WHR), the association between plasma DHEAS and WBGD was still observed ($r = .31$, $P < .005$). Comparing subjects at the third tertile versus those at the first and second tertiles of plasma age-adjusted DHEAS concentration, the former group showed a weaker association between WBGD and age ($r = -.38$, $P < .05$) than the latter group ($r = -.43$, $P < .002$). The difference between the two regression lines was also significant ($P < .03$). After controlling for sex, body fat, and WHR, the association between plasma DHEAS and WBGD was dependent on the age of the subjects, being strong in adults (n = 30, age < 50 years, $r = .69$, $P < .001$), weak in old subjects (n = 30, age 51 to 99 years, $r = .23$, $P < .05$), and absent in centenarians ($r = -.05$, $P < .88$). With the subjects divided by sex throughout the different age groups, the univariate association between plasma DHEAS and WBGD was present in females ($r = .43$, $P < .01$) but not in males ($r = .17$, $P < .32$). Plasma total testosterone and insulin-like growth factor-1 (IGF-1) concentrations declined with advancing age and were significantly correlated with DHEAS and WBGD. In a multivariate analysis with WBGD as the dependent variable, a model including age, sex, body fat, WHR, DHEAS, total testosterone, and IGF-1 explained 66% of WBGD variability, with DHEAS significantly and independently associated with WBGD ($P < .004$). In conclusion, the negative relationship between advancing age and insulin action seems related to plasma DHEAS concentration. Differences in plasma total testosterone and IGF-1 concentrations may provide a further contribution to the relationship between DHEAS and WBGD.

AGING IS ASSOCIATED WITH a decline in insulin action[1,2] and an increasing prevalence of non–insulin-dependent diabetes mellitus.[3] The Baltimore Longitudinal Study of Aging (742 subjects aged 17 to 92 years) showed that the age-related decline in glucose tolerance is mainly explained by the secondary influences of an increase in fatness, a decline in fitness, and a change toward an upper-body fat distribution.[3] These findings have been recently confirmed and extended by the European Group for Insulin Resistance.[4] In a large sample ($N = 1,146$) of healthy subjects, the study demonstrated that insulin-mediated glucose disposal declines with advancing age as a consequence of change in body composition (more fatness) and a more central body fat distribution.

Recent evidence indicates that plasma dehydroepiandrosterone sulfate (DHEAS) concentrations decline with advancing age[5,6] and that such a change is associated with an increase in body fatness and a decline in fitness.[7-9] It has also been demonstrated that DHEAS and fasting plasma insulin levels are negatively correlated,[7-8,10] and that DHEAS may improve insulin action through an increase in insulin binding to its own receptor.[11] Thus, one cannot exclude an age-related decline in plasma DHEAS as being partially responsible for the decline in insulin-mediated glucose disposal with age. Unfortunately, previous studies addressing the relation between insulin action and plasma DHEAS concentration have been made in subjects with a limited age range.

In the present study, we tested the hypothesis that impaired insulin-mediated glucose disposal with age is associated with the decline in plasma DHEAS concentration. In subjects with a wide age range (21 to 106 years), we measured plasma DHEAS, total testosterone, and IGF-1 concentrations, insulin action by euglycemic glucose clamp, and substrate oxidation by indirect calorimetry.

SUBJECTS AND METHODS

Subjects

Seventy-five subjects (42 men and 33 women) with a wide age range (21 to 106 years) were studied (Table 1). Premenopausal women were all studied in the luteal phase. All subjects were normotensive, taking no medications, nonsmokers, and had no evidence of metabolic or cardiovascular disease. Oral glucose tolerance[12] (75 g glucose) was tested in all of the volunteers before study enrollment. Subjects with a family history of non–insulin-dependent diabetes mellitus, obesity, or hypertension were excluded from the study. All tests were conducted in the morning and after an overnight fast (≥12 hours). Subjects had been on a similar standard weight-maintaining diet containing 150 g carbohydrate per day for at least 7 days before the tests. After a clear explanation of the potential risks of the study, all subjects provided informed consent to participate in the study, which was approved by the Ethics Committee of our institution.

Anthropometric Determinations

Weight and height were measured by standard techniques. Fat-free mass (FFM) was measured using a four-terminal bioimpedance analyzer (RJL Spectrum Bioelectrical Impedance-BIA 101/SC Akern; RJL System, Florence, Italy).[13] All subjects were measured in the supine position after an overnight fast and with an empty bladder. Prediction of FFM by BIA was made with equations validated for a wide age range in the elderly.[13] In centenarians, validation of BIA has been reported

From the Department of Geriatric Medicine and Metabolic Diseases, Institute of Endocrinology, II University of Naples, Naples, Italy.

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Address reprint requests to Giuseppe Paolisso, MD, Department of Geriatric Medicine and Metabolic Diseases, Servizio di Astanteria Medica, Piazza Miraglia 2, I-80138 Napoli, Italy.

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Table 1. Clinical Characteristics of the Subjects (N = 75)

	Mean	Range
Sex (female/male)	33/42	
Age (yr)	61.8	21-106
BF (%)	25	20-28
FFM (kg)	44.1	35.2-54.3
WHR	0.82	0.70-0.94
FPG (mmol/L)	4.9	3.7-5.9
FPI (pmol/L)	83.8	69-102
WBGD (μmol/kg FFM/min)	30.6	16.6-6.1
DHEAS (nmol/L)	33.3	2.3-112.3
Total testosterone (nmol/L)	10.9	1.6-27.7
IGF-1 (μg/L)	138.78	11-565

Abbreviations: BF, body fat; FPG, fasting plasma glucose; FPI, fasting plasma insulin.

previously.[14] Body mass index was calculated as body weight divided by height squared. Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest (normally the umbilical level) and hip circumference at the level of trochanter. Both were measured to the nearest 0.5 cm with a plastic tape measure, and the waist to hip ratio (WHR) was calculated.

Metabolic Tests

Insulin action was measured using the euglycemic-hyperinsulinemic glucose clamp technique. In this test, a fixed insulin infusion rate (7.1 pmol/kg/min for 120 minutes, Humulin R; Eli-Lilly, Florence, Italy) and a variable amount of glucose (as a 20% solution) were administered. Simultaneous indirect calorimetry was performed using an open-circuit, ventilated-hood system (Deltatrac Monitor; Datex, Helsinki, Finland). The respiratory quotient (Rq) and substrate oxidation rate were calculated from the oxygen consumption, carbon dioxide production, and urinary nitrogen excretion rate according to Ferrannini.[15]

Analytical Methods

Plasma glucose level was immediately measured by the glucose oxidase method (Beckman Autoanalyzer; Fullerton, CA). Blood samples for insulin, DHEAS, and total testosterone measurements were collected in heparinized tubes. After centrifugation, plasma insulin (Sorin Biomedical, Milan, Italy; coefficient of variation [CV] 3.3% ± 0.2%), DHEAS (Sorin; CV, 4.9% ± 0.3%), total testosterone (Clinical Assay, Milan, Italy; CV, 3.8% ± 0.3%), and insulin-like growth factor-1 (IGF-1) (RIA with extraction; DSI, Webster, TX; minimum detection limit, 0.8 μl/L; intraassay CV, 3.9%) were determined by radioimmunoassay.

Calculations and Statistical Analyses

To investigate age-related differences, the subjects ($N = 75$) were categorized in three groups: (1) adults (< 50 years, n = 30), (2) old subjects (51 to 99 years, n = 30), and (3) centenarians (> 100 years, n = 15).

Whole-body glucose disposal (WBGD) was calculated during the final 60 minutes of the clamp according to the formula WBGD = glucose infusion rate + pool correction, where the pool correction takes into account the change in the whole-body glucose pool, as estimated from the change in plasma glucose pool, as estimated from the change in plasma glucose concentration.[16] This correction was always less than 5% of the glucose infusion rate during the glucose clamp. This calculation is valid when there is no entry of glucose into the plasma from the liver.[16] In nondiabetic subjects, hepatic glucose output has been found to be fully suppressed during a glucose clamp at this insulin infusion rate.[17] Further, in preliminary clamps, an insulin infusion rate of 7.1 pmol/kg/min fully suppressed (but without negative numbers) hepatic glucose output at all ages. Nonoxidative glucose metabolism (NOGM) was calculated as WBGD oxidative glucose metabolism calculated by indirect calorimetry.[15] Substrate oxidation and NOGM were not determined in centenarians.

For predicting the adequacy of sample size to investigate the relationship of age, DHEAS, total testosterone, and WBGD, the nQuery test was used. A sample size of 75 subjects was found to be statistically large enough. Further, analysis of 95% regression confidence intervals showed narrow ranges, thus confirming the nQuery test prediction. To approximate normal distributions, plasma insulin, DHEAS, total testosterone, and IGF-1 concentrations were logarithmically transformed and used in all calculations. ANOVA with Scheffe's test was used to test for age and sex differences in all variables studied. Analysis of covariance (ANCOVA) was also used to adjust each variable for a single covariate. Pearson product-moment correlations were calculated to test associations among variables. Partial correlation analysis was used to test associations between two variables independent of a covariate. Multivariate linear regression analyses tested the independent association of each variable with WBGD. Statistical analyses were made using the SOLO (BMDP, Cork, Ireland) software package. All values are presented as the mean ± SD.

RESULTS

Along with the insulin infusion, steady-state plasma glucose (range, 4.8 to 5.0 mmol/L) and insulin (range, 560 to 600 pmol/L) were kept within a narrow range (CV < 4.0%), without statistically significant differences among the ages ($P = .56$) and between the sexes ($P = .73$).

In the whole group ($N = 75$), advancing age was significantly associated with a decline in WBGD ($r = -.29$, $P < .01$) and fasting plasma DHEAS concentrations (Fig 1; $r = -.77$, $P < .001$). Plasma DHEAS concentration was positively correlated with WBGD (Fig 1; $r = .57$, $P < .001$), Rq (n = 60, $r = .25$, $P < .05$), NOGM (n = 60, $r = .37$, $P < .005$), and FFM ($r = .29$, $P < .01$) and negatively correlated with body fat ($r = -.47$, $P < .001$), WHR ($r = .22$, $P < .05$), and fasting plasma insulin ($r = -.35$, $P < .001$). After controlling for age, sex, body fat, and WHR, plasma DHEAS concentration was still correlated, albeit weakly, with WBGD ($r = .31$, $P < .005$) and NOGM (n = 60, $r = .29$, $P < .03$). Furthermore, plasma DHEAS concentration and WHR were weakly correlated after adjustment for sex ($r = .23$, $P < .05$). The influence of age- and sex-adjusted plasma DHEAS concentration on the univariate association between WBGD and age is shown in Fig 2. Subjects with an elevated (third tertile) plasma DHEAS concentration (n = 25) showed a weak ($r = -.38$, $P < .05$) association between WBGD and age, whereas in subjects with a low (first and second tertiles) plasma DHEAS concentration (n = 50), a steeper regression line ($r = -.43$, $P < .002$) between WBGD and age was found. The difference between the two regression lines was also significant ($P < .03$).

Fasting plasma total testosterone concentration was negatively correlated with age ($r = -.59$, $P < .001$), body fat ($r = -.31$, $P < .008$), WHR ($r = -.24$, $P < .05$), and fasting plasma glucose ($r = -.23$, $P < .05$) and insulin ($r = -.33$, $P < .004$) concentrations, while positive correlations with DHEAS ($r = .68$, $P < .001$), WBGD ($r = .30$, $P < .005$), and NOGM (n = 60, $r = .30$, $P < .03$) were found. After adjusting for age, sex, body fat, and WHR, fasting plasma total testoster-

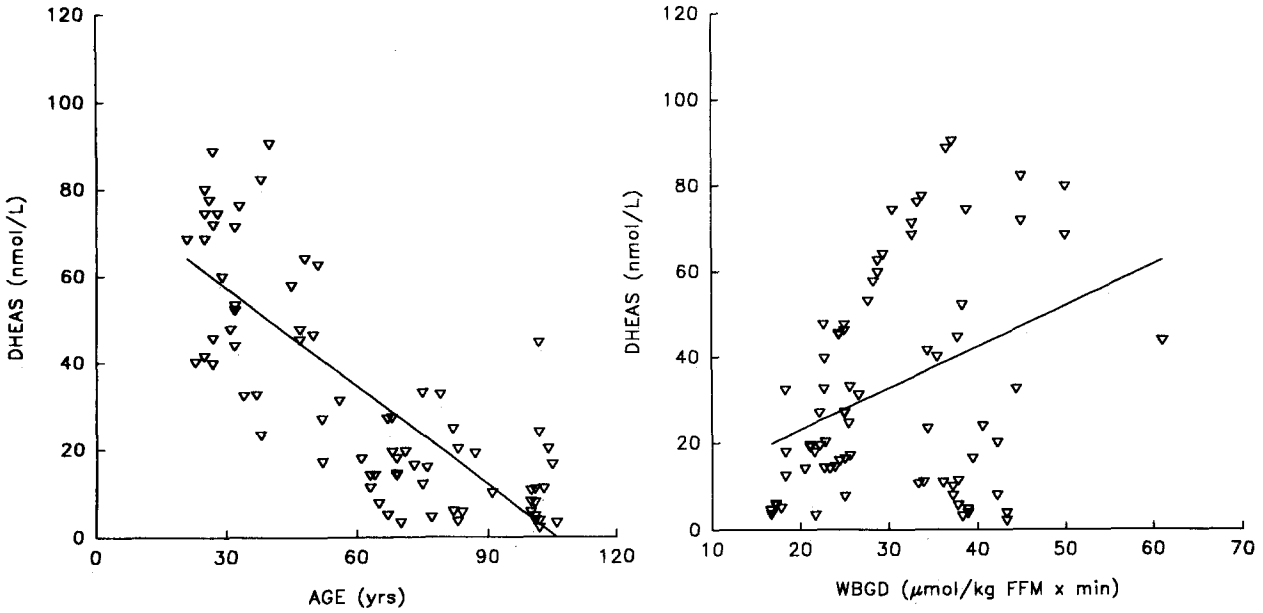

Fig 1. Correlation of plasma DHEAS with age ($r = -.77$, $P < .001$) and with WBGD ($r = .57$, $P < .001$).

one was weakly correlated with WBGD ($r = .27$, $P < .01$). The fasting plasma DHEAS/testosterone concentration was also correlated with WBGD ($r = .28$, $P < .009$). Such a correlation persisted, even if weakened, after adjustment for age, sex, body fat, and WHR ($r = .24$, $P < .05$).

Fasting plasma total IGF-1 concentration correlated with age ($r = -.67$, $P < .001$), body fat ($r = -.40$, $P < .003$), FFM ($r = .32$, $P < .003$), WHR ($r = -.08$, $P < .45$), fasting plasma glucose ($r = -.31$, $P < .04$) and plasma DHEAS ($r = .71$, $P < .001$; Fig 3) concentrations, WBGD ($r = .34$, $P < .002$), and NOGM (n = 60, $r = .33$, $P < .03$). After adjusting for age, sex, body fat, and WHR, fasting plasma total IGF-1 was still significantly correlated with DHEAS ($r = .36$, $P < .003$) and WBGD ($r = .38$, $P < .001$).

Multivariate regression analyses with WBGD as the dependent variable are reported in Table 2. Independently of age, sex, body fat, and WHR, plasma DHEAS, total testosterone, and IGF-1 concentrations were all associated with WBGD. Interestingly, in the more complex model, the relationship between plasma DHEAS concentration and WBGD was found to be independent of the anthropometric and hormonal variables. In this model, plasma DHEAS concentration explained 21% of

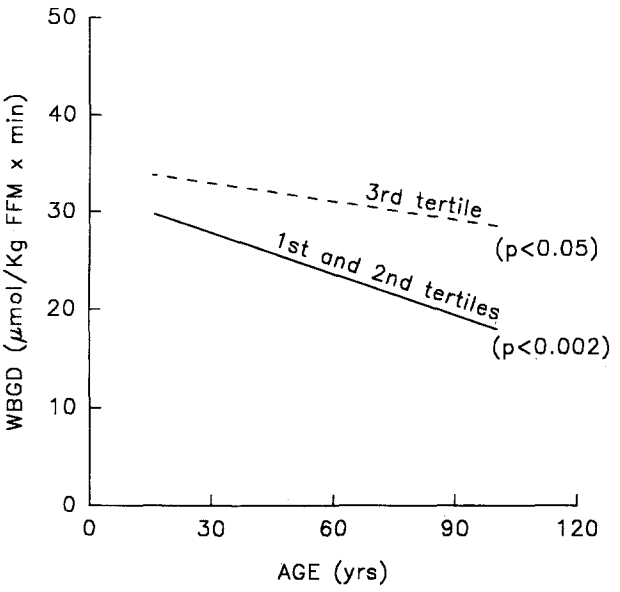

Fig 2. Correlation between WBGD and age in subjects at the third tertile of plasma age- and sex-adjusted DHEAS concentration (- - - -; $r = -.38$, $P < .05$, n = 25) and subjects at the first and second tertiles of plasma age- and sex-adjusted DHEAS concentration (--------; $r = -.43$, $P < .002$, n = 50). All regression lines are shown.

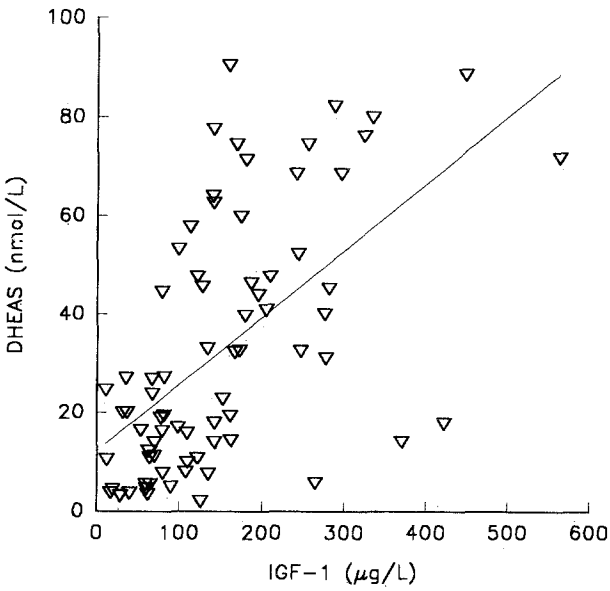

Fig 3. Correlation of plasma DHEAS with plasma IGF-1 ($r = -.71$, $P < .001$).

Table 2. Multiple Regression Analyses With WBGD as the Dependent Variable

Variable	t	P	
Age (yr)	−2.46	.01	
Sex	2.85	.04	
BF (%)	−0.35	.41	
WHR	−3.23	.001	
DHEAS (nmol/L)	3.67	.001	
			R^2 = 59%
Age (yr)	−1.83	.05	
Sex	−0.17	.80	
BF (%)	−4.48	.005	
WHR	−1.99	.05	
Total testosterone (nmol/L)	6.43	.001	
			R^2 = 42%
Age (yr)	−2.80	.05	
Sex	0.81	.42	
BF (%)	−4.64	.001	
WHR	−1.94	.05	
IGF-1 (μg/L)	3.07	.003	
			R^2 = 40%
Age (yr)	−4.0	.001	
Sex	0.10	.95	
BF (%)	−4.40	.002	
WHR	−2.04	.01	
IGF-1 (μg/L)	1.90	.05	
DHEAS (nmol/L)	3.00	.004	
Total testosterone (nmol/L)	0.80	.42	
			R^2 = 66%

Abbreviation: BF, body fat.

WBGD variability. Finally, the impact of the plasma DHEAS to testosterone ratio on WBGD was also tested. In a model including age, sex, body fat, WHR, and the plasma DHEAS to testosterone ratio, the latter parameter explained 11% of WBGD variability.

Sex-Related Differences

Age (73.7 ± 26.9 *v* 62.4 ± 25.6 years, P = NS), body fat (25.8 ± 1.5 *v* 25.3 ± 1.5 kg/m^2, P = NS), FFM (45.2 ± 4.5 *v* 41.6 ± 3.0 kg, P = NS), fasting plasma glucose (4.8 ± 0.5 *v* 4.9 ± 0.4 mmol/L, P = NS), insulin (83 ± 8.7 *v* 83 ± 7.0 pmol/L, P = NS), and IGF-1 (134 ± 100 *v* 114 ± 104 μg/L, P = .12) concentrations, WBGD (31.1 ± 4.4 *v* 29.9 ± 5.3 μmol/kg FFM/min, P = NS), and NOGM (20.4 ± 2.1 *v* 17.8 ± 3.1 μmol/kg FFM/min, P = NS) were not statistically different between men and women, respectively. In contrast, WHR was lower in women than in men (0.80 ± 0.03 *v* 0.86 ± 0.05, P < .02). Fasting plasma DHEAS tended to be greater in men than in women (44.0 ± 27.5 *v* 19.7 ± 20.1 nmol/L), but the difference did not reach statistical significance (P < .07). Fasting plasma total testosterone (16.6 ± 5.0 *v* 3.6 ± 1.3 nmol/L, P < .001) was significantly greater in men than in women.

In men (n = 42), age was significantly correlated with body fat (r = .49, P < .008), FFM (r = −.45, P < .005), DHEAS (r = −.80, P < .001), total testosterone (r = −.74, P < .001), WBGD (r = −.37, P < .01), and NOGM (n = 38, r = −.32, P < .05). In univariate analysis, the association between plasma DHEAS concentration and WBGD (r = .17, P = .32) was absent. Nevertheless, this latter relationship was strengthened by controlling for age, body fat, and WHR (r = .33, P < .01). Again in univariate analysis, plasma total testosterone was correlated with WBGD (r = .60, P < .005). In the multivariate analysis (n = 42) with WBGD as the dependent variable, age (P < .001), body fat (P < .001), WHR (P < .05), DHEAS (P < .05), and plasma testosterone (P < .02) were all significantly associated with WBGD. This model explained 72% of the variability of WBGD in men.

In women, premenopausal (n = 12) versus postmenopausal (n = 21) subjects had higher fasting plasma DHEAS concentrations (89.6 ± 23.2 *v* 26.5 ± 18.5 nmol/L, P < .01) but similar plasma total testosterone concentrations (4.1 ± 1.6 *v* 3.5 ± 1.2 nmol/L, P = NS). In the entire group of women (n = 33), age was significantly correlated with body fat (r = .41, P < .02), FFM (r = −.34, P < .05), DHEAS (r = −.64, P < .001), WBGD (r = −.36, P < .04), and NOGM (n = 25, r = −.39, P < .05), but not with total testosterone (r = −.24, P = .17). WGBD was correlated with fasting plasma DHEAS (r = .43, P < .01), but not with plasma total testosterone (r = .29, P = .10). Multivariate linear analysis (n = 33) with WBGD as the dependent variable demonstrated that age (P < .002) and DHEAS (P < .005), but not body fat (P < .31), WHR (P < .11), and plasma testosterone (P < .20), were significantly and independently associated with WBGD. Such a model explained 51% of WBGD variability in women.

Does a Relationship Between Plasma DHEAS and WBGD Exist in Centenarians?

Centenarians had plasma DHEAS concentrations (12.4 ± 11.8 nmol/L) similar to those of old subjects (16.5 ± 8.8 nmol/L, P = NS) but lower than those of adults (60.8 ± 21.3 nmol/L, P < .005). Independently of sex, body fat (percent), and WHR, the association between DHEAS and WBGD was present in adults (n = 30, r = .69, P < .001), weak in old subjects (n = 30, r = .23, P < .05), and absent in centenarians (n = 15, r = −.05, P = .88).

DISCUSSION

Our study confirms that fasting plasma DHEAS concentration and WBGD decline with advancing age and demonstrates that (1) changes in plasma DHEAS concentration are associated with the age-related decline in WBGD; (2) such an association is much more important in subjects with elevated plasma DHEAS than in those with low concentrations; (3) plasma total testosterone concentration is positively related to WBGD only in men; (4) plasma DHEAS concentration is associated with plasma IGF-1 concentration, and the latter is also correlated with WBGD; (5) the relationship between plasma DHEAS or total testosterone concentrations and WBGD is present in adults and old subjects but is lost in centenarians; and (6) throughout the different age groups, the association between plasma DHEAS concentration and WBGD is stronger in women than in men.

Several factors have been proposed as contributing to the age-related impairment of glucose disposal in skeletal muscle: (1) a change in body composition, principally an increase in body fat and a reduction in lean body mass[1]; (2) physical inactivity, which involves not only a decrease in peripheral insulin sensitivity but also a decline in B-cell sensitivity to glucose[18]; (3) higher fasting plasma free fatty acid concentra-

tions with a secondary increase in Randle cycle activity[19]; (4) an enhanced diffusion distance of the capillary supply to skeletal muscle and a change in the proportion of different gradient muscle fiber types within tissue[1,20]; (5) higher plasma free-radical concentrations, which can negatively affect insulin action[21]; and (6) inhibition of receptor tyrosine kinase activity.[22] In addition, an altered metabolic/hormonal milieu associated with advancing age might also negatively affect the relationship between aging and WBGD. DHEAS is the steroid with the highest serum concentration in human,[5] and its modulator role in WBGD has already been the object of numerous investigations. In animals, DHEAS administration has been associated with improved glucose tolerance and insulin sensitivity and with a decline in fasting plasma insulin concentration.[23-25] In humans, contrasting data have been reported. In lean healthy men, Nestler et al[26] found no changes in glucose disposal after DHEA treatment. Morales et al[27] treated aged subjects with DHEAS (50 mg/d), showing a trend toward improvement in insulin action, but without reaching statistical significance. Nevertheless, in this latter study, insulin action was measured by the minimal-model technique, whereas in our study the glucose clamp method was used. In postmenopausal women of the Rancho Bernardo Study, a direct relationship between plasma DHEAS and diabetes was observed.[28] In contrast, Buffington et al[11] demonstrated that patients with adrenal hyperandrogenemia have elevated insulin binding likely secondary to an alteration in insulin receptor number or affinity through interaction with other membrane receptors, ie, IGF-1 and -2. An effect of plasma DHEAS on plasma IGF-1 concentration cannot be ruled out. Interestingly, in our study, we found a significant correlation between plasma DHEAS and IGF-1 concentrations. Such data were not unexpected, since an increase in plasma IGF-1 after DHEAS administration has been already demonstrated.[27] It has been suggested that the effect of DHEAS on IGF-1 may operate outside the known regulatory mechanism of the growth hormone (GH) IGF-1.[27] In particular, it was suggested that elevated plasma DHEAS concentration may exert a stimulatory action on either the hepatic production of IGF-1 or the generation of GH receptors, thereby enhancing the effectiveness of ambient GH levels for IGF-1 production.[27] Furthermore, Buffington et al[11] showed that pyruvate dehydrogenase activity is also enhanced in subjects with elevated plasma DHEAS; thus, they hypothesized that not only insulin-receptor events but also postreceptor activities are stimulated by DHEAS. Most recently, it has also been suggested that plasma DHEAS may modulate intracellular calcium entry, thus having antihypertensive effects.[28] Since elevated calcium content is associated with insulin resistance,[29] one cannot exclude that plasma DHEAS might improve insulin action, counteracting intracellular calcium content. The impact of fasting plasma DHEAS concentration on glucose metabolism is also confirmed by data showing an inverse relationship between plasma DHEAS and insulin concentrations.[8-10] In particular, many studies have provided evidence that plasma DHEAS is decreased in pathological states characterized by hyperinsulinemia and insulin resistance such as obesity,[30] hypertension,[31] and untreated non–insulin-dependent diabetes mellitus.[32,33] This might be due to the inhibitory effect of hyperinsulinemia on plasma DHEAS concentration.[34] Furthermore, pharmacological agents known to affect insulin action and insulin levels, such as metformin,[35] benfluorex,[36] and diltiazem,[37] are also useful for enhancing plasma DHEAS concentration.

In our study, an association between age-related differences in plasma DHEAS concentration and WBGD were found. Thus, one can hypothesize that plasma DHEAS concentration may affect age-related changes in WBGD. Several evidences strengthen this hypothesis. First, subjects with a low (first and second tertile) plasma age-adjusted DHEAS concentration had a steeper regression line between WBGD and age than subjects with an elevated (third tertile) plasma age-adjusted DHEAS concentration. Such results might be interpreted as evidence that an elevated plasma DHEAS concentration has a protective role against the age-related decline in WBGD. Second, in multivariate analyses, plasma DHEAS concentration was significantly associated with WBGD independently of other significant factors such as age and body fat. The relationship between WBGD and fasting plasma DHEAS concentration was present in women, whereas in men it was present only after controlling for body fat and WHR. Such data are in agreement with those reported by Haffner and Valdez,[9] but the reasons for these sex-related differences are still obscure and deserve further investigation.[9]

DHEAS is also the precursor of sex hormones, and among them, testosterone has been shown to have a positive impact on WBGD.[9-10] More recently, low plasma sex hormone–binding globulin and testosterone concentrations have been shown to predict the development of non–insulin-dependent diabetes mellitus.[38] Indeed, in our study, plasma testosterone declined with advancing age, but it was positively correlated with WBGD. Thus, one cannot exclude the possibility that DHEAS may also affect WBGD through a change in plasma testosterone concentration. The plasma DHEAS to testosterone ratio may be a useful index for evaluating the plasma DHEAS concentration as a steroid reservoir. Nevertheless, in a multivariate analysis with age, sex, body fat, and WHR as covariates, the plasma DHEAS to total testosterone ratio explained only 11% of WBGD variability, compared with 23%, 16%, and 18% of plasma DHEAS and testosterone and IGF-1, respectively. Thus, one can conclude that both plasma DHEAS and testosterone could be related to WBGD, but such a relationship may work through different pathways.

An indirect and new finding of our study is that centenarians have plasma DHEAS concentrations not different from those of old subjects. Thus, one can speculate that the age-related decline in adrenal and gonadic functions is not progressive, reaching a steady-state with advancing age. A previous study has demonstrated that centenarians have a preserved insulin action.[37] It has been hypothesized that in such a population a preserved adrenal function may cooperate in avoiding the decline in insulin-mediated glucose disposal with age.[37] Our study demonstrates that the relationship between plasma DHEAS and WBGD is lost in centenarians. Such data may be justified by the fact that centenarians have WBGD similar to that of adults despite lower plasma DHEAS and testosterone concentrations.

In conclusion, the negative relationship between advancing age and insulin action also seems to be associated with plasma DHEAS concentration. The potential role of plasma DHEAS seems stronger in women than in men. Finally, in centenarians, the plasma DHEAS concentration is not lower than in old

subjects, but the association between DHEAS and WBGD is lost. These data indicate that plasma DHEAS concentration is associated with the differences in insulin action that occur with advancing age.

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