J. Steroid Biochem. Molec. Biol. Vol. 40, No. 4–6, pp. 599–605, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

METABOLISM AND ACTIONS OF DEHYDROEPIANDROSTERONE IN HUMANS

JOHN E. NESTLER,* JOHN N. CLORE and WILLIAM G. BLACKARD
Division of Endocrinology and Metabolism, Medical College of Virginia/Virginia Commonwealth University, MCV Station, Box 111, Richmond, VA 23298-0111, U.S.A.

Summary—Dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one; DHA) and DHA-sulfate are abundantly produced adrenal steroids, whose serum concentrations exceed those of other adrenal steroids. Serum concentrations of DHA and DHA-sulfate, in contrast to other adrenal steroids, exhibit a progressive age-related decline. The mechanism(s) for this selective decline in serum DHA and DHA-sulfate levels and the biologic function of these steroids remain unknown. Studies examining insulin's regulation of adrenal androgens are reviewed. These studies show that experimentally-induced hyperinsulinemia lowers serum DHA and DHA-sulfate levels, and suggest that insulin reduces serum concentrations of these steroids by inhibiting production rather than by increasing clearance. Studies examining the actions of short-term pharmacologic DHA administration to young nonobese and obese men are also reviewed. These studies suggest that DHA may possess hypolipidemic and, possibly, anti-obesity properties. They have failed, however, to demonstrate any effect of DHA on tissue insulin sensitivity.

INTRODUCTION

Dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one; DHA) and DHA-sulfate are intriguing steroids. DHA-sulfate is the most abundantly circulating adrenal steroid [1], yet its physiologic role and that of its parent steroid, DHA, remain unknown. In addition, the metabolism of these steroids is unique among adrenal steroids. Serum DHA and DHA-sulfate levels peak at age 25–30 years and decline progressively thereafter, so that by age 60 years serum levels of these steroids are only 5–10% of what they were during youth [1, 2]. In contrast, serum concentrations of cortisol and other adrenal steroids remain relatively unchanged with aging [3]. The mechanism(s) of this selective age-related decline in serum DHA and DHA-sulfate levels is not known. Furthermore, the fall in serum concentrations of DHA and DHA-sulfate occurs as the incidence of atherosclerosis, obesity and diabetes rise, suggesting that higher levels of DHA or DHA-sulfate may be protective against the development of these degenerative processes. These characteristics bespeak an important biologic function for DHA, and suggest that its relative lack may contribute to some of the degenerative changes that accompany the aging process.

Over the past two decades numerous studies have been conducted examining the effects of DHA in animals. Many of these studies have shown that DHA administration (1) improves serum lipids and retards the development of atherosclerosis [4–8], (2) results in lower body weight without affecting appetite or food intake [5, 9–31], (3) prevents the development of diabetes in genetically diabetic (*-db/db*) or obese (*-ob/ob*) mice [15–19] and (4) increases tissue sensitivity to insulin in aged normal mice [17]. Studies examining the effects of DHA in man, however, are limited. In this paper we will review studies of ours examining both the regulation of DHA and DHA-sulfate metabolism by insulin [32–34] and possible biologic actions of these steroids in man [35–37].

METHODS AND RESULTS

Effects of hyperinsulinemia on DHA and DHA-sulfate metabolism

Our interest in DHA was stimulated by the unexpected finding of a decline in serum DHA-sulfate levels in normal women during experimentally-induced hyperinsulinemia [32]. To study the effects of acute hyperinsulinemia on serum androgens, 5 normal women received a 0.1 U/kg (0.72 nmol/kg) insulin bolus dose

Proceedings of the VIIIth International Congress on Hormonal Steroids, The Hague, The Netherlands, 16–21 September 1990.

*To whom correspondence should be addressed.

followed by an insulin infusion at a rate of 10 mU/kg/min (72 pmol/kg/min) for 12 h (achieving a mean serum insulin concentration of 11,500–14,350 pmol/l), while the serum glucose concentration was kept constant at the fasting level via a variable glucose infusion (i.e. hyperinsulinemic–euglycemic clamp).

Serum testosterone and progesterone levels remained relatively constant during the insulin infusion. In contrast, serum DHA-sulfate concentrations decreased progressively, declining significantly by 39% at 12 h (3.91 vs 6.35 μmol/l at zero time) (Fig. 1). This steady decline in serum DHA-sulfate levels was shown not to be due to diurnal variation, suppression of adrenocorticotropin (ACTH) release, or alterations in serum prolactin levels [32]. Subsequently, a fall in serum DHA-sulfate levels when serum insulin levels were raised into the physiologic range (700–1450 pmol/l) has also been demonstrated by 3 independent groups of investigators [38–40].

To determine whether this fall in serum DHA-sulfate levels might have been due to insulin-stimulated (1) hydrolysis of DHA-sulfate to DHA, (2) conversion of DHA/DHA-sulfate to androstenedione and/or (3) urinary excretion of these steroids, 10 additional men were studied by the hyperinsulinemic–euglycemic clamp technique and during a control saline infusion [33]. Each man received a 0.1 U/kg (0.72 nmol/kg) insulin bolus dose, followed by a 10 mU/kg/min (72 pmol/kg/min) insulin infusion for 4 h. An average insulin level of 12390 $\pm$ 259 pmol/l was achieved, while the serum glucose level was maintained at the fasting level of 5.0 $\pm$ 0.1 mmol/l.

In these men serum DHA-sulfate levels also declined progressively during experimentally-induced hyperinsulinemia, and were only 79.1 $\pm$ 3.2% of the baseline value by the end of the study (hour 6) ($P < 0.05$). Serum DHA levels fell concurrently, and were 52.9 $\pm$ 10.2% of the baseline value by the end of the study ($P < 0.05$). Since serum DHA-sulfate and DHA levels fell concurrently, hyperinsulinemia does not appear to reduce serum DHA-sulfate levels by increasing the hydrolysis of DHA-sulfate to DHA.

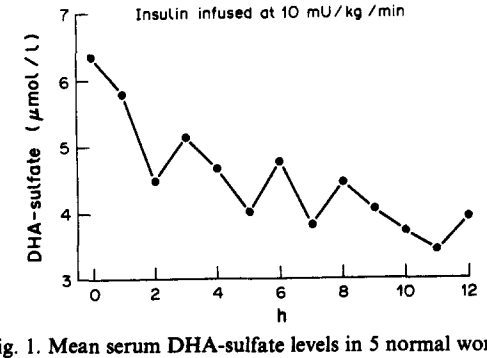

Fig. 1. Mean serum DHA-sulfate levels in 5 normal women studied by the hyperinsulinemic–euglycemic clamp technique. Adapted from results reported in Ref. [32].

DHA is converted by the enzyme 3β-hydroxysteroid dehydrogenase to androstenedione, and enhanced conversion of DHA to androstenedione by insulin could theoretically explain the fall in serum DHA/DHA-sulfate levels during hyperinsulinemia. If this were so, one might expect serum androstenedione levels to rise as serum DHA/DHA-sulfate levels fell. In this study, however, serum androstenedione levels also fell progressively during experimentally-induced hyperinsulinemia, and were only 48.1 $\pm$ 3.3% of the baseline value at the end of the study ($P < 0.05$). This suggests that the decline in serum DHA and DHA-sulfate levels was also not due to insulin-stimulated conversion of DHA/DHA-sulfate to androstenedione.

Notably, no change in serum DHA-sulfate, DHA or androstenedione levels occurred in paired control studies, during which 0.45% saline was infused at rates matched exactly to the rates of the dextrose and insulin infusions during the hyperinsulinemic clamp studies. Thus, neither dilution nor diurnal variation were responsible for the insulin-mediated decline in serum levels of these steroids.

Finally, we also considered the possibility that hyperinsulinemia might lower serum DHA and DHA-sulfate levels by increasing urinary excretion of these steroids. Indeed, despite decreasing serum DHA and DHA-sulfate levels during hyperinsulinemia, urinary DHA-sulfate and DHA-glucuronide excretions were increased by 50% ($P < 0.05$) and 86% ($P < 0.05$), respectively, when compared to urinary excretion of these steroids during the control studies. In contrast, urinary excretion of unconjugated DHA was unchanged. Quantitatively, however, increased urinary excretion of conjugated DHA during hyperinsulinemia could account for only about 5% of the concomitant fall in serum DHA-sulfate concentrations.

These observations indicate that hyperinsulinemia reduces serum DHA-sulfate, DHA and androstenedione levels in men, and suggest that neither insulin-stimulated hydrolysis of DHA-sulfate nor increased conversion of DHA/DHA-sulfate to androstenedione are likely

mechanisms for the fall in serum DHA-sulfate levels. Moreover, although hyperinsulinemia increases urinary excretion of conjugated DHA, the contribution of this mechanism to the insulin-induced fall in serum DHA-sulfate levels is negligible. Thus, it is likely that insulin, in addition to its stimulation of urinary steroid excretion, must either decrease adrenal DHA-sulfate production and/or increase DHA-sulfate catabolism.

Recent *in vivo* [40] and *in vitro* [41] studies suggest that hyperinsulinemia inhibits DHA production. Since hyperinsulinemia appears to specifically decrease adrenal androgen production without affecting adrenal production of glucocorticoids [32], it seems likely that hyperinsulinemia exerts its effect by inhibiting 17,20-lyase activity, which is a branch point between androgen production and the production of glucocorticoids and mineralocorticoids. Preliminary results from an *in vivo* study of ours show that the ACTH-stimulated molar ratio of 17α-hydroxyprogesterone to androstenedione rises significantly after an insulin infusion but remains unchanged after a control saline infusion [34]. These results suggest that the acute elevation of serum insulin into the high physiologic range can indeed inhibit adrenal 17,20-lyase activity.

Metabolic effects of DHA administration to normal man

This decline in serum DHA and DHA-sulfate levels in response to hyperinsulinemia was intriguing in view of recent reports which demonstrated that DHA administration prevented the development of diabetes mellitus in genetically diabetic (*-db/db*) or obese (*-ob/ob*) mice [15–19] and increased tissue sensitivity to insulin in aged normal mice [17]. We speculated that in response to insulinopenia the body might raise DHA and DHA-sulfate levels in order to increase its sensitivity to insulin, whereas during hyperinsulinemia serum DHA and DHA-sulfate would be reduced [32]. In this manner DHA and/or DHA-sulfate would act as an endogenous regulator of tissue sensitivity to insulin.

In addition, DHA had been reported to exert beneficial effects on lipids [4–6] and obesity [9–31] in animals. In man an inverse correlation exits between fetal serum DHA-sulfate and low-density lipoprotein (LDL) levels [43], while a more recent study demonstrated an inverse correlation between DHA-sulfate levels and death from cardiovascular disease in adult men [44]. It seemed plausible that DHA might exert some of its beneficial effects on lipids and obesity by altering tissue sensitivity to insulin.

To test the hypotheses that DHA administration would increase tissue sensitivity to insulin, decrease serum cholesterol levels, and decrease body fat content, we conducted a prospective, randomized, double-blind study in which 5 normal men ingested oral DHA [1600 mg/day (554.7 mmol/day)] and 5 normal men ingested placebo for 28 days [35]. The DHA and placebo groups were matched for age, weight and body mass index. Before and after DHA or placebo administration, tissue sensitivity to insulin, serum lipid levels and anthropometric parameters were determined. To assess compliance, serum DHA-sulfate levels were checked weekly. Serum DHA-sulfate levels did not change in the placebo group, but rose 2.5- to 3.5-fold in the DHA group [the mean value rose from 10.9 ± 1.9 μmol/l on day 0 to 38.8 ± 11.2 μmol/l on day 28 ($P < 0.05$)].

Serum androstenedione concentrations were similar in the placebo and DHA groups on day 0 (5.5 ± 0.2 vs 4.3 ± 0.6 nmol/l, $P = N$), and serum androstenedione levels did not change in the placebo group during the study. In the DHA group, however, the mean serum androstenedione concentration increased 2-fold from 4.3 ± 0.6 to 8.6 ± 1.2 nmol/l ($P < 0.004$). Serum concentrations of estrone, estradiol, total testosterone, free testosterone and sex hormone-binding globulin did not change in either the placebo or DHA group.

Serum concentrations of high-density lipoprotein (HDL) cholesterol, very low density lipoprotein (VLDL) cholesterol, and triglycerides did not change in either the placebo or DHA group. In the DHA group, however, the mean serum total cholesterol concentration fell by 7.1% from 4.82 ± 0.21 to 4.48 ± 0.29 mmol/l ($P < 0.05$), whereas no change occurred in the placebo group. This fall in serum total cholesterol in the DHA group was due almost entirely to a 7.5% fall in serum LDL cholesterol concentrations from 3.21 ± 0.11 to 2.97 ± 0.14 mmol/l ($P < 0.01$). Again, no change in serum LDL cholesterol levels occurred in the placebo group.

In addition, serum apolipoprotein B (apoB) levels fell in all 5 men who ingested DHA, with a fall of 24.0% in the mean value (from 1229 ± 108 to 934 ± 56 μg/ml; $P < 0.01$) [37]. In contrast, in the placebo group no significant

Table 1. Anthropometric data in normal men before and after 28 days of oral placebo or DHA [1600 mg/day (554.7 mmol/day)] administration

	Day 0	Day 28
	Placebo group ($n = 5$)	
Weight (kg)	75.7 ± 7.0	74.3 ± 7.0
Body fat (%)	16.9 ± 3.5	16.7 ± 2.2
	DHA group ($n = 5$)	
Weight (kg)	77.5 ± 3.4	78.2 ± 3.5
Body fat (%)	15.9 ± 3.7	10.9 ± 1.2

Adapted from results presented in Ref. [35].

change in the mean value occurred. It should be noted, however, that in contrast to the LDL cholesterol results, the magnitude of apoB reduction between the DHA and placebo groups was not statistically different.

No statistically significant change in anthropometric measurements occurred in either the placebo or DHA group (Table 1). In the DHA-treated group, however, despite no change in overall body weight, the percentage of body fat (determined by hydrostatic weighing) appeared to decrease an average of 31% in 4 of the 5 men studied (Table 1). In contrast, no change in any anthropometric parameter was observed in the men given placebo.

We recently utilized a slightly different single-blind study design to determine whether obese men might be more susceptible to DHA's putative anti-obesity actions [36]. Six young obese men were administered a placebo daily for 28 days, and then switched over to 1600 mg (554.7 mmol/day) of DHA daily for another 28 days. Inconsistent effects of DHA on weight and body fat mass were noted. In 2 men body fat mass was lower after DHA administration, but for the group as a whole neither total weight nor body fat mass changed during the study. In this study DHA also failed to affect serum lipids. The discrepancies between our two studies may have been due to differences in study design (a placebo group studied in parallel with a DHA group [35] vs a single group receiving serially first placebo and then DHA [36]) or the daily dose of DHA administered on a total weight basis (20.8 ± 1.0 mg/kg in nonobese men [35] vs 16.7 ± 0.9 mg/kg in obese men [36]; $P < 0.015$).

In neither our study of young nonobese [35] nor obese men [36] did DHA administration affect either fasting serum insulin or glucose levels or tissue sensitivity to insulin (as determined by either the hyperinsulinemic–euglycemic clamp technique [45] or Bergman's modified minimal model technique [46], respectively).

DISCUSSION

Our studies show that acute hyperinsulinemia results in a reduction in serum DHA and DHA-sulfate levels in man. The mechanism(s) subserving these reductions remains unknown, although studies examining DHA/DHA-sulfate clearance have failed to show increased catabolism of these steroids during experimentally-induced hyperinsulinemia [33], and evidence exists that insulin may inhibit DHA production [34, 41, 42]. Since suprapharmacologic serum insulin levels were attained in our studies, the physiologic relevance of our findings is unclear. Nonetheless, it should be noted that other investigators have reported a similar decline in serum DHA-sulfate levels during physiologic elevations of serum insulin levels [38–40]. Although speculative, the increased insulin resistance that accompanies aging [47]—and, consequently, the progressive increase in insulinemia—may in some way contribute to the steady decline in serum DHA and DHA-sulfate levels as one grows older. Thus, insulin may function as a physiologic regulator of adrenal function. This nonpituitary control mechanism may partly explain why serum DHA and DHA-sulfate levels fall with aging, while serum levels of other adrenal androgens and glucocorticoids, as well as adrenal responsiveness to ACTH, remain stable [3].

Although we hypothesized that DHA might function as a physiologic regulator of tissue sensitivity to insulin, short-term administration of DHA to healthy young men does not appear to alter fasting serum insulin or glucose levels or tissue sensitivity to insulin. It should be emphasized, however, that the apparent lack of effect of DHA on tissue insulin sensitivity should be interpreted with caution. The techniques for measuring tissue insulin sensitivity employed in our studies [35, 36], although state of the art, may miss a subtle but significant improvement in insulin sensitivity. For example, it has recently been reported that a mean intraindividual coefficient of variation in insulin sensitivity (S_I) of 26% was observed in 9 subjects studied on 3 occasions by Bergman's modified minimal model technique [48].

Furthermore, DHA's beneficial actions on glucose tolerance may involve mechanisms other than tissue insulin sensitivity, such as improved insulin release or beneficial effects on hepatic glucose output. Our studies were performed in young nondiabetic men whose serum

DHA and DHA-sulfate concentrations were at peak levels. Thus, these studies do not exclude the possibility that DHA might alter insulin sensitivity if administered for a longer duration, to a group of individuals with preexisting insulin resistance (e.g. Type II diabetic patients), or to individuals whose serum DHA and DHA-sulfate levels are low (e.g. the elderly). Supporting this possibility, it should be noted that even in animal models DHA does not exert an anti-diabetic action in normal young animals—only in animals with a genetic susceptibility [(*-db/db*) or (*-ob/ob*)] for developing diabetes [15–19] or in aged animals [17]. Studies in elderly individuals (whose basal DHA/DHA-sulfate levels are low) or in individuals with diabetes mellitus (who are resistant to insulin) still need to be conducted.

Of our observations, the one that has engendered the most public interest is the association of DHA administration with a reduction in body fat mass. This is not surprising, since obesity is such a common disorder and effective therapies remain elusive. We caution, however, that in our studies DHA exerted a variable effect on body fat mass, and no statistically significant effect of DHA on body composition was observed [35, 36]. Nonetheless, we should note that these studies were performed in young healthy men, whose serum DHA and DHA-sulfate levels were presumably at their zenith, and that studies in elderly individuals, whose basal DHA and DHA-sulfate levels are low, still need to be conducted.

The most promising therapeutic effect of DHA may lie in its hypolipidemic and, possibly, anti-atherogenic properties. The reductions in serum LDL cholesterol and apoB levels which we observed in our study of nonobese men [35] are particularly striking, since young normocholesterolemic men were studied in whom basal serum DHA and DHA-sulfate concentrations were presumably at peak levels. The lack of effect of DHA on serum lipids in young obese men [36] remains unexplained. Furthermore, several animal studies have shown a protective effect of DHA against the development of aortic [6, 7] and coronary [8] atherosclerosis without a significant change in serum lipids. In one of these studies, which employed a cholesterol-fed heart-transplanted rabbit model of accelerated atherosclerosis, coronary luminal stenosis in rabbits fed DHA was reduced by 72% compared to paired control rabbits not fed DHA [8]. Although DHA may confer protection against the development of coronary atherosclerosis via a subtle hypolipidemic action, the possibility exists that DHA may exert anti-atherogenic effects through other mechanisms as well (e.g. interpolation into cellular membranes, alterations in the physicochemical properties of lipoproteins, suppression of superoxide radical generation etc.).

In summary, a steroid produced as abundantly as DHA, and whose metabolism is regulated so distinctly from other steroids, is likely to serve an important biologic function. Although numerous animal studies suggest such functions, their applicability to human physiology remains uncertain. A renewed interest in DHA has emerged over the past decade, which will hopefully result in an increasing number of human studies designed to delineate DHA's metabolic actions in man.

Acknowledgements—This work was supported in part by NIH Grants DK18903 and PR00065, a Fellowship Grant from the Virginia Affiliate of the American Diabetes Association and a Feasibility Grant from the American Diabetes Association. We thank Drs Cornelius Barlascini, Keith Usiskin and Devin Welty and the staff of the General Clinical Research Center of the Medical College of Virginia for their assistance in performing the studies. We also thank Dr Jerome Strauss III of the University of Pennsylvania for some of the steroid measurements and Drs Yadon Arad and Henry Ginsberg of Columbia University for the apoB determinations.

REFERENCES

1. Orentreich N., Brind J. L., Rizer R. L. and Vogelman J. H.: Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J. Clin. Endocr. Metab.* **59** (1984) 551–555.
2. Migeon C. J., Keller A. R., Lawrence B. and Shepard II T. H.: Dehydroepiandrosterone and androsterone levels in human plasma. Effect of age and sex; day-to-day and diurnal variations. *J. Clin. Endocr. Metab.* **17** (1957) 1051–1062.
3. Parker L. N.: *Adrenal Androgens in Clinical Medicine*. Academic Press, San Diego, CA (1989) pp. 118–134.
4. Ben-David M., Dikstein S., Bismuth G. and Sulman F. G.: Anti-hypercholesterolemic effect of dehydroepiandrosterone in rats. *Proc. Soc. Exp. Biol. Med.* **125** (1967) 1136–1140.
5. Kurzman I. D., MacEwen E. G. and Haffa A. L. M.: Reduction in body weight and cholesterol in spontaneously obese dogs by dehydroepiandrosterone. *Int. J. Obesity* **14** (1990) 95–104.
6. Gordon G. B., Bush D. E. and Weisman H. F.: Reduction of atherosclerosis by administration of dehydroepiandrosterone. A study in the hypercholesterolemic New Zealand White rabbit with aortic intimal injury. *J. Clin. Invest.* **82** (1988) 712–720.
7. Arad Y., Badimon J. J., Badimon L., Hembree W. C. and Ginsberg H. N.: Dehydroepiandrosterone feeding prevents aortic fatty streak formation and cholesterol accumulation in cholesterol-fed rabbit. *Arteriosclerosis* **9** (1989) 159–166.

8. Eich D. M., Johnson D. E., Nestler J. E., Daijin K., Hess M. L. and Wechsler A. S.: Inhibition of cardiac allograft atherosclerosis by dehydroepiandrosterone. *Clin. Res.* **38** (1990) 2A.
9. Yen T. Y., Allan J. A., Pearson D. V. and Acton J. M.: Prevention of obesity in Avy/a mice by dehydroepiandrosterone. *Lipids* **12** (1977) 409–413.
10. Schwartz A. G.: Inhibition of spontaneous breast cancer formation in female C3H(Avy/a) mice by long-term treatment with dehydroepiandrosterone. *Cancer Res.* **39** (1979) 1129–1132.
11. Schwartz A. G., Hard G. C., Pashko L. L., Abou-Gharbia M. and Swern D.: Dehydroepiandrosterone: an anti-obesity and anti-carcinogenic agent. *Nutr. Cancer* **3** (1981) 46–53.
12. Nyce J. W., Magee P. N., Hard G. C. and Schwartz A. G.: Inhibition of 1,2-dimethylhydrazine-induced colon tumorigenesis in BALB/c mice by dehydroepiandrosterone. *Carcinogenesis (Lond.)* **5** (1984) 57–62.
13. Pashko L. L. and Schwartz A. G.: Effect of food restriction, dehydroepiandrosterone, or obesity on the binding of 3H-7,12-dimethylbenz(*a*)anthracene to mouse skin DNA. *J. Geront.* **38** (1983) 8–12.
14. Cleary M. P., Seidenstat R., Tannen R. H. and Schwartz A. G.: The effect of dehydroepiandrosterone on adipose tissue cellularity in mice. *Proc. Soc. Exp. Biol. Med.* **171** (1982) 276–284.
15. Coleman D. L., Leiter E. H. and Schwizer R. W.: Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. *Diabetes* **21** (1982) 830–833.
16. Coleman D. L., Leiter E. H. and Applezweig N.: Therapeutic effects of dehydroepiandrosterone metabolites in diabetes mutant mice (C57BL/KsJ-db/db). *Endocrinology* **115** (1984) 239–243.
17. Coleman D. L., Schwizer R. W. and Leiter E. H.: Effect of genetic background on the therapeutic effects of dehydroepiandrosterone (DHEA) in diabetes-obesity mutants and in aged normal mice. *Diabetes* **33** (1984) 26–32.
18. Coleman D. L.: Antiobesity effects of etiocholanolones in diabetes (db), viable yellow (Avy), and normal mice. *Endocrinology* **117** (1985) 2279–2283.
19. Coleman D. L.: Therapeutic effects of dehydroepiandrosterone and its metabolites in diabetes-obesity mutants. In *Proceedings of the 18th Steenbock Symposium "Hormones, Thermogenesis and Obesity"* (Edited by H. A. Lardy and F. Stratman). Elsevier, New York (1989) pp. 377–384.
20. Weindruch R., McFeeters G. and Walford R. L.: Food intake reduction and immunologic alterations in mice fed dehydroepiandrosterone. *Expl Geront.* **19** (1984) 297–304.
21. Lucas J. A., Ahmed S. A., Casey L. M. and MacDonald P. C.: Prevention of autoantibody formation and prolonged survival in New Zealand black/New Zealand white F1 mice fed dehydroepiandrosterone. *J. Clin. Invest.* **75** (1985) 2091–2093.
22. Tannen R. H. and Schwartz A. G.: Reduced weight gain and delay of Coomb's positive hemolytic anemia in NZB mice treated with dehydroepiandro- sterone (DHEA). *Redn Proc.* **41** (1982) 463 (Abstr. No. 1131).
23. Cleary M. P., Billheimer J. and Schwartz A. G.: Effect of dehydroepiandrosterone in Zucker rats: a possible futile cycle. *Gerontology* **22** (1982) 136.
24. Cleary M. P., Shepherd A. and Jenks B.: Effect of dehydroepiandrosterone on growth in lean and obese Zucker rats. *J. Nutr.* **114** (1984) 1242–1251.
25. Shepherd A. and Cleary M. P.: Metabolic alterations after dehydroepiandrosterone treatment in Zucker rats. *Am. J. Physiol.* **246** (1984) E123–128.
26. Cleary M. P., Fox N., Lazin B. and Billheimer J. T.: A comparison of the effects of dehydroepiandrosterone treatment to ad libitum and pair-feeding in the obese Zucker rat. *Nutr. Res.* **5** (1985) 1247–1257.
27. Cleary M. P., Shepherd A., Zisk J. and Schwartz A.: Effect of dehydroepiandrosterone on body weight and food intake in rats. *Nutr. Behav.* **1** (1983) 127–136.
28. Tagliaferro A. R. and Davis J. R.: The effect of dehydroepiandrosterone (DHEA) on caloric intake, body weight, and resting metabolism. *Fedn Proc.* **42** (1983) 326.
29. Casazza J. P., Schaffer W. T. and Veech R. L.: The effect of dehydroepiandrosterone on liver metabolites. *J. Nutr.* **116** (1986) 304–310.
30. Pashko L. L., Fairman D. K. and Schwartz A. G.: Inhibition of proteinuria development in aging Sprague–Dawley rats and C57BL/6 mice by long-term treatment with dehydroepiandrosterone. *J. Geront.* **41** (1986) 433–438.
31. Cleary M. P., Hood S. S., Chando C., Hansen C. T. and Billheimer J. T.: Response of sucrose-fed BHE rats to dehydroepiandrosterone. *Nutr. Res.* **4** (1984) 485–494.
32. Nestler J. E., Clore J. N., Strauss III J. F. and Blackard W. G.: The effects of hyperinsulinemia on serum testosterone, progesterone, dehydroepiandrosterone sulfate, and cortisol levels in normal women and in a woman with hyperandrogenism, insulin resistance, and acanthosis nigricans. *J. Clin. Endocr. Metab.* **64** (1987) 180–184.
33. Nestler J. E., Usiskin K. S., Barlascini C. O., Welty D. F., Clore J. N. and Blackard W. G.: Suppression of serum dehydroepiandrosterone sulfate levels by insulin: an evaluation of possible mechanisms. *J. Clin. Endocr. Metab.* **69** (1989) 1040–1046.
34. McClanahan M. A., Clore J. N., Blackard W. G. and Nestler J. E.: Insulin inhibits adrenal 17,20-lyase activity. *Clin. Res.* **30** (1990) 979A.
35. Nestler J. E., Barlascini C. O., Clore J. N. and Blackard W. G.: Dehydroepiandrosterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity in normal men. *J. Clin. Endocr. Metab.* **66** (1988) 57–61.
36. Usiskin K. S., Butterworth S., Clore J. N., Arad Y., Ginsberg H. N., Blackard W. G. and Nestler J. E.: Lack of effect of dehydroepiandrosterone in obese men. *Int. J. Obesity* **14** (1990) 457–463.
37. Nestler J. E., Barlascini C. O., Clore J. N., Blackard W. G., Ginsberg H. N. and Arad Y.: Effects of dehydroepiandrosterone (DHEA) on serum apolipoprotein B (apoB) levels and low-density lipoprotein (LDL) particle size in normal men. *Clin. Res.* **37** (1989) 22A.
38. Diamond M. P., Grainger D., Gill A. L., Zych K., Knab G. and DeFronzo R. A.: Exogenous and endogenous elevations of circulating insulin levels are associated with acute reduction in levels of dehydroepiandrosterone sulfate (DHAS), but not androstenedione (A), testosterone (T), or free testosterone (FT). In *Scientific Program and Abstracts for the 36th Annual Meeting of the Society for Gynecologic Investigation* (1989) p. 293 (Abstr. 424).
39. Hubert G. D., Schriock E. D., Givens J. R. and Buster J. E.: Insulin suppression of adrenal androgen concentrations during glucose tolerance testing in normal women. In *Scientific Program and Abstracts for the 37th Annual Meeting of the Society for Gynecologic Investigation* (1990) p. 112 (Abstr. 31).
40. Falcone T., Finegood D. T., Fantus G. and Morris D.: Androgen response to endogenous insulin secretion during the frequently sampled intravenous glucose tolerance test in normal and hyperandrogenic women. *J. Clin. Endocr. Metab.* **71** (1990) 1653–1657.
41. Farah M. J., Givens J. R. and Kitabchi A. E.: Bimodal correlation between the circulating insulin level and the production rate of dehydroepiandrosterone: positive correlation in controls and negative correlation in the polycystic ovary syndrome with acanthosis nigricans. *J. Clin. Endocr. Metab.* **70** (1990) 1075–1081.

42. Kramer R. E., Buster J. E. and Andersen R. N.: Differential modulation of ACTH-stimulated cortisol and androstenedione secretion by insulin. *J. Steroid Biochem.* **36** (1990) 33–42.
43. Parker Jr C. R., Simpson E. R., Bilheimer D. W., Leveno K., Carr B. R. and Macdonald P. C.: Inverse relation between low-density lipoprotein-cholesterol and dehydroepiandrosterone sulfate in human fetal plasma. *Science* **208** (1980) 512–514.
44. Barrett-Connor E., Khaw K-T. and Yen S. S. C.: A prospective study of dehydroepiandrosterone sulfate, motality, and cardiovascular disease. *N. Engl. J. Med.* **315** (1986) 1519–1524.
45. Rizza R. A., Mandarino L. J. and Gerich J. E.: Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am. J. Physiol.* **240** (1981) E630–E639.
46. Yang Y. J., Youn J. H. and Bergman R. N.: Modified protocols improve insulin sensitivity estimation using the minimal model. *Am. J. Physiol.* **253** (1987) E595–E602.
47. Grunberger G., Taylor S. I., Dons R. F. and Gorden P.: Insulin receptors in normal and disease states. *Clinics Endocr. Metab.* **12** (1983) 191–219.
48. Buchanan T., Murray J., Watanabe R. and Bergman R.: Short-term variations in insulin sensitivity but not glucose effectiveness in normal men. *Clin. Res.* **37** (1989) 127A.