

Relationship between Dehydroepiandrosterone Sulfate and Ischemic Heart Disease in Men

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Abstract:

Thirty two patients with ischemic heart disease (aged 40- 69 years) involved in this study during their admission Ibn-Albitar, Ibn-Alnafees Hospitals\ Baghdad and the Iraqi Center for Heart Surgery, Medical City. Age matched twenty seven healthy men also included as control group. The results obtained are: serum triglycerides (TG) and very low density lipoprotein (VLDL) shows a significant ($p<0.05$) increase in patients with I.H.D as compared with control group in age range 50-59 years. As well as, no significant correlation was found in I.H.D and control groups between dehydroepiandrosterone sulfate (DHEA-S) and lipid profile, although there was a slight negative correlation between DHEA-S levels and each of TG and VLDL in I.H.D group.

Introduction:

A number of prospective studies comparing serum DHEA or DHEA-S levels in cardiovascular disease cases versus controls have been published. Most of these studies were nested case-control designs, which provide useful information on risk factors for various cardiovascular disease endpoints, but generally have the caveat of overestimating the relative risk associated with a given factor. Results have been inconclusive in both men and women. In women, a study found lower DHEA-S levels in ischemic heart disease cases compared with controls (Haffner *et al.*, 1996), whereas this association was not found in the an other study (Hill *et al.*, 2002). In men, results are even more contradictory, as one study found lower DHEA-S levels in myocardial infarction cases compared with controls (Lacroix *et al.*, 1992), while another smaller size study actually found increased DHEA-S in myocardial infarction cases (Hautanen *et al.*, 1994). Two other studies did not find a significant association (Contoreggi *et al.*, 1990; Newcomer *et al.*, 1994).

Epidemiological prospective population studies have examined the relationship of endogenous serum DHEA-S levels with cardiovascular disease mortality rates. Prospective population studies on DHEA-S and mortality rates in men as opposed to the statistically non-significant studies in women, most studies in men (five out of seven) found that elevated levels of DHEA-S were associated with lower mortality rates, either from cardiovascular disease or any other cause (Barrett-Connor *et al.*, 1986; Barrett- Connor and Good man- Gruen, 1995; Beer *et al.*, 1996; Trivedi and Khaw, 2001; Mazat *et al.*, 2001), while the two others generated non-significant results (Legrain *et al.*, 1995; Tilvis *et al.*, 1999).

Several other longitudinal, cross-sectional and retrospective analyses have examined the relationship between DHEA-S levels and the presence or the extent of various aspects of cardiovascular disease. Several studies have identified plasma DHEA or DHEA-S as being cardioprotective (Ishihara *et al.*, 1992; Mitchell *et al.*, 1994; Herrington, 1995; Bernini *et al.*, 2001). In both men and women, elevated DHEA-S levels were associated with retarded progression of atherosclerosis measured by coronary artery angiography (Herrington, 1995), ultrasound carotid wall thickness (Bernini *et al.*, 2001) and pulse wave velocity aorta calcification (Ishihara *et al.*, 1992). On the other hand, other groups did not confirm these findings using coronary angiography in two male samples (Hauner *et al.*, 1991; Phillips *et al.*, 1994). Retrospective studies have examined whether myocardial infarction survivors were

characterized by reduced DHEAS levels compared with healthy controls (Zumoff *et al.*, 1982; Slowinska *et al.*, 1989; Mitchell *et al.*, 1994). Two of these studies demonstrated that DHEA-S levels were lower in myocardial infarction survivors compared with healthy controls (Slowinska *et al.*, 1989; Mitchell *et al.*, 1994), while the third one found the opposite (Zumoff *et al.*, 1982). In a Japanese studies, the researchers have found, as expected, that DHEA levels were negatively correlated with the older men, the lower their DHEA levels. They also found significant negative correlation between DHEA levels and carotid-artery wall thickness and between DHEA levels and the total carotid "plaque score", which could be calculated from the measurements of wall thickness. In other words, lower levels of DHEA were significantly correlated with higher values of these reliable indicators of atherosclerosis. Also it was observed that DHEA levels were significantly lower in the diabetic men who had cardiovascular disease (CVD) than in those who did not (Baulieu *et al.* 2000; Fukui *et al.*, 2005). Ng and colleagues (2003) have shown a DHEA increases human macrophage foam cell formation, a potentially pro-atherogenic effect. This effect appears to be mediated via the androgen receptor and involves the upregulation of lipoprotein-processing enzymes.

The reasons for the numerous discrepancies among studies are unclear at the present time. It has been suggested that they may be due to the possible confounding effect of smoking on both DHEA-S and cardiovascular disease, or to differences in the analytical methods used or to the cardiovascular endpoint selected (Nafziger *et al.*, 1991; Mitchell *et al.*, 1994), although some studies cited above have already avoided these caveats. It has also been hypothesized that differences may be attributable to population variability. For example, the prospective study by Mazat *et al.* (2001), demonstrated that the relative risk of 8-year mortality associated with low DHEA-S was 3.4 times higher in males under 70 years compared with older men (odds ratios of 6.5 versus 1.9 respectively), suggesting substantial heterogeneity in this population according to age (Mazat *et al.*, 2001). Adjustment for BMI in most studies may have attenuated the relationship of DHEA-S to cardiovascular disease endpoints. Finally, most studies have relied on a single measure of plasma DHEA-S performed several years before the disease events. Diurnal variations in the levels of this hormone and possible alterations in frozen samples over time may have had an impact on the associations (Wu Fc and Von Eckardstein, 2003).

Subjects and Methods:

Fifty nine cases included in this work, thirty two patients with ischemic heart disease aged 40- 69 years (mean age 54.31) and twenty seven healthy men aged 40-69 years (mean age 54.40). This study was carried out in the Ibn-Albitar, Ibn-Alnafees Hospitals\ Baghdad and the Iraqi Center for Heart Surgery, Medical City \ Baghdad. Five milliliters of venous blood was obtained by antecubital vein-puncture using G 23 needle were drawn from ischemic heart disease patients and control subjects between 8:30-10A.M after 12 hour fasting. The blood was allowed to clot in plain test tube at room temperature. The serum was aspirated after centrifugation at 3000 rpm for 10 minutes, divided into aliquots in plastic tubes and stored at -20° until used for measurement of the concentration of DHEA-S hormone and serum lipid profile such as TC, TG, HDL, LDL, VLDL and atherogenic index. Total cholesterol and high density lipoprotein kit for quantitative colorimetric determination of TC and HDL-C in serum was supplied by Biolabo SA, France, this method presented by Tietz, 1999. Also serum triglycerides level was measured by Biolabo SA, France kit, this method presented by Fossati and Principe, 1982, moreover dehydroepiandrosterone Sulfate kit for the direct immunoenzymatic determination of DHEA-S concentration in serum

was purchased from DiaMetra- Italy, this method presented by (Abraham *et al.*, 1976). The data expressed as mean $\pm$ S.E. SPSS version 10 for window was used for all statistical analyses. Statistical significance was assessed by ANOVA, P- values of less than (0.05 – 0.01) was considered significant. Regression analysis was chosen as a statistical tool to investigate the effect of DHEA-S on the measured parameters and to find the correlation simple linear regression was used and the correlation coefficient (r) was calculated.

Results:

There was no significant difference in levels of DHEA-S between patients with ischemic heart disease and control group. But when compared among different age groups within studied groups there was a significant ($p < 0.05$) increase of DHEA-S in age range 40- 49 years compared with age range 60- 69 years within I.H.D group as well as there was a significant ($p < 0.05$) increase of DHEA-S in age range 40- 49 years as compared with other age groups within control group as shown in table (1).

Table (1): Dehydroepiandrosterone sulfate ($\mu\text{g/ml}$) levels in ischemic heart disease and control group according to age. Mean $\pm$ S.E.

AGE RANGE(YEARS)	ISCHEMIC HEART DISEASE N=32	CONTROL N=27
40-49	1.55 $\pm$ 0.29*	1.72 $\pm$ 0.28†
50-59	1.18 $\pm$ 0.18	0.92 $\pm$ 0.22
60-69	0.81 $\pm$ 0.21	0.47 $\pm$ 0.06

* denote a significant ($p < 0.05$) difference compared to age range 60-69 years group

† denote a significant ($p < 0.05$) difference compared to other age groups.

The results of the concentration lipid profile tests were compared among the studied groups at each range of age, the results indicated that:

In age range 40-49 years, serum cholesterol, T.G, HDL, LDL, VLDL and atherogenic index did not differ significantly between studied groups as shown in table (2). On the other hand, in age range 50-59 years, the serum cholesterol, HDL, LDL and atherogenic index showed no significant difference between studied groups. But the serum T.G and VLDL showed a significant ($p < 0.05$) difference increase in I.H.D patients as compared with control group at the same age group. No significant differences in all parameter of lipid profile were observed between studied groups in age range 60-69 years (Table 2). The same table also shows a significant ($p < 0.05$) increase in LDL in age group 50- 59 years as compared with age range 60- 69 years within I.H.D group.

Table (2): Serum Cholesterol, Triglyceride, HDL, LDL, VLDL and Atherogenic index levels according to age in I.H.D and control group. Mean $\pm$ S.E

GROUPS	SUBJECT NO.	AGE MEAN (YEARS)	AGE RANGE (YEARS)	CHOL MG\DL	T.G MG\DL	HDL MG\DL	LDL MG\DL	VLDL MG\DL	ATHER O. INDEX
I.H.D	10	45.4	40-49	186.70 $\pm$ 16.44	157.69 $\pm$ 16.70	37.39 $\pm$ 2.83	117.80 $\pm$ 14.00	31.51 $\pm$ 3.34	5.18 $\pm$ 0.50
	12	54.5	50-59	206.27 $\pm$ 10.54	166.85 $\pm$ 21.13	38.13 $\pm$ 1.77	134.78 $\pm$ 7.88	33.35 $\pm$ 4.22	5.45 $\pm$ 0.35
				174.56	172.42	37.96	102.13	34.47	4.78

	10	63	60-69	± 11.10	± 32.25	± 2.39	± 9.04	± 6.45	± 0.55
Control	10	45.3	40-49	173.79 ± 8.55	143.09 ± 11.82	40.40 ± 2.10	104.78 ± 6.34	28.61 ± 2.36	4.35 ± 0.20
	10	55.3	50-59	177.34 ± 6.79	118.79 ± 10.55	38.94 ± 2.47	114.65 ± 5.37	23.75 ± 2.10	4.64 ± 0.25
	7	65.1	60-69	167.71 ± 9.70	114.14 ± 13.84	42.71 ± 2.84	107.31 ± 10.49	22.82 ± 2.76	3.97 ± 0.29

† denote a significant difference ($p < 0.05$) compared to control in age range 50-59 year.

‡ denote a significant difference ($p < 0.05$) compared to age range 50- 69 years within I.H.D.

Chol: cholesterol, **T.G:** triglyceride, **HDL:** high density lipoproteins, **LDL:** low density lipoproteins, **VLDL:** very low density lipoproteins and **I.H.D:** ischemic heart disease

The relationship between the evaluation of DHEA-S level and lipid profile parameters was also studied. No significant difference was found in correlation between DHEA-S level and lipid profile in I.H.D and control group (Figure 1 and Figure 2, respectively) although, there was a slight negative correlation between DHEA-S levels and each of TG and VLDL in I.H.D group as shown in figure (1)

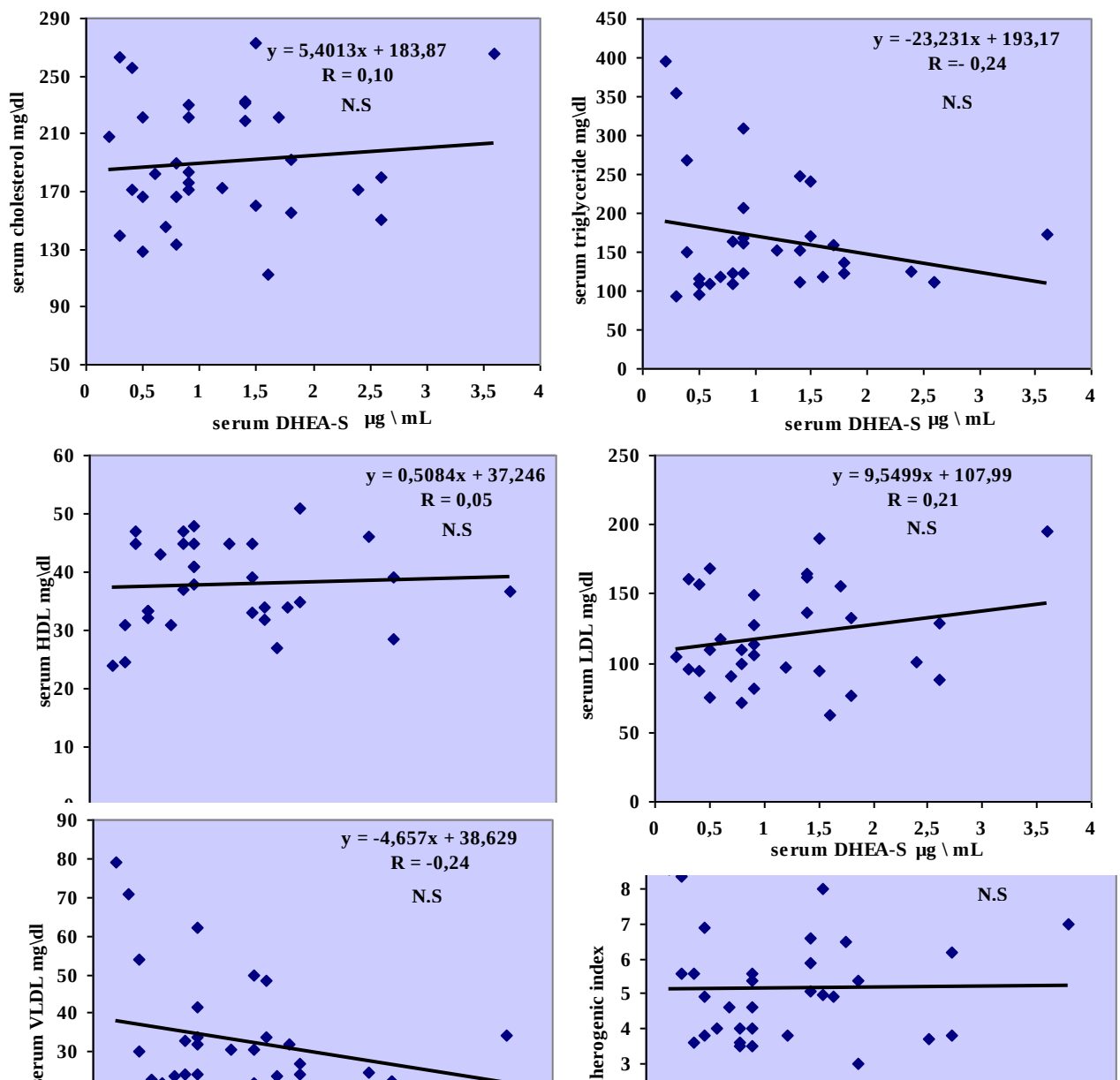


Figure (1): Correlation between dehydroepiandrosterone sulfate and lipid profile in ischemic heart disease group

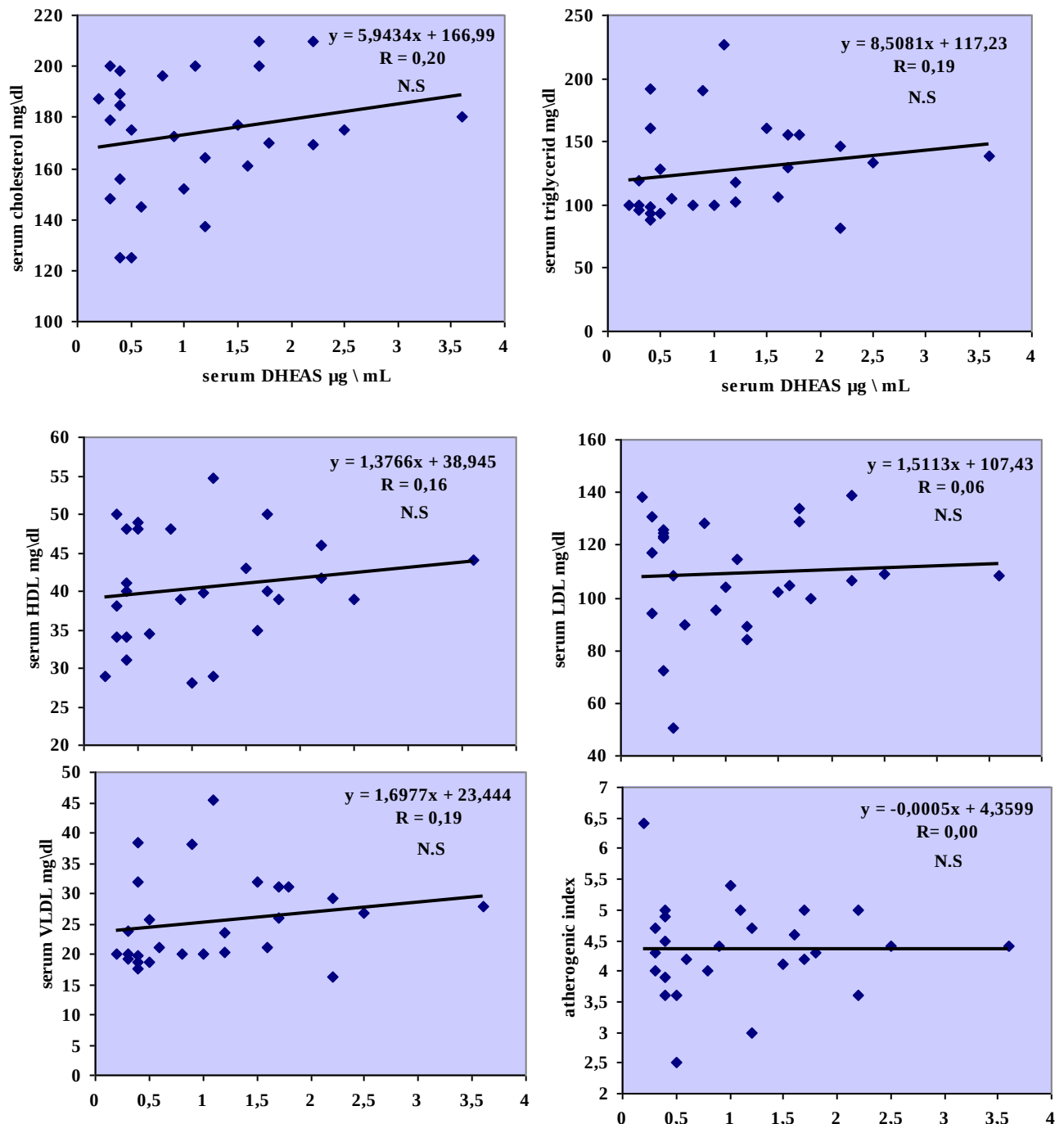


Figure (2): Correlation between dehydroepiandrosterone sulfate and lipid profile in control group

Discussion:

The incidence of atherosclerosis and its related disease increases with aging (Reaven *et al.*, 1999). Moreover, aging is associated with several important biochemical modifications in the arterial wall (Nakamura *et al.*, 1999) as well as in hormonal status such as dehydroepiandrosterone, which may be implicated in the increased incidence of cardiac vascular disease.

Comparisons of DHEA-S levels in this study with those from other samples are problematic because differences may result from variation in how the samples are defined. Although we have not found any other reports of DHEA-S levels for a nationally representative samples, data from various community- based samples suggest that comparisons between different countries differ depending on sex, age and the samples itself.

Multiple prospective studies of middle- age and elderly subjects have investigated the relationship between DHEA-S levels and atherosclerosis. It has been reported that low DHEA-S levels, as found in the elderly, are associated with increased cardiovascular morbidity and mortality (LaCroix *et al.*, 1992; Hayashi *et al.*, 2000; Moriyama *et al.*, 2000; Feldman *et al.*, 2001; Bjørnerem *et al.*, 2004; Fukui *et al.*, 2004; Fukui *et al.*, 2007 and Khaw *et al.*, 2007). However, several other studies failed to detect such association (Baulieu *et al.*, 2000; Kiechl *et al.*, 2000; Trivedi and Khaw., 2001; Nair *et al.*, 2006; Arnlov *et al.*, 2006; Hougaku *et al.*, 2006 and Araujo *et al.*, 2007), but other studies showed elevated levels of DHEA-S in patients with cardio vascular disease (CVD) compared with control group (Zumoff *et al.*, 1982; Hautanen *et al.*, 1994).

Simoncini *et al.* (2003) reported that the DHEA is able to directly regulate the human vascular wall by controlling nitric oxide synthesis in endothelial cells, which improved vessel dilation. As well as there were several plausible mechanisms for a beneficial effect of DHEA-S on cardiovascular morbidity or mortality, these include prevention of platelets aggregation (Jesse *et al.*, 1991), inhibition of macrophage accumulation in the intima as well as proliferation of smooth muscle cells from the media into the intima (Dworkin *et al.*, 1986), interference with arterial uptake of cholesterol (Leszczynski *et al.*, 1989), or conversion of DHEA to estrogen, because the estrogen inhibition of aromatase (estrogen- converting enzyme) and able to inhibit cholesterol accumulation and intimal hyperplasia (Hayashi *et al.*, 2000).

On the other hand, Hautanen *et al.* (1994) using data from the Helsinki heart study (a trial involving dyslipidemic men with a mean age of 48 years), found that after adjustment for smoking and age, men with high DHEA-S levels had a two fold increased risk of myocardial infarction, as compared with men with lower levels. DHEA has been reported to increase human macrophage foam cell formation, which is potentially proatherogenic (Ng *et al.*, 2003). Moreover, Zumoff *et al.* (1982) reported that prior myocardial infarction patients showed significantly elevated

plasma concentration of estrone (E_1), DHEA and DHEA-S compared with normal controls. In contrast, Bjørnerem *et al.* (2004) reported that men with chronic diseases had lower levels of DHEA-S compared with healthy men.

A number of studies have examined the relationships between endogenous DHEA or DHEA-S and serum levels of lipids and lipoprotein. Results from these studies have been somewhat inconsistent (Villareal *et al.*, 2000; Arlt *et al.*, 2001; Kawano *et al.*, 2003; Lovas *et al.*, 2003), our study also failed to show any significant correlation between serum lipid profile and DHEA-S levels in different studied groups.

Examining the effects of DHEA replacement in subjects with previously documented hypercholesterolemia did not lead to the finding of significant effects of the treatment in a 12-week randomized placebo-controlled study (Kawano *et al.*, 2003). But other studies have reported a significant inverse correlation between DHEA-S and LDL-C (Okamoto, 1996). Indeed, some studies have also found the elevated plasma DHEA or DHEA-S levels may be related to a favorable plasma lipid-lipoprotein profile through positive relation with the HDL-C concentration (Haffner *et al.*, 1993; Abbassi *et al.*, 1998), and negative association with total cholesterol (Tchernof *et al.*, 1997).

There were several variables such as differences in age as well as the degree of obesity and abdominal fat accumulation may have represented important confounding factors in the evaluation of these associations.

Accordingly, the associations between serum DHEA and variables of the lipid-lipoprotein profile could be explained to a large extent by concomitant variations in adiposity and abdominal body fat accumulation (Tchernof *et al.*, 1997). Interestingly, at least in men, the associations of DHEA with variables of the lipid profile are similar to those of testosterone with these variables (Tchernof *et al.*, 1997); namely, elevated plasma androgens appear to be related to a favorable plasma lipid lipoprotein profile in men (Tchernof and Despres, 2000), which supports the notion that DHEA may be related to the lipid profile through its conversion to androgenic steroids. Therefore, the fail in concurring any association between DHEA-S and lipid profile in our study, may be related to decrement the conversion DHEA to androgen or estrogen, while, Arlt *et al.* (2001) who reported the conversion of DHEA to androgen or estrogen can only take place after removal of the sulfate group, i.e. the conversion from DHEA-S to DHEA, by widespread tissue sulfatase activity. Of note, the DHEA/DHEA-S ratio was significantly lower in the older age group. This might reflect reduced sulfatase activity in older men and thus reduced availability of DHEA for peripheral bioconversion.

References

- Abbassi, A.; Duthie, E. H.; Sheldahl, L.; Wilson, C.; Sasse, E. and Rudman, I. (1998). Association of dehydroepiandrosterone sulfate, body composition and physical fitness in independent community-dwelling older men and women. *J. Am. Geriatr. Soc.*, 46: 263–273.
- Abraham, G. E.; Maroulis, G. B.; Buster, J. E.; Chang, R. J.; Marshall, J. R. (1976). Effect of dexamethasone on serum cortisol and androgen levels in hirsute patients. *Obstet. Gynecol.*, 47: 395- 402.
- Araujo, A. B.; Kupelian, V.; Page, S. T.; Handelsman, D. J.; Bremner, W. J.; McKinlay, J. B. (2007). Sex Steroids and All-Cause and Cause-Specific Mortality in Men. *Arch. Intern. Med.*, 167:1252-1260.
- Arlt, W.; Callies, F.; Koehler, I.; van Vlijmen, J. C.; Fassnacht, M. and Strasburger, C. J. (2001). Dehydroepiandrosterone supplementation in healthy men

with an age-related decline of dehydroepiandrosterone secretion. *J. Clin. Endocrinol. Metab.*, 86: 4686–4692.

Arnlov, J.; Pencina, M. J.; Amin, S.; Nam, B.; Benjamin, E. J.; Murabito, J. M.; Wang, T. J.; Knapp, P.E.; D'Agostino, R. B.; Bhasin, S.; and Vasan, R. S. (2006). Endogenous Sex Hormones and Cardiovascular Disease Incidence in Men. *Ann. Intern. Med.*, 145:176-184.

Barrett-Connor, E. and Goodman-Gruen, D. (1995). The epidemiology of DHEAS and cardiovascular disease. *Annals of the New York Academy of Sciences*, 774: 259-270.

Barrett-Connor, E.; Khaw, K. T. and Yen, S. A. (1986). Prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl. J. Med.* 315: 1519-1524.

Baulieu, E. E.; Thomas, G.; Legrain, S.; Lahlou, N.; Roger, M.; Debuire, B.; Veronique, F.; Girard, L.; Hervy, M.; Latourj, F.; Leaudk, M.; Mokranel, A.; Pitti- errandim, H.; Trivallef, C.; et al., (2000). Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: Contribution of the DHEAge Study to a sociobiomedical issue. *PNAS*, 97:4279-4284.

Beer, C.; Lafont, S.; Debuire, B, Dartigues, J. F. and Beaulieu, E. E. (1996). Relationships of dehydroepiandrosterone sulfate in the elderly with functional, psychological, and mental status, and shortterm mortality: a French community-based study. *PNAS*, 93: 13410-13415.

Bernini, G. P.; Moretti, A.; Sgro, M.; Argenio, G. F., Barlascini, C. O. and Cristofani, R. (2001). Influence of endogenous androgens on carotid wall in postmenopausal women. *Menopause*, 8: 43-50.

Bjørnerem, Å.; Straume, B.; Midtby, M.; Fønnebø, V.; Sundsfjord, J.; Svartberg, J.; Acharya, G.; Øian, P.; And Rosvold Berntsen, G. K. (2004). Endogenous sex hormone in relation to age, sex, life style factors and chronic diseases in a general population: the tromso study. *J. Clin. Endocrinol. Metab.*, 89: 6039–6047.

Contoreggi, C. S.; Blackman, M. R.; Andres, R.; Muller, D. C.; Lakatta, E. G.and Fleg, J. L. (1990). Plasma levels of estradiol, testosterone, and DHEAS do not predict risk of coronary artery disease in men. *J. Androl.*, 11: 460-470.

Dworkin, C. R.; Gorman, S. D.; Pashko, L. L.; Cristofalo, V. J. and Schwartz, A. G. (1986). Inhibition of growth of HeLa and WI-38 cells by dehydroepiandrosterone and its reversal by ribo- and deoxyribonucleosides. *Life Sci.*, 38: 1451-1457.

Feldman, A. H.; Johannes, B. C.; Araujo, A. B.; Moher, B. A.; Longcope C. and Mckinlay B. H. (2001). Low dehydroepiandrosterone and ischemic heart disease in middle- aged men: prospective results from the Massachusetts male aging study. *Am. J. Epidemiol*, 153: 79-89.

Fossati P.; Prencipe L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem*, 28: 2077- 2080.

Fukui, M.; Kitagawa, Y.; Nakamura, N.; Kadono, M.; Hasegawa, G. and Yoshikawa, T. (2004). Association between urinary albumin excretion and serum dehydroepiandrosterone sulfate concentration in male patients with type 2 diabetes. *Diabetes Care*, 27: 2893-2897.

Fukui, M.; Kitagawa, Y.; Nakamura, N.; Kadono, M.; Yoshida, M.; Hirata, C.; Wada, K.; Hasegawa, G. and Yoshikawa, T. (2005). "Serum dehydroepiandrosterone sulfate concentration and carotid atherosclerosis in men with type 2 diabetes". *Atherosclerosis*, 181: 339-344.

- Fukui, M.; Kitagawa, Y.; Ose, H.; Hasegawa, G.; Yoshikawa, T. and Nakamura, N. (2007).** Role of endogenous androgen against insulin resistance and atherosclerosis in men with type 2 diabetes. *Current Diabetes Reviews*, 3: 25-31.
- Haffner, S. M.; Mykka"nen, L.; Valdez, R. A. and Katz, M. S. (1993).** Relationship of sex hormones to lipids and lipoproteins in nondiabetic men. *J. Clin. Endocrinol. Metab.*, 77: 1610-1615.
- Haffner, S. M; Moss, S. E.; Klein, B. E. and Klein, R. (1996).** Sex hormones and DHEA-SO₄ in relation to ischemic heart disease mortality in diabetic subjects. The Wisconsin epidemiologic study of diabetic retinopathy. *Diabetes Care*. 19: 1045-1050.
- Hauner, H.; Stangl, K.; Burger, K.; Busch, U.; Blomer, H. and Pfeiffer, E. F. (1991).** Sex hormone concentrations in men with angiographically assessed coronary artery disease – relationship to obesity and body fat distribution. *Klinische Wochenschrift*, 69: 664-668.
- Hautanen, A.; Manttari, M.; Manninen, V.; Tenkanen, L.; Huttunen, J. K.; Frick, M. H. and Adlercreutz, H. (1994).** Adrenal androgens and testosterone as coronary risk factors in the Helsinki heart study. *Atherosclerosis*, 105: 191-200.
- Hayashi, T.; Esaki, T.; Muto, E.; Kano, H.; Asai, Y.; Thakur, N. K.; Sumi, D.; Jayachandran, M. and Iguchi, A. (2000).** Dehydroepiandrosterone retards atherosclerosis formation through its conversion to estrogen: the possible role of nitric oxide. *Arterioscler. Thromb. Vasc. Biol.*, 20:782-792.
- Herrington, D. M. (1995).** Dehydroepiandrosterone and coronary atherosclerosis. *Annals of the New York Academy of Sciences*, 774: 271-280.
- Hill, G. S.; Maguire, A.; Ding, J.; Crouse, J. R.; Cauley, J. A.; Zacur, H. (2002).** Endogenous postmenopausal hormones and carotid atherosclerosis: a case-control study of the atherosclerosis risk in communities cohort. *Ameri. J. Epidemiol.* 155: 43-445.
- Hougaku, H.; Flag, J. L.; Najjar, S. S.; Lakatt E. G.; Harman, S. M.; Blackman, M. R. and Metter E. J. (2006).** Relationship between androgenic hormones and arterial stiffness, based on longitudinal hormone measurements. *Am. J. Physiol. Endocrinol. Metab.* 290:E234-E242.
- Ishihara, F.; Hiramatsu, K.; Shigematsu, S.; Aizawa, T.; Niwa, A. and Takasu, N. (1992).** Role of adrenal androgens in the development of arteriosclerosis as judged by pulse wave velocity and calcification of the aorta. *Cardiology*, 80: 332-338.
- Kawano, H.; Yasue, H.; Kitagawa, A.; Hirai, N.; Yoshida, T. and Soejima, H. (2003).** Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. *J. Clin. Endocrinol. Metab.*, 88: 3190–3195.
- Khaw, K. T.; Dowsett, M.; Folkard, E.; Bingham S.; Wareham, N.; Luben, R.; Welch, A. and Day, N. (2007).** Endogenous testosterone and mortality due to all causes, cardiovascular disease and cancer in men: European prospective investigation into cancer in norfolk (EPIC- Norfolk) prospective population study. *Circulation*, 116: 2694- 2701.
- Kiechl, S.; Willeit, J.; Bonora, E.; Schwarz, S. and Xu, Q. (2000).** No association between dehydroepiandrosterone sulfate and development of atherosclerosis in a prospective population study (Bruneck study). *Arteriocsler. Thromb. Vasc. Biol.*, 20:1094-1100.
- LaCroix, A. Z.; Yano, K. and Reed, D. M. (1992).** Dehydroepiandrosterone sulfate, incidence of myocardial infarction, and extent of atherosclerosis in men. *Circulation*, 86:1529–1535.

- Legrain, S.; Berr, C.; Frenoy, N.; Gourlet, V.; Debuire, B. and Baulieu, E. E. (1995).** Dehydroepiandrosterone sulfate in a long-term care aged population. *Gerontology*, 41: 343-351.
- Leszczynski, D. E.; Schafer, R. M.; Perkins, E. G.; Jerrell, J. P. and Kummerow, F. A. (1989).** Esterification of dehydroepiandrosterone by human plasma HDL. *Biochim. Biophys. Acta*, 1014:90-97.
- Lovas, K.; Gebre-Medhin, G.; Trovik, T. S.; Fougner, K. J.; Uhlving, S. and Nedrebo, B. G. (2003).** Replacement of dehydroepiandrosterone in adrenal failure: no benefit for subjective health status and sexuality in a 9-month, randomized, parallel group clinical trial. *J. Clin. Endocrinol. Metab.*, 88: 1112-1118.
- Mazat, L.; Lafont, S.; Berr, C.; Debuire, B.; Tessier, J.; Dartigues, J. and Baulieu, E. (2001).** Prospective measurement of dehydroepiandrosterone sulfate in a cohort of elderly subjects: relationship to gender, subjective health, smoking habits and 10 year mortality. *PNAS*, 98: 8145-8150.
- Mitchell, L. E.; Sprecher, D. L.; Borecki, I. B.; Rice, T.; Laskarzewski, P. M. and Rao, D. C. (1994).** Evidence for an association between dehydroepiandrosterone sulfate and nonfatal, premature myocardial infarction in males. *Circulation*, 89: 89-93.
- Moriyama, Y.; Yasue, H.; Yoshimura, M.; Mizuno, Y.; Nishiyama, K.; Tsunoda, R.; Kawano, H.; Kugiyama, K.; Ogawa, H.; Saito, Y.; And Nakao, K. (2000).** The Plasma Levels of Dehydroepiandrosterone Sulfate Are Decreased in Patients with Chronic Heart Failure in Proportion to the Severity. *J. Clin. Endocrinol. Metab.* 85: 1834-1840.
- Nafziger, A. N.; Herrington, D. M. and Bush, T. L. (1991).** Dehydroepiandrosterone and dehydroepiandrosterone sulfate: their relation to cardiovascular disease. *Epidemiologic Reviews*, 13: 267-293.
- Nair, K.; Rizza, R.; OBrien, P.; Dhatriya, K.; Short, K.; Nehra, A.; Vittone, J.; *et al.* (2006).** DHEA in elderly women and DHEA or testosterone in elderly men. *N. E. J. of Medicine*, 355: 1647-1659.
- Nakamura, T.; Fox-Robichaud, A.; Kashiwagi, R. A.; Kojima, H.; Fujimiya, M. and Wong, N. C. (1999).** Transcription factors and age-related decline in apolipoprotein A-I expression. *J. Lipid Res.*, 40: 1709-1718.
- Newcomer, L. M.; Manson, J. E.; Barbieri, R. L.; Hennekens, C. H. and Stampfer, M. J. (1994).** Dehydroepiandrosterone sulfate and the risk of myocardial infarction in US male physicians: a prospective study. *Ameri. J. Epidemiol.* 140: 870-875.
- Ng, M. K. C.; Nakhla, S.; Baoutina, A.; Jessup, W.; Handelsman, D. J. and Celermajer, D. S. (2003).** Dehydroepiandrosterone, an adrenal androgen, increases human foam cell formation. *J. Am. Coll. Cardiol.*, 42: 1967-1974.
- Okamoto, K. (1996).** Relationship between dehydroepiandrosterone sulfate and serum lipid levels in Japanese men. *J. Epidemiol.*, 6: 63- 67.
- Phillips, G. B.; Pinkernell, B. H. and Jing, T. Y. (1994).** The association of hypotestosteronemia with coronary artery disease in men. *Arteriosclerosis and Thrombosis*, 14: 701-706.
- Reaven, P. D., C.; Napoli, S. M. and Witztum, J. L. (1999).** Lipoprotein modification and atherosclerosis in aging. *Exp. Gerontol.*, 34: 527-537.
- Simoncini, T.; Mannella, P.; Fornari, L.; Varone, G.; Caruso, A. and Genazzani, A. R. (2003).** Dehydroepiandrosterone modulates endothelial nitric oxide synthesis via direct genomic and non genomic mechanisms. *Endocrinology*, 144: 3449-3455.
- Slowinska-Srzednicka, J.; Zgliczynski, S.; Ciswicka-Sznajderman, M.; Srzednicki, M.; Soszynski, P.; Biernacka, M. (1989).** Decreased plasma

dehydroepiandrosterone sulfate and dihydroepiandrosterone concentrations in young men after myocardial infarction. *Atherosclerosis*, 79: 197-203.

Tchernof, A. and Despre's, J. P. (2000). Sex steroid hormones, sex hormonebinding globulin, and obesity in men and women. *Horm. Metab. Res.*, 32: 526-536.

Tchernof, A.; Labrie, F.; Be' langer, A.; Bouchard, C.; Prud'homme, D. and Moorjani, S. (1997). Relationships between endogenous steroid hormone, sex hormone-binding globulin and lipoprotein levels in men: contribution of visceral obesity, insulin levels and other metabolic variables. *Atherosclerosis* 1997 133 235–244.

Tietz, N. W. (1999). Text book of clinical chemistry, 3^{ed} Ed. C. A. Burtis, E. R. Ashwood, W. B. Saunders. P. 809-861.

Tilvis, R.S.; Ka"ho"hen, M. and Ha"rko"nen, M. (1999). Dehydroepiandrosterone sulfate, diseases and mortality in a general aged population. *Aging Clin. Exp. Res.* 11:30-34.

Trivedi, D.P. and Khaw, K.T. (2001). Dehydroepiandrosterone sulfate and mortality in elderly men and women. *J. Clin. Endocrinol. Metab.* 86: 4171–4177.

Villareal, D. T.; Holloszy, J. O. and Kohrt, W.M. (2000). Effects of DHEA replacement on bone mineral density and body composition in elderly women and men. *Clin. Endocrinol.*, 53 561-568.

Wu, F. C. and Von Eckardstein, A. (2003). Androgens and coronary artery disease. *Endocrine Reviews*, 24: 183-217.

Zumoff, B.; Troxler, R. G.; Connor, J. O.; Rosenfeld, R. S.; Kream, J.; Levin, J.; Hickman, J. R.; Solan, A. M.; Wolker, w.; Cook, R. L. and Fukushima, D. K. (1982). Abnormal hormone levels in men with coronary artery disease. *Arteriosler. Thromb. Vasc. Boil.* 2: 58-67.