[21] E.M. Makarov, O.V. Makarova, T. Achsel, R. Luhrmann, The human homologue of the yeast splicing factor prp6p contains multiple TPR elements and is stably associated with the U5 snRNP via protein–protein interactions, J. Mol. Biol. 298 (2000) 567–575.
[22] W. Seghezzi, K. Chua, F. Shanahan, O. Gozani, R. Reed, E. Lees, Cyclin E associates with components of the pre-mRNA splicing machinery in mammalian cells, Mol. Cell. Biol. 18 (1998) 4526–4536.
[23] K. Ishitani, T. Yoshida, H. Kitagawa, H. Ohta, S. Nozawa, S. Kato, p54nrb acts as a transcriptional coactivator for activation function 1 of the human androgen receptor, Biochem. Biophys. Res. Commun. 306 (2003) 660–665.
[24] L.D. D'Andrea, L. Regan, TPR proteins: the versatile helix, Trends Biochem. Sci. 28 (2003) 655–662.
[25] Y. Xing, C.V. Johnson, P.T. Moen Jr., J.A. McNeil, J. Lawrence, Nonrandom gene organization: structural arrangements of specific pre-mRNA transcription and splicing with SC-35 domains, J. Cell Biol. 131 (1995) 1635–1647.
[26] T. Misteli, D.L. Spector, RNA polymerase II targets pre-mRNA splicing factors to transcription sites in vivo, Mol. Cell 3 (1999) 697–705.
[27] D. Auboeuf, A. Honig, S.M. Berget, B.W. O'Malley, Coordinate regulation of transcription and splicing by steroid receptor coregulators, Science 298 (2002) 416–419.
[28] T. Misteli, Cell biology of transcription and pre-mRNA splicing: nuclear architecture meets nuclear function, J. Cell Sci. 113 (2000) 1841–1849.
[29] A. Tomura, K. Goto, H. Morinaga, M. Nomura, T. Okabe, T. Yanase, R. Takayanagi, H. Nawata, The subnuclear three-dimensional image analysis of androgen receptor fused to green fluorescence protein, J. Biol. Chem. 276 (2001) 28395–28401.
[30] M. Saitoh, R. Takayanagi, K. Goto, A. Fukamizu, A. Tomura, T. Yanase, H. Nawata, The presence of both the amino- and carboxyl-terminal domains in the AR is essential for the completion of a transcriptionally active form with coactivators and intranuclear compartmentalization common to the steroid hormone receptors: a three-dimensional imaging study, Mol. Endocrinol. 16 (2002) 694–706.
[31] A.J. Bendall, C. Abate-Shen, Roles for Msx and Dlx homeoproteins in vertebrate development, Gene 247 (2000) 17–31.
[32] J.T. Bryan, M.I. Morasso, The Dlx3 protein harbors basic residues required for nuclear localization, transcriptional activity and binding to Msx1, J. Cell Sci. 113 (2000) 4013–4023.
[33] M.M. Sohocki, S.J. Bowne, L.S. Sullivan, S. Blackshaw, C.L. Cepko, A.M. Payne, S.S. Bhattacharya, S. Khaliq, S. Qasim Mehdi, D.G. Birch, W.R. Harrison, F.F. Elder, J.R. Heckenlively, S.P. Daiger, Mutations in a new photoreceptor-pineal gene on 17p cause Leber congenital amaurosis, Nat. Genet. 24 (2000) 79–83.
[34] S. Grizot, F. Fieschi, M.C. Dagher, E. Pebay-Peyroula, The active N-terminal region of p67phox. Structure at 1.8 Å resolution and biochemical characterizations of the A128V mutant implicated in chronic granulomatous disease, J. Biol. Chem. 276 (2001) 21627–21631, Epub 22001 Mar 21621.
[35] Y. Masuhiro, Y. Mezaki, M. Sakari, K. Takeyama, T. Yoshida, K. Inoue, J. Yanagisawa, S. Hanazawa, W. O'Malley, B.S. Kato, Splicing potentiation by growth factor signals via estrogen receptor phosphorylation, Proc. Natl. Acad. Sci. USA 102 (2005) 8126–8131, Epub 2005 May 8126.
[36] D.J. Elliott, D.S. Bowman, N. Abovich, F.S. Fay, M. Rosbash, A yeast splicing factor is localized in discrete subnuclear domains, Embo J. 11 (1992) 3731–3736.
[37] T. Misteli, J.F. Caceres, D.L. Spector, The dynamics of a pre-mRNA splicing factor in living cells, Nature 387 (1997) 523–527.
[38] G. Dellaire, E.M. Makarov, J.J. Cowger, D. Longman, H.G. Sutherland, R. Luhrmann, J. Torchia, W.A. Bickmore, Mammalian PRP4 kinase copurifies and interacts with components of both the U5 snRNP and the N-CoR deacetylase complexes, Mol. Cell. Biol. 22 (2002) 5141–5156.
[39] G. Jenster, H.A. van der Korput, J. Trapman, A.O. Brinkmann, Identification of two transcription activation units in the N-terminal domain of the human androgen receptor, J. Biol. Chem. 270 (1995) 7341–7346.
[40] N.L. Chamberlain, D.C. Whitacre, R.L. Miesfeld, Delineation of two distinct type 1 activation functions in the androgen receptor amino-terminal domain, J. Biol. Chem. 271 (1996) 26772–26778.
[41] J. Reid, S.M. Kelly, K. Watt, N.C. Price, I.J. McEwan, Conformational analysis of the androgen receptor amino-terminal domain involved in transactivation. Influence of structure-stabilizing solutes and protein–protein interactions, J. Biol. Chem. 277 (2002) 20079–20086, Epub 22002 Mar 20014.
[42] R. Kumar, E.B. Thompson, Transactivation functions of the N-terminal domains of nuclear hormone receptors: protein folding and coactivator interactions, Mol. Endocrinol. 17 (2003) 1–10.

Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Diabetes Research and Clinical Practice 73 (2006) 322–328
DIABETES RESEARCH AND CLINICAL PRACTICE
www.elsevier.com/locate/diabres

The Gly146Ala variation in human *SF-1* gene: Its association with insulin resistance and type 2 diabetes in Chinese

Wei Liu [a], Min Liu [a], WuQiang Fan [a,b,*], Hajime Nawata [c], Toshihiko Yanase [b]

[a] *Department of Medicine and Endocrinology, Renji Hospital, Shanghai 2nd Medical University, #1630 DongFang Road, PuDong, Shanghai 210127, China*
[b] *Department of Medicine and Bioregulatory Science, Graduate School of Medical Science, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan*
[c] *Graduate School of Medical Science, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan*

Received 23 August 2005; accepted 20 February 2006
Available online 27 March 2006

Abstract

Aims: While steroidogenic factor 1 (SF-1) is traditionally an essential nuclear receptor for steroidogenic tissues, current emerging studies revealed that the receptor is also closely implicated in metabolism. Mutations of *SF-1* gene cause metabolic disorders like obesity in both human and mice. The aim of the present study is to examine whether the *Gly*146*Ala* variation in the gene for *SF-1*, that is known to impair SF-1 function and related to adrenal disorders, affects susceptibility to type 2 diabetes.
Methods: Hundred and fifty-one type 2 diabetic subjects and 141 non-diabetic control subjects of Han Chinese were recruited and the *SF-1* genotype were analyzed by PCR-RFLP method.
Results: The *Gly*146*Ala* variation occurs frequently in the Han Chinese. Allele *Ala* frequency in the control subjects (27.3%) was significantly lower than that in type 2 diabetic subjects (37.1%, $\chi^2 = 6.37, p = 0.01$). The *Gly/Ala* and *Ala/Ala* genotypes frequencies were also higher in diabetic subjects. In both the diabetic and control populations, subjects carrying allele *Ala*, as compared to those not, had higher fasting insulin levels and higher HOMA values.
Conclusions: The *SF-1 Gly*146*Ala* variation may constitute a susceptible factor for development of type 2 diabetes and impairment of insulin actions.

Keywords: Steroidogenic factor 1; Type 2 diabetes; Single nucleotide polymorphism; HOMA

1. Introduction

Steroidogenic factor 1 (SF-1), also known as adrenal 4 binding protein and formally designed as nuclear receptor subfamily 5 group A number 1 (NR5A1), is a monomeric nuclear receptor, that transcriptionally regulates a vast array of genes involved in adrenal and gonadal development, sex differentiation, steroidgenesis and reproduction through out the hypothalamic-pituitary-adrenal/gonad axis [1]. Disruption of *sf-1* gene in mice leads to complete adrenal and gonadal agenesis, impaired function of pituitary gonadatropes and

Abbreviations: AgRP, agouti-related peptide; BMI, body mass index; DBP, diastolic blood pressure; f-IRI: fasting immunoreactive insulin; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; HPA, hypothalamic-pituitary-adrenal; LDL-C, low density lipoprotein cholesterol; NPY, neuropeptide Y; PCR-RFLP, polymerase-chain reaction plus restriction fragment length polymorphism; POMC, pro-opiomelanocortin; SBP, systolic blood pressure; SF-1, steroidogenic factor 1; TC, total cholesterol; TG, triglyceride; VMH, ventromedial hypothalamic; WHR, waist-to-hip ratio

* Corresponding author. Tel.: +86 21 5875 2345x3447; fax: +86 21 5839 4262.
E-mail address: fan_wq@yahoo.com (W. Fan).

0168-8227/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.diabres.2006.02.007

abnormalities of ventromedial hypothalamic nucleus (VMH) [1,2]. Mutations of the gene in human cause congenital adrenal and gonadal disorders as well [3–7].

While SF-1 plays vital roles in steroidogenesis, emerging studies linked the gene to metabolic dishomeostasis. Recent discovery of phospholipids as endogenous ligands for SF-1, make the receptor as a possible nuclear lipid sensor [8,9]. *sf*-1 knockout mice develop late-onset obesity when rescued by adrenal transplantation [10]. All the three adult human *SF-1* gene mutation patients have mild to severe obesity [3,6,7]. Thus impaired *SF-1* function may cause metabolic disorders in both mouse and human.

Functional alterations of human SF-1 caused by mutations may have limited clinical significance since mutations occur very rarely. In contrast, a nonsynonymous single nucleotide polymorphism variation, *Gly*146*Ala* of human *SF-1*, which bears a slightly impaired transactivation, was found occurring frequently in a Japanese population, and had potential clinical importance [11].

To further clarify potential implications of *SF-1* gene function in human metabolic disorders, we analyzed the relationship between human *SF-1* *Gly*146*Ala* polymorphism and type 2 diabetes and insulin action in Han Chinese.

2. Materials and methods

2.1. Subjects

A total of 292 Chinese subjects were enrolled in the study. They included 151 subjects with type 2 diabetes and 141 non-diabetic normal control subjects. The diabetic subjects were randomly recruited from patients attending the outpatient clinic of the department of Medicine and Endocrinology, Renji Hospital, Shanghai 2nd Medical University. The diagnosis of type 2 diabetes was based on World Health Organization [12]. Non-diabetic subjects were recruited from an unselected population undergoing routine health checkups at the Renji Hospital. To enhance statistical power to detect association, inclusion criteria as follows were used for non-diabetic subjects: (1) >55 years of age; (2) HBA1C values <6.0%; (3) no family history of type 2 diabetes. All the subjects enrolled in this study were of full Chinese Han ethnicity. The study complied the recommendations of the Declaration of Helsinki, and was performed after obtaining the written informed consent from all the subjects and was approved by the ethics committee of the Shanghai 2nd Medical University.

2.2. Biological measurements

Whole blood samples were drawn in the fasting state, and the fasting plasma glucose (FPG) levels, concentrations of serum lipids, fasting plasma immunoreactive insulin (f-IRI) concentrations (or, C-peptide alternatively, for those who are receiving any sorts of insulin therapy), and HbA1c levels were determined in each subject by standard laboratory techniques calibrated by the uniform standards. Genomic DNA was extracted by kit of QIAamp Blood Maxi Kit (QIAGEN, CA), following the manufacture's protocol. Insulin resistance HOMA: homeostasis model assessment of insulin resistance (HOMA-IR) = fasting insulin (μU/ml) × fasting glucose (mmol/L)/22.5; β-cell function (HOMA-β) = 20 × fasting insulin (μU/ml)/[fasting glucose (mmol/L)-3.5] [13].

2.3. Genotyping of the Gly146Ala polymorphism in SF-1 gene

The *Gly*146*Ala* polymorphism in human *SF-1* gene was genotyped by the polymerase-chain reaction plus restriction fragment length polymorphism (PCR-RFLP) method [11]. In brief, leukocyte genomic DNA was amplified by polymerase-chain reaction with primer flanking exon 4 of human *SF-1* gene, and the PCR products were then digested overnight at 37 °C with the restriction enzyme of *Sph*I. The sequences of the primers are: forward: 5′ CTT AGA GAG GGT GAG TCT GA 3′; reverse: 5′ CTG AAG CCA GTG GGA AGG AT 3′. The annealing temperature was 60 °C. SphI recognizes only the Ala allele and the digestion give rise to one (773 bp) fragment for the Gly allele and two (541 and 232 bp) fragments for the Ala allele. Digest products were resolved by 2% ethidium bromide stained-agarose gel electrophoresis.

2.4. Statistical analysis

Clinical variables are expressed as mean ± standard error of the mean (S.E.M.). Differences in the clinical characteristics between subjects with and without the polymorphism were evaluated by two-tailed Student's *t*-test. The proportions of genotype or alleles were compared by χ^2 analysis. Odds ratio (OR) and 95% confidence interval (CI), non-adjusted and adjusted for age, gender and body mass index (BMI) were calculated by logistic regression analysis. A p value <0.05 was considered as statistically significant. All statistics were analyzed using SPSS for Windows Version 11.0.

3. Results

The characteristics of the type 2 diabetic and non-diabetic control subjects are summarized in Table 1. The clinical features of the type 2 diabetic subjects and non-diabetic control subjects classified according to *SF-1* genotype were shown in Tables 3 and 4, respectively.

The *Gly*146*Ala* human *SF-1* polymorphic variation was relatively frequent in the Han Chinese. 66 of the 141 (46.8%) non-diabetic control subjects contained one or two allele *Ala* (55 *Gly*/*Ala*, 11 *Ala*/*Ala*). The total allele *Ala* frequency was 27.3%, which is much higher

Table 1
Characteristics of the type 2 diabetic (T2DM) and non-diabetic (NDM) subjects enrolled

Characteristics	T2DM	NDM	*p*
n	151	141	–
Gender (male/female)	61/90	34/107	0.003
Age (years)	61.17 ± 0.86	67.31 ± 0.76	0.001
Diabetes onset age	54.95 ± 1.15	–	–
Family history (+/−)	115/36	–	–
BMI (kg/m^2)	26.60 ± 0.37	21.30 ± 0.19	1.13E−28
Waist-to-hip ratio	0.89 ± 0.004	0.82 ± 0.005	6.03E−20
FPG (mmol/l)	9.43 ± 0.49	4.54 ± 0.05	6.12E−22
f-IRI (pmol/l)	21.84 ± 2.34	10.71 ± 0.42	2.80E−05
HOMA-IR	9.29 ± 1.83	2.23 ± 0.09	4.27E−04
HOMA-β	105.19 ± 8.92	258.47 ± 48.09	1.24E−03
SBP (mmHg)	136.03 ± 1.80	128.00 ± 1.33	4.70E−04
DBP (mmHg)	83.39 ± 0.84	81.70 ± 0.80	0.15
TC (mmol/l)	4.93 ± 0.09	5.06 ± 0.07	0.067
TG (mmol/l)	1.73 ± 0.09	1.52 ± 0.09	0.099
HDL-C (mmol/l)	1.35 ± 0.03	1.81 ± 0.04	7.38E−18
LDL-C (mmol/l)	3.18 ± 0.08	3.06 ± 0.08	0.32

than that observed in a healthy Japanese population [11]. Table 2 shows that the allele frequency for the *Ala* variant of the *Gly*146 → *Ala* polymorphism of *SF-1* was significantly higher in the type 2 diabetes group (37.1%) than that in the control group (27.3%, $\chi^2 = 6.37$, $p = 0.01$). A consistent higher genotype frequency of *Gly/Ala* (42.4% in diabetic group versus 39.0% in non-diabetic group) and *Ala/Ala* (15.9% in diabetic group versus 7.8% in non-diabetic group) were also observed ($\chi^2 = 6.22$, $p = 0.04$).

These data indicates that the *Ala*146 *SF-1* variant is associated with increased risk for the development of type 2 diabetes. The OR is 1.57; and 95% CI is 1.11–2.23. The relative risk value is 1.23. This association still exists after adjustment for age, sex, and BMI (OR: 1.56; 95% CI: 1.10–2.31).

The genotypic distribution of the *SF-1 Gly*146*Ala* polymorphism were in Hardy–Weinberg equilibrium both in the non-diabetic and the type 2 diabetic subjects.

In both diabetic and non-diabetic populations, subjects with and without the *Gly*146*Ala* polymorphism were assessed for insulin resistance and β-cell function by the homeostasis model assessment (HOMA-IR and HOMA-β) as described in the Methods. Values obtained from HOMA have been shown to correlate well with those from the glucose clamp technique [14]. For diabetic patients who are receiving any kinds of insulin therapy, fating C-peptide was alternatively measured to assess the endogenous insulin secretion.

Among diabetic patients, although fasting glucose levels were not different between the two sub-groups subjects who carry the *Ala*146 variant or not respectively (Table 3), fasting insulin levels were noticed significantly elevated in the subjects carrying one or two *Ala*146 allele (25.94 ± 3.60) as compared to those *Gly/Gly* subjects (15.01 ± 0.88, $p = 0.02$). The high fasting insulin levels led to significantly higher HOMA-IR and HOMA-β values in subjects carrying the *Ala*146 variant (Table 3).

Type 2 diabetes per se affects on insulin and glucose level, and overt diabetic patients received treatments in forms of diet, oral hypoglycemic agent and/or insulin. Although the treatment profiles are not significantly different between the Ala carrying and non-carrying T2DM subjects (Table 3), those treatments might still to some extent impact on the secretion or sensitivity (or both) of insulin. Therefore we carried out a further analysis to investigate any possible effect of the *Gly*146*Ala* polymorphism on insulin resistance in non-diabetic subjects as well. As shown in Table 4,

Table 2
Genotypic and allelic prevalence of the *SF-1 Gly*146*Ala* polymorphism in type 2 diabetic (T2DM) and non-diabetic (NDM) subjects

	Genotype [*n*, (%)]			χ^2	*p*	Alleles [*n*, (%)]		χ^2	*p*
	Gly/Gly	*Gly/Ala*	*Ala/Ala*			*Gly*	*Ala*		
T2DM [151]	63 (41.7)	64 (42.4)	24 (15.9)	6.22	0.04	190 (62.9)	112 (37.1)	6.37	0.01
NDM [141]	75 (53.2)	55 (39.0)	11 (7.8)			205 (72.7)	77 (27.3)		

Table 3
Clinical characteristics of those type 2 diabetic subjects with and without *Ala*146 variant of the *Gly*146*Ala* polymorphism of *SF-1*

Characteristics	*Gly/Gly*	*Gly/Ala* + *Ala/Ala*	*p*
n	63	88	–
Gender (male/female)	24/39	37/51	0.63
Age (years)	62.07 ± 0.81	60.51 ± 1.17	0.37
Diabetes onset age	54.74 ± 1.80	55.12 ± 1.50	0.87
Family history (+/−)	49/14	65/23	0.58
BMI (kg/m^2)	26.20 ± 0.57	26.90 ± 0.49	0.35
WHR	0.90 ± 0.007	0.89 ± 0.006	0.47
FPG (mmol/l)	9.10 ± 0.47	9.68 ± 0.79	0.57
f-IRI (pmol/l)	15.01 ± 0.88	25.94 ± 3.60	0.02
HOMA-IR	5.38 ± 0.43	11.78 ± 2.95	0.04
HOMA-β	76.78 ± 5.56	123.32 ± 13.69	0.01
SBP (mmHg)	139.37 ± 2.40	133.68 ± 2.57	0.12
DBP (mmHg)	84.65 ± 1.31	82.51 ± 1.11	0.22
TC (mmol/l)	4.94 ± 0.15	4.93 ± 0.13	0.93
TG (mmol/l)	1.75 ± 0.14	1.73 ± 0.12	0.90
HDL-C (mmol/l)	1.37 ± 0.05	1.33 ± 0.04	0.53
LDL-C (mmol/l)	3.21 ± 0.14	3.16 ± 0.11	0.76
Treatments			0.50
Diet only	0	1	
Oral agent only	32	49	
Insulin only	8	15	
Oral agent + insulin	23	24	

while the differences haven't reached statistical significance due to relative large S.E.M. values, the tendency of higher fasting insulin levels, higher HOMA-IR and HOMA-β values are obvious in those *Ala*146 carrying subjects; suggesting the *Ala*146 variation of *SF-1* may confer resistance to insulin action in healthy subjects as well.

Table 4
Clinical characteristics of those non-diabetic subjects with and without *Ala*146 variant of the *Gly*146*Ala* polymorphism of *SF-1*

Characteristics	*Gly/Gly*	*Gly/Ala* + *Ala/Ala*	*p*
n	75	66	–
Gender (male/female)	21/54	13/53	0.25
Age (years)	67.51 ± 1.07	67.03 ± 1.05	0.76
BMI (kg/m^2)	21.20 ± 0.27	21.40 ± 0.3	0.74
WHR	0.83 ± 0.007	0.81 ± 0.008	0.06
FPG (mmol/l)	4.49 ± 0.08	4.60 ± 0.06	0.32
f-IRI (pmol/l)	10.17 ± 0.91	11.26 ± 0.72	0.20
HOMA-IR	2.11 ± 0.09	2.35 ± 0.15	0.10
HOMA-β	209.82 ± 16.19	308.61 ± 97.69	0.15
SBP (mmHg)	130.19 ± 1.73	125.52 ± 2.03	0.08
DBP (mmHg)	82.53 ± 1.12	80.74 ± 1.15	0.27
TC (mmol/l)	5.09 ± 0.11	5.24 ± 0.11	0.18
TG (mmol/l)	1.66 ± 0.15	1.37 ± 0.09	0.12
HDL-C (mmol/l)	1.81 ± 0.06	1.82 ± 0.05	0.83
LDL-C (mmol/l)	2.96 ± 0.11	3.18 ± 0.12	0.09

While mutations of *SF-1* gene cause obesity both in human and mice, a predicted effect of *Gly*146*Ala* polymorphism on obesity was not observed. In both the diabetic group and the non-diabetic group, body mass index (BMI), as well as waist-to-hip (WHR), was not affected by the presence of *Ala*146 allele (Tables 3 and 4).

Although *SF-1 Gly*146*Ala* affected the susceptibility to type 2 diabetes, it appears that the variation is not related with an early onset of diabetes, the diabetes onset age was similar between subjects with and without the *Ala*146 allele, neither the prevalence of family history differed between the two subgroups (Table 3).

Among both diabetic and control subjects, there were no significant differences in age, sex, blood pressure and plasma lipid profile between the two subgroups with and without the *Ala*146 variation (Tables 3 and 4).

4. Discussion

While SF-1 is traditionally a key factor for steroidogenic processes and is crucial for development and function of adrenal and gonads in both sexes, accumulating findings are linking the nuclear receptor to metabolism disorders. Mutations of the gene cause obesity in both human and rodents. Added to these findings, the present study, to our knowledge, for the first time revealed a higher *Gly*146*Ala* polymorphism frequency in type 2 diabetes population as compared with a non-diabetic control population, and thus further suggests a potential implication of *SF-1* gene in metabolic diseases.

The effects of SF-1 on metabolism might firstly rely on its vital role in the hypothalamus, especially the nucleus of ventromedial hypothalamic nucleus (VMH). Hypothalamus is believed to be the center of energy homeostasis and metabolism regulation [15]. This area of science became one of the most dramatically progressing field since the discovery of leptin [16]. Body fat is regulated by a complex neuroendocrine system through which information about the status of stored energy is converted to key CNS circuit using changing levels of hormones such as leptin and insulin. Leptin, which is in proportion to body fat mass, circulates to hypothalamus, stimulates PMOC neurons and represses AgRP/NPY neurons, and results in inhibited appetites and increased energy burning. Added to this well established hypothalamic role in energy homeostasis and body weight control, Rossetti and colleagues recently have proposed that, in addition to hormones (leptin and insulin), fuels such as glucose

and lipid are also afferent endocrine factors that regulate neuronal activity in the hypothalamus, and play critical roles in controlling blood glucose [17,18]. Thus hypothalamus is crucial for both energy and glucose homeostasis, and might be directly involved in onset of both obesity and diabetes. Among various nuclei of hypothalamus, VMH is one of the most important that plays vital roles in regulation of appetite and body weight. Lesions in VMH cause hyperphagia and obesity. *sf-1* KO mice have marked abnormalities in the VMH [19], which persists in the adult animals and contributes to the onset of obesity of those mice [10]. SF-1 is the most definitive molecular marker for the VMH, and has a distinct role in the VMH terminal differentiation and development [20,21]. SF-1 may also impact on either hormone (leptin and insulin) or fuels induced hypothalamic neuro-endocrine circuits, and functional alterations of the receptor caused by mutations or polymorphic variations may thus contribute to metabolism disorders by interfering with central regulation of energy and glucose homeostasis. Intriguingly, specific knockout of *sf-1* gene in hypothalamus is sufficient to induce obesity in mice [22].

A recent discovery of phospholipids as endogenous ligands for SF-1 [8,9] is very striking; important moleculars involved in insulin signaling, like PIP3, is thus a cognate ligand for SF-1. Given the dominant hypothalamic expression of SF-1 and critical role of hypothalamic insulin signaling in both energy and glucose homeostasis [23], this becomes particularly significant. Insulin, as above-mentioned, is a major afferent humoral information regarding the sufficiency of body fat stores to central nervous system (CNS). Reduced CNS insulin signaling contributes to the pathogenesis of metabolic disorders [23]. SF-1 in hypothalamus may works as a sensor of local insulin signaling. Phospholipids as SF-1 ligand also makes the receptor a possible nuclear lipid sensor, and potentially have important role in intracellular metabolism regulation.

Another possible way via which SF-1 exert its role in metabolism might rely on its well-known role in steroidogenesis. One identified cause of obesity, especially visceral obesity and metabolic complications like diabetes and dyslipidemia is exposure to excessive levels of glucocorticoids. Systematic glucocorticoid excess in Cushing syndrome causes visceral obesity, hyperglycemia and the metabolic syndrome [24,25]. Local overproduction of glucocorticoids in adipose tissue causes visceral obesity, insulin-resistant diabetes, and hyperlipidemia [26]. *Gly*146*Ala SF-1* variation may affect the diabetes onset via its role on adrenal steroidogenesis function, since the polymorphism is associated with pathological background of the adrenal; it produces a more susceptible state to adrenal disorders, and was evidenced to related with occurrence of these disorders in human being [11].

SF-1 critically regulates a big cluster of genes through out the hypothalamic-pituitary-adrenal (HPA) axis [27]; is critical for a functional HPA axis. A normal HPA axis is not only a prerequisite for proper glucocorticoid production (which affects insulin action), but also important for a normal circadian rhythm. HPA maintains consciousness and modulates sleep [28]. A recent study with the CLOCK transcriptional factor mutant mice showed that altering circadian rhythm also results in pathophysiological changes resembling the metabolic syndrome [29]. And importantly, disturbance of HPA axis function in human is associated with not only sleep diseases, but metabolic syndrome as well [28,30]. In this regard, a caveat to be considered in the interpretation of our present clinical data arising here is that, we, in the present study, couldn't include the analysis for the adrenal functional statuses (glucocorticoidogenesis), and especially the HPA functions status for subjects with different *SF-1* genotype. The issue is the major subject of one of our ongoing studies.

The higher fasting insulin levels and HOMA values observed in both diabetic and non-diabetic subjects carrying *Ala*146 allele is likely, secondary due to *SF-1 Gly*146*Ala*'s role in metabolism, although a direct effect of the receptor on islet β-cell function is not excludable. Our data warrants careful evaluations of issues of hyperinsulinemia and insulin resistance in the previously reported human subjects and mice model of *SF-1* mutations, where mild to severe obesity was observed. The data also suggest that diabetic individuals with *Ala*146 variant are likely to experience a worse insulin action and severe form of the disease.

In conclusion, the present study revealed a significant higher frequency of human *SF-1* gene *Gly*146*Ala* variation in type 2 diabetes subjects as compared to the non-diabetic subjects in Han Chinese; the allele *Ala*146 is associated with an elevated risk for the development of type 2 diabetes. The variation is also related with a higher level of plasma insulin, and severer insulin resistance. While the Chinese Han ethic group is more homogenous than Caucasians [31], and the influence of population stratification is likely smaller in the present study, studies with larger populations and those carried out in various races are definitely of great essentiality to further clarify the relationship between

the *Gly*146*Ala SF-1* polymorphism and type 2 diabetes and insulin resistance.

Acknowledgements

We thank Professor Min Luo, Guo Li and Tianhong Luo (Shanghai Institute of Endocrinology, Ruijin Hospital, Shanghai) for their continuing support and help. This study was supported by a grant of the "Natural Science Research Foundation Project of Shanghai Second Medical University" 02XJ21016.

References

[1] K. Morohashi, T. Omura, Ad4BP/SF-1, a transcription factor essential for the transcription of steroidogenic cytochrome P450 genes and for the establishment of the reproductive function, FASEB J. 10 (1996) 1569–1577.

[2] K.L. Parker, B.P. Schimmer, Steroidogenic factor 1: a key determinant of endocrine development and function, Endocrinol. Rev. 18 (1997) 361–377.

[3] J.C. Achermann, M. Ito, M.P.C. Hindmarsh, J.L. Jameson, A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans, Nat. Genet. 22 (1999) 125–126.

[4] J.C. Achermann, G. Ozisik, M. Ito, U.A. Orun, K. Harmanci, B. Gurakan, et al., Gonadal determination and adrenal development are regulated by the orphan nuclear receptor steroidogenic factor-1, in a dose-dependent manner. J. Clin. Endocrinol. Metab. 87 (2002) 1829–1833.

[5] A. Biason-Lauber, E.J. Schoenle, Apparently normal ovarian differentiation in a prepubertal girl with transcriptionally inactive steroidogenic factor 1 (NR5A1/SF-1) and adrenocortical insufficiency. Am. J. Hum. Genet. 67 (2000) 1563–1568.

[6] R.V. Correa, S. Domenice, N.C. Bingham, A.E. Billerbeck, W.E. Rainey, K.L. Parker, et al., A microdeletion in the ligand binding domain of human steroidogenic factor 1 causes XY sex reversal without adrenal insufficiency. J. Clin. Endocrinol. Metab. 89 (2004) 1767–1772.

[7] T. Hasegawa, M. Fukami, N. Sato, N. Katsumata, G. Sasaki, K. Fukutani, et al., Testicular dysgenesis without adrenal insufficiency in a 46, XY patient with a heterozygous inactive mutation of steroidogenic factor-1, J. Clin. Endocrinol. Metab. 89 (2004) 5930–5935.

[8] Y. Li, M. Choi, G. Cavey, J. Daugherty, K. Suino, A. Kovach, et al., Crystallographic identification and functional characterization of phospholipids as ligands for the orphan nuclear receptor steroidogenic factor-1, Mol. Cell. 17 (2005) 491–502.

[9] I.N. Krylova, E.P. Sablin, J. Moore, R.X. Xu, G.M. Waitt, J.A. MacKay, et al., Structural analyses reveal phosphatidyl inositols as ligands for the NR5 orphan receptors SF-1 and LRH-1, Cell 120 (2005) 343–355.

[10] G. Majdic, M. Young, E. Gomez-Sanchez, P. Anderson, L.S. Szczepaniak, R.L. Dobbins, et al., Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity, Endocrinology 143 (2002) 607–614.

[11] F. WuQiang, T. Yanase, L. Wei, K. Oba, M. Nomura, T. Okabe, et al., Functional characterization of a new human Ad4BP/SF-1 variation, G146A, Biochem. Biophys. Res. Commun. 311 (2003) 987–994.

[12] K.G. Alberti, P.Z. Zimmet, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation, Diab. Med. 15 (1998) 539–553.

[13] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28 (1985) 412–419.

[14] E. Bonora, G. Targher, M. Alberiche, G. Formentini, F. Calcaterra, S. Lombardi, et al., Predictors of insulin sensitivity in Type 2 diabetes mellitus, Diab. Med. 19 (2002) 535–542.

[15] M.W. Schwartz, D. Porte Jr., Diabetes, obesity, and the brain, Science 307 (2005) 375–379.

[16] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, J.M. Friedman, Positional cloning of the mouse obese gene and its human homologue, Nature 372 (1994) 425–432.

[17] W. He, T.K. Lam, S. Obici, L. Rossetti, Molecular disruption of hypothalamic nutrient sensing induces obesity, Nat. Neurosci. 9 (2006) 227–233.

[18] T.K. Lam, R. Gutierrez-Juarez, A. Pocai, L. Rossetti, Regulation of blood glucose by hypothalamic pyruvate metabolism, Science 309 (2005) 943–947.

[19] Y. Ikeda, X. Luo, R. Abbud, J.H. Nilson, K.L. Parker, The nuclear receptor steroidogenic factor 1 is essential for the formation of the ventromedial hypothalamic nucleus, Mol. Endocrinol. 9 (1995) 478–486.

[20] P.V. Tran, M.B. Lee, O. Marin, B. Xu, K.R. Jones, L.F. Reichardt, et al., Requirement of the orphan nuclear receptor SF-1 in terminal differentiation of ventromedial hypothalamic neurons, Mol. Cell. Neurosci. 22 (2003) 441–453.

[21] Y. Shima, M. Zubair, S. Ishihara, Y. Shinohara, S. Oka, S. Kimura, et al., Ventromedial hypothalamic nucleus-specific enhancer of Ad4BP/SF-1 gene, Mol. Endocrinol. 19 (11) (2005) 2812–2823.

[22] L. Zhao, M. Bakke, N.A. Hanley, G. Majdic, N.R. Stallings, P. Jeyasuria, et al., Tissue-specific knockouts of steroidogenic factor 1, Mol. Cell. Endocrinol. 215 (2004) 89–94.

[23] D. Porte Jr., D.G. Baskin, M.W. Schwartz, Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from *C. elegans* to humans, Diabetes 54 (2005) 1264–1276.

[24] P. Bjorntorp, R. Rosmond, Obesity and cortisol, Nutrition 16 (2000) 924–936.

[25] B.L. Wajchenberg, Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome, Endocrinol. Rev. 21 (2000) 697–738.

[26] H. Masuzaki, J. Paterson, H. Shinyama, N.M. Morton, J.J. Mullins, J.R. Seckl, et al., A transgenic model of visceral obesity and the metabolic syndrome, Science 294 (2001) 2166–2170.

[27] W. Fan, T. Yanase, Y. Wu, H. Kawate, M. Saitoh, K. Oba, et al., Protein kinase A potentiates adrenal 4 binding protein/steroidogenic factor 1 transactivation by reintegrating the subcellular dynamic interactions of the nuclear receptor with its cofactors, general control nonderepressed-5/transformation/transcription domain-associated protein, and suppressor, dosage-sensitive sex reversal-1: a laser confocal imaging study in living KGN cells. Mol. Endocrinol. 18 (2004) 127–141.

[28] R. Pasquali, V. Vicennati, The abdominal obesity phenotype and insulin resistance are associated with abnormalities of the

hypothalamic-pituitary-adrenal axis in humans. Horm. Metab. Res. 32 (2000) 521–525.
[29] F.W. Turek, C. Joshu, A. Kohsaka, E. Lin, G. Ivanova, E. McDearmon, et al., Obesity and metabolic syndrome in circadian Clock mutant mice. Science 308 (2005) 1043–1045.
[30] T.M. Buckley, A.F. Schatzberg, On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders. J. Clin. Endocrinol. Metab. 90 (2005) 3106–3114.
[31] K. Xiang, Y. Wang, T. Zheng, W. Jia, J. Li, L. Chen, et al., Genome-wide search for type 2 diabetes/impaired glucose homeostasis susceptibility genes in the Chinese: significant linkage to chromosome 6q21-q23 and chromosome 1q21-q24, Diabetes 53 (2004) 228–234.

Geriatr Gerontol Int 2006; 6: 194–198

Serum concentrations of dehydroepiandrosterone sulfate (DHEA-S) in oldest old Japanese women correlate with cognitive activity rather than activities of daily living

Toshihiko Yanase,[1] Kazuo Muta[2] and Hajime Nawata[3]

[1]Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, [2]Muta Hospital, and [3]Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Background: Dehydroepiandrosterone (DHEA) and its interconvertible sulfate ester, DHEA sulfate (DHEA-S), mainly produced by the adrenal glands, are progressively decreased with aging and are proven markers of longevity. Although serum level of DHEA (-S) has been shown to be decreased in dementing diseases, the issue remains controversial. We investigated the physiological significance of DHEA-S in oldest old Japanese women in respect of activities of daily living (ADL) or cognitive activities.

Methods: Cross-sectional study of 50 women aged 90–103 years old. Serum concentrations of DHEA-S levels were measured by radioimmunoassay. ADL and cognitive activity were evaluated by the Bathel index and revised Hasegawa's dementia rating scale (HDS-R), respectively. Univariate or multivariate regression analyzes were used for statistics.

Results: Of the 50 subjects, 80% exceeded the lowest level of the normal range for women in their forties. Serum concentrations of DHEA-S were significantly correlated with HDS-R but not with the Barthel index.

Conclusion: Relatively higher levels of serum DHEA-S in oldest old women may reflect the longevity of this population and the levels might be associated with cognitive activity rather than ADL.

Keywords: activities of daily living (ADL), aging, dementia, dehydroepiandrosterone-sulfate (DHEA-S).

Introduction

Dehydroepiandrosterone (DHEA) and its interconvertible sulfate ester, DHEA sulfate (DHEA-S) are mainly produced by the adrenal glands, with production decreasing progressively with aging. After the age of 80 years, DHEA-S levels drop to 10–20% of the peak levels of women in their second decade.[1] DHEA and DHEA-S have recently received much attention because their serum levels were shown to be an important marker for longevity[2] and because a number of beneficial or anti-aging effects of DHEA-S on, for example, dementia, obesity, lipid metabolism, diabetes mellitus, atherosclerosis, osteoporosis, carcinogenesis and immune responses have been clarified.[1,3] Indeed, several preliminary trials to treat aged people with DHEA within a year appear promising; replacement of

Accepted for publication 15 May 2006.

Correspondence: Dr Toshihiko Yanase, Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Email: yanase@intmed3.med.kyushu-u.ac.jp

 doi: 10.1111/j.1447-0594.2006.00346.x

DHEA (50 mg) in men and women (50 years old) for 6 months in a double-blind trial showed a significant increase in the sense of well-being both in men and women.[4] Baulieu *et al.* performed a large scale double-blind study in which 280 healthy individuals (women and men, 60–79 years old) were daily given orally either 50 mg DHEA or a placebo for a year; a significant increase in most libido parameters and bone mineral density and the improvement of skin status was noted in the women.[5]

As for dementia, Sunderland *et al.* first reported decreased serum concentration of DHEA-S in Alzheimer's disease (AD) patients compared to controls.[6] However, subsequent similar trials revealed that serum DHEA or DHEA-S appeared inconstant as a biological marker for AD.[7] Other reports, including a pervious one of ours, have reported that not only with AD patients, but also patients with cerebrovascular dementia (CVD) have lower concentrations of DHEA-S compared with age- and gender-matched controls.[8,9] Further, a recent, randomized, double-blind study involving 6 month's DHEA treatment of AD showed no significant improvement of cognitive function.[10] On the other hand, studies regarding the relationship between serum DHEA-S levels and activities of daily living (ADL) performances in elderly people are also conflicting.[11–13] Therefore, the physiological significance of DHEA-S in the elderly remains controversial. There are no reports investigating whether serum DHEA-S levels are closely associated with either or both of ADL performance or cognitive activity.

The oldest old people are a good model to study the association of DHEA-S with longevity in respect of mental and physical activities. However, DHEA-S levels in the Japanese oldest old population have not been well studied. In the present study, to ascertain more about the significance of serum DHEA-S in oldest old women, we evaluated the relationship between the serum concentration of DHEA-S and ADL and cognitive activity of this population; here, aged more than 90 years old.

Materials and methods

Subjects

Fifty women over 90 years old, who resided in the nursing ward of Muta Hospital (Fukuoka, Japan) participated in this study. The blood samples were collected with subjects in a supine position at 8 AM on the day when regular check-ups of blood chemistry were undertaken. Functional independence in respect of ADL was evaluated by the Barthel index score (full score, 100).[14] A higher score indicates greater independence. Cognitive activity was evaluated by revised Hasegawa's dementia rating scale (HDS-R; full score, 30).[15] A higher score indicates less dementia. These evaluations are routine work for elderly patients in Muta Hospital. ADL evaluators were blind to hormonal levels and those who performed hormone assays were blind to ADL scores.

Based on clinical diagnoses, the 50 oldest old women consisted of 10 with hypertension, five with mild heart failure, nine with after-effect of cerebrovascular disease, 16 with various degrees of dementia (13 with senile dementia of Alzheimer's type, one with relatively early onset AD, and two with cerebrovascular dementia) and 10 with disease-free state. However, by HDS-R evaluations, 45 women who scored less than 20 were assumed to have actual dementia.

Radioimmunoassay

The concentrations of DHEA-S and DHEA in serum were measured by SRL (Tokyo, Japan) using Coat-A Count DHEA-SO4 and DHEA (Diagnostic Products Corporation, Los Angels, CA, USA) kits, respectively, as previously reported.[7] Normal ranges of serum DHEA-S concentration by decade in females based on SRL's evaluation were 85–299 (20–29 years old), 64–203 (30–39 years old), 25–195 (40–49 years old), 11–116 (50–59 years old) and 5–100 μg/dL (over 60 years old; see Fig. 1).

Statistics

Correlations among age, serum concentrations of DHEA-S (or DHEA), Barthel index score and HDS-R were evaluated by univariate or multivariate regression analyses. Statistical evaluation was performed using SPSS 11.5 software (Windows; SPSS, Chicago, IL, USA) with the level of significance set at 5%.

Results

Serum concentrations of DHEA-S in the oldest old women subjects ranged 2–141 μg/dL (Fig. 1). A significant age-associated change of serum DHEA-S level was not observed ($r = -0.0174$, $P = 0.91$). Furthermore, 80% (40/50) of all women exceeded the lowest level of the normal range of women in their forties, while five subjects exceeded even the lowest level of the normal range of women in their twenties.

In the Bathel index, 10 subjects scored 0/100, whereas the other 40 subjects scored from 12/100 to 100/100. On the other hand, on HDS-R, 16 subjects scored 0/30 while the other 34 subjects scored from 1/30 to 24/30. Indeed, the 45 women who scored less than 20 were assumed to have actual dementia. Interestingly, univariate regression analysis revealed that the serum DHEA-S level significantly correlated with HDS-R ($R = 0.392$, $P < 0.05$) but not with the Barthel index ($R = -0.009$; Fig. 2). We also measured serum concentrations of DHEA because DHEA is expected to be an active form

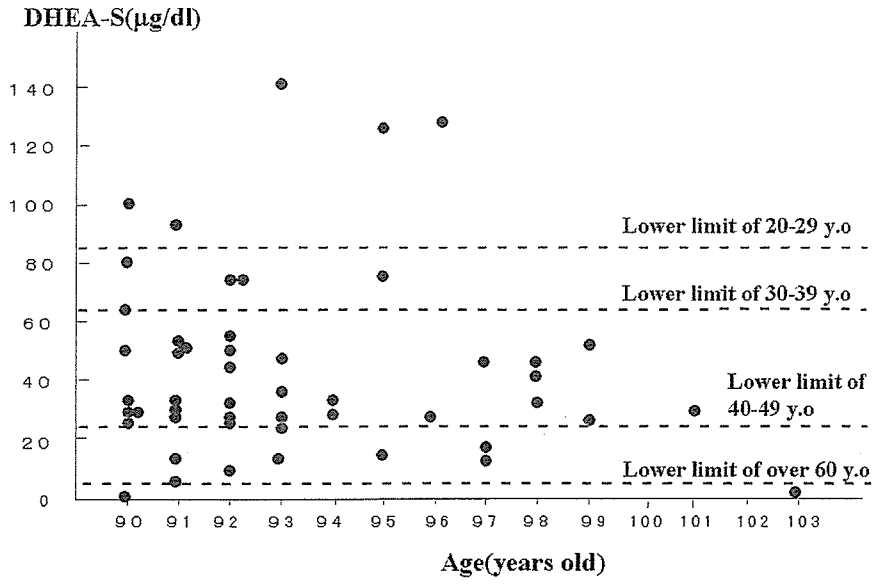

Figure 1 Scattergram of serum dehydroepiandrosterone sulfate (DHEA-S) concentrations versus age in oldest old women ($n = 50$). The dashed line indicates the lower limit of DHEA-S concentrations according to age.

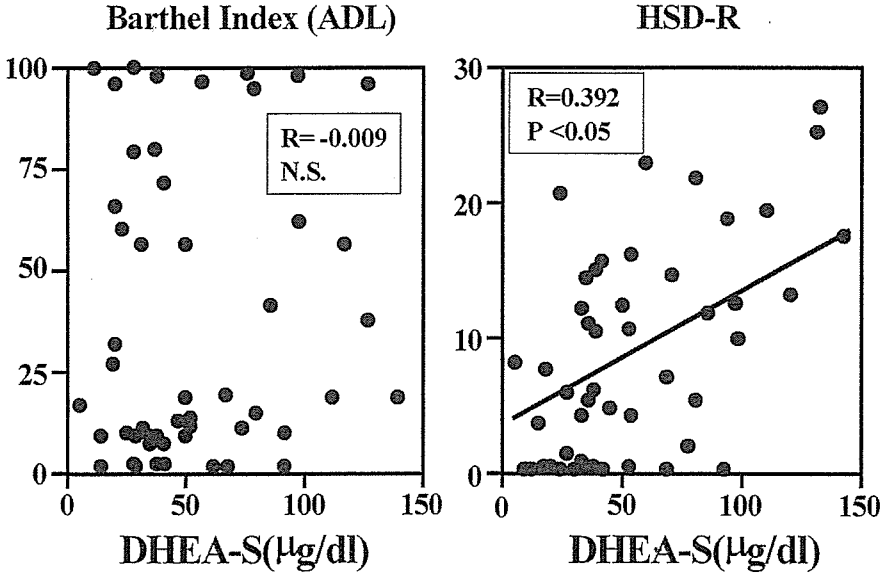

Figure 2 Correlation between DHEA-S concentrations and Barthel index scores (left), and Hasegawa's dementia rating scale (right).

of DHEA-S and pass through the blood brain (B–B) barrier,[16] unlike DHEA-S.[17] The results for DHEA were the same as those of DHEA-S; namely, that DHEA significantly correlated with HDS-R ($R = 0.367$, $P < 0.05$) but not with Bathel's index ($R = -0.017$). DHEA and DHEA-S-values showed good correlation with each other as expected ($R = 0.792$, $P < 0.001$).

Multivariate regression analysis also revealed that only HDS-R was a factor significantly correlated to DHEA-S or DHEA at a significance level of 5%. There was no significant correlation between the Barthel index and HDS-R on both univariate and multivariate regression analyses, suggesting that dementia did not essentially affect the ADL performance scales.

Discussion

In the present study, we measured serum concentrations of DHEA-S in 50 oldest old females and evaluated the relationships with ADL and mental activity, estimated by the Barthel index and HDS-R, respectively. Most of our subjects, all over 90 years of age, showed levels above the lowest normal levels for serum DHEA-S in women in their forties. The relatively higher level of serum DHEA-S levels in the oldest old female population may provide a plausible basis for the longevity of our subjects since Roth *et al.* has reported that relatively higher serum DHEA-S levels are associated with a significantly higher survival rate in humans.[2]

The de novo synthesis of DHEA in the brain has been demonstrated in the presence of steroidogenic enzymes[18] and by the fact that brain DHEA content is not affected by adrenalectomy or even castration.[19] Administration of DHEA or DHEA-S to mice has been shown to enhance memory retention[20] and to block the memory-impairing effects of scopolamine or benzodiazepine.[21,22] Also, DHEA has a trophic effect on cholinergic neurons and cultured neurological cells.[20,23] DHEA binds specifically to synaptosomal membranes and acts as an allosteric antagonist of GABA (γ-aminobutyric acid) receptors.[24] Although the relationship between such neuromodulatory functions and neuronal excitability or memory enhancement by DHEA is not well understood, these findings suggest the functional importance of DHEA-S on the central nervous system, and possibly in the prevention of dementia. Decreased concentrations of DHEA may fail to protect partly degenerated or at-risk brain cells, which might thus disturb memory retention. However, the role of serum DHEA-S levels in dementing diseases including AD is controversial.[5–9] Well-Engerer *et al.* directly determined steroid concentrations in various regions of the aged human brain by gas chromatography-mass spectrometry; demonstrating that high levels of key proteins were implicated in the formation of plaques and neurofibrillary tangles and were correlated with decreased brain levels of DHEA-S suggesting a possible neuroprotective role for DHEA in AD.[25] Moreover, the presence of DHEA, DHEA-S and their metabolites such as 7α-, 7β, or 16α-hydroxy DHEA in human cerebrospinal fluid (CSF) has been reported.[26] Although the physiological significance of this remains unclear, relatively higher DHEA and lower DHEA-S levels in CSF were observed in patients with AD and CVD compared with levels in controls.[26]

Further investigations are required to ascertain whether such reductions of brain DHEA content in patients with AD can be linked with decreases of serum DHEA-S. At present, whatever the causes of variations in serum DHEA-S levels are, it is evident that it would be convenient if we could accurately estimate cognitive and physical activities of elderly people by serum DHEA-S levels. Our results showing a relatively good correlation between serum DHEA-S concentrations and HDS-R might support the hypothesis that DHEA-S protects against dementia.

On the other hand, DHEA-S levels were not correlated with ADL evaluated by the Barthel index. In respect to our study, dementia did not appear to affect ADL estimations because the Barthel index and HDS-R were statistically independent of each other. Ravaglia *et al.* reported that in healthy men over 90 years of age, there was a positive relationship between serum DHEA-S levels and functional independence as evaluated by the Katsz scale of ADL.[11] In contrast, several other studies have reported an inverse relation between serum DHEA-S levels and functional independence (ADL) in nursing home men[12] and women.[13] While reasons for these conflicting results remain unclear, it might depend on differences in contents of ADL estimation scales or differences in study populations.

In summary, while further studies are needed to elucidate these relationships, our results suggest that DHEA-S levels in serum in oldest old Japanese females may be more related with cognitive function than with ADL. Trials involving DHEA treatment using different doses to groups of oldest old Japanese would test this hypothesis.

Acknowledgments

This work was partly supported by the Research Grant for Longevity Sciences from the Ministry of Health and Welfare.

References

1 Nawata H, Yanase T, Goto K *et al.* Mechanism of action of anti-aging DHEA-S and the replacement of DHEA-S. *Mech Ageing Dev* 2002; **123**: 1101–1106.
2 Roth GS, Lane MA, Ingram DK *et al.* Biomarkers of caloric restriction may predict longevity in humans. *Science* 2002; **297**: 811.
3 Kroboth PD, Salek FS, Pittenger AL *et al.* DHEA and DHEA-S: a review. *J Clin Pharmacol* 1999; **39**: 327–348.
4 Morales AJ, Nolan JJ, Nelson JC *et al.* Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. *J Clin Endocrinol Metab* 1994; **78**: 1360–1367.
5 Baulieu EE, Thomas G, Legrain S *et al.* Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. *Proc Natl Acad Sci USA* 2000; **97**: 4279–4284.
6 Sunderland T, Merril CR, Harrington MG *et al.* Reduced plasma dehydroepiandrosterone concentrations in Alzheimer's disease. *Lancet* 1989; **2**: 570.
7 Yanase T, Nawata H. Chapter 6, Dehydroepiandrosterone (DHEA) and Alzheimer's disease. In: Ronald RW, ed. *Health Promotion and Aging: the Role of Dehydroepiandrosterone (DHEA)*. Amsterdam: Harwood Academic Publishers, 1998; 63–70.
8 Nasman B, Olsson T, Backstom T *et al.* Serum dehydroepiandrosterone sulfate in Alzheimer's disease and in multi-infarct dementia. *Biol Psychiatry* 1991; **30**: 684–690.
9 Yanase T, Fukahori M, Taniguchi S *et al.* Serum dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) in Alzheimer's disease and in cerberovascular dementia. *Endocrine J* 1996; **43**: 119–123.
10 Wolkowitz OM, Kramer JH, Reus VI *et al.* DHEA treatment of Alzheimer's disease: a randomized, double-blind, placebo-controlled study. *Neurology* 2003; **60**: 1071–1076.
11 Ravaglia G, Forti P, Maioli F *et al.* The relationship of dehydroepiandrosterone sulfate (DHEAS) to endocrine-metabolic parameters and functional status in the oldest old. Results from an Italian study on healthy free living over-ninety year olds. *J Clin Endocrinol Metab* 1996; **81**: 1173–1178.

12 Rudman D, Shetty KR, Mattson DE. Plasma dehydroepiandrosterone sulfate in nursing home men. *J Am Geriatr Soc* 1990; **38**: 421–427.
13 Breuer B, Trungold S, Martucci C *et al.* Relationships of sex hormone levels to dependence in activities of daily living in the frail elderly. *Maturitas* 2001; **39**: 147–159.
14 Group NINDS. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA. Stroke Study Group. *N Engl J Med* 1995; **333**: 1581–1587.
15 Hosokawa T, Yamada Y, Isagoda A *et al.* Psychometric equivalence of the Hasegawa dementia scale-revised with the mini-mental state examination in stroke patients. *Percept Mot Skills* 1994; **79**: 664–666.
16 Baulieu EE, Robel P. Dehydroepiandrosterone and dehydroepiandrosterone sulfate as neuroactive neurosteroids. *J Endocrinol* 1996; **150**: S221–S239.
17 Kishimoto Y, Hoshi M. Dehydroepiandrosterone sulfate in rat brain: incorporation from blood and metabolism in vivo. *J Neurochem* 1972; **19**: 2207–2215.
18 Brown RC, Liu Y, Papadopoulos VDHEA. Biosynthesis, regulation and function in the central nervous system. In: Morfin R, ed. *DHEA and the Brain*. London: Taylor & Francis, 2000; 129–146.
19 Mellon SH. Neurosteroids: biochemistry, mode of action, and clinical relevance. *J Clin Endocrinol Metab* 1994; **78**: 1003–1008.
20 Roberts E, Bologa L, Flood JF *et al.* Effect of dehydroepiandrosterone and its sulfate on brain tissue in culture and on memory in mice. *Brain Res* 1987; **406**: 357–362.
21 Flood JF, Roberts E. Dehydroepiandrosterone sulfate improves memory in aging mice. *Brain Res* 1988; **448**: 178–181.
22 Flood JF, Smith GE, Roberts E. Dehydroepiandrosterone and its sulfate enhances memory retention in mice. *Brain Res* 1988; **447**: 269–278.
23 Bologa L, Sharma J, Roberts E. Dehydroepiandrosterone and its sulfated derivative reduce neuronal death and enhance astrocyte differentiation in brain. *J Neurosci Res* 1987; **17**: 226–234.
24 Majewska MD, Demirgoren S, Spivak CE *et al.* The neurosteroid dehydroepiandrosterone sulfate is an allosteric antagonist of the GABAA receptor. *Brain Res* 1990; **526**: 143–146.
25 Well-engerer S, David J-P, Sazdovitch V *et al.* Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. *J Clin Endocrinol Metab* 2002; **87**: 5138–5142.
26 Kim SB, Hill M, Kwak YT *et al.* Neurosteroids: cerebrospinal fluid levels for Alzheimer's disease and vascular dementia diagnostics. *J Clin Endocrinol Metab* 2003; **88**: 5199–5206.

AJH 2006; 19:1206–1212

Evaluation of a New Carotid Intima-Media Thickness Measurement by B-Mode Ultrasonography Using an Innovative Measurement Software, Intimascope

Toshihiko Yanase, Shigeru Nasu, Yoshihiro Mukuta, Yutaka Shimizu, Tetsu Nishihara, Taijiro Okabe, Masatoshi Nomura, Toyoshi Inoguchi, and Hajime Nawata

Carotid intima-media thickness (IMT), an indicator of atherosclerosis and coronary heart disease (CHD) is usually evaluated by eye measurement under B-scope carotid artery ultrasonography. However, the axial resolution of this system is ≥0.1 mm, which causes difficulties in respect to accuracy and reproducibility. We evaluated a newly developed B-scope carotid artery ultrasonography programmed by an innovative measurement software, Intimascope (Media Cross Co. Ltd., Tokyo, Japan), which measures IMT with 10 times higher axial resolution at an estimated scale of 0.01 mm. Intraobserver or interobserver coefficient of variation (CV) of the computer-based average IMT (aver-IMT) value and 3-point IMT value were much smaller than the corresponding value by conventional eye-measurement method (3-point value). We measured IMT of 427 asymptomatic subjects undergoing medical checkups (243 men and 184 women, 23 to 73 years of age). Although the mean values of aver-IMT and 3-point IMT of 427 subjects were comparable with that of the eye measurement method, the aver-IMT showed the smallest SD (standard deviation) and CV values. In both men and women, multivariate regression analysis revealed significant contributions of age and LDL-C to the aver-IMT value. Univariate regression analysis revealed that the aver-IMT value of total subjects showed the highest correlation coefficient values with most risk factors and risk assessment score, Framingham Risk Assessment, or Prospective Cardiovascular Munster study (PROCAM) Risk Score. These results may suggest superiority of computer-based aver-IMT over 3-point IMT by either computer-based or eye measurement method. Carotid aver-IMT measurement using the new Intimascope software may provide a more precise and reproducible index of atherosclerosis than does conventional IMT measurement.

Am J Hypertens 2006;19:1206–1212 © 2006 American Journal of Hypertension, Ltd.

Key Words: IMT, carotid, software.

Intima-media thickness (IMT) determined by B-mode ultrasonography is comparable to the thickness of intima-media complexes as determined by histologic examination.[1,2] The extent of carotid atherosclerosis is strongly associated with the presence of coronary heart disease (CHD) and is a marker for the early phase of the atherosclerotic process[3–10]; thus carotid IMT has now become a convenient and noninvasive method for evaluating structural changes in the arterial wall. However, it has usually been evaluated by the eye-measurement (manual) method using a devices such as slide calipers, making it somewhat difficult to obtain reproducible results by multiple observers or indeed even by a single observer. Furthermore the axial resolution of this system is at least in the order[11] of 0.1 mm, thus presenting further difficulties in respect to precision and reproducibility.

To resolve these problems of accuracy and reproducibility, a new ultrasonic diagnostic equipment (SDU-2200,

Received September 16, 2005. First decision May 14, 2006. Accepted May 16, 2006.

From the Department of Medicine and Bioregulatory Science (TY, TO, MN, TI, HN), Third Department of Internal Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; Human Dry Dock Center Wellness (SN), Fukuoka, Japan; and Shimadzu Corporation (YM, YS, TN), Kyoto, Japan.

Address correspondence and reprint requests to Dr. Toshihiko Yanase, Department of Medicine and Bioregulatory Science (Third Department of Internal Medicine), Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; e-mail: yanase@intmed3.med.kyushu-u.ac.jp

0895-7061/06/$32.00
doi:10.1016/j.amjhyper.2006.05.010

Shimadzu) programmed by an innovative measurement software for IMT (Intimascope, Media Cross Co. Ltd., Tokyo, Japan) was developed. This system was designed to avoid errors in the smooth estimation of the boundary of the intima and adventitia. It also enables the averaging of approximately 250 points of IMT values in the measurement segment with a high axial resolution in the order of 0.01 mm.

In the present study, we report the high utility of this computer-automated measurement system as an evaluation tool for atherosclerosis based on a comparative study with manual method and an extensive study of 427 asymptomatic subjects. In addition, we examined the relationships between carotid IMT and risk assessment scores for CAD and with various atherosclerosis-related parameters.

Subjects and Methods

Subjects

For intraobserver variation, 10 examiners measured IMT of three different subjects (all male; 35, 37 and 38 years of age). For interobserever variation, IMT of 11 volunteers (nine men, 33 to 48 years of age; and two women, 25 and 35 years of age) were evaluated by 10 different measurers to assess interobserver variations by methods using computer-automated IMT measurement and conventional eye-measurement IMT measurement.

The subject group comprised 427 individuals (243 men, age 23 to 73 years, average 48±9 (mean ± SD; 184 women, 25 to 73 years, average 46 ± 8) who consulted the Human Dry Dock Center, Wellness (Fukuoka, Japan) for a routine health evaluation. A summary of the subject profiles is shown in Table 1. Evaluations included measurement of body mass index (BMI), body fat ratio, blood chemistry analyses, electrocardiography, chest cardiography, evaluations of serum chemistry and carotid artery ultrasonography. By interview, smoking index (number of cigarettes/day), and family history of myocardial infarction were also evaluated. All studies were undertaken as part of routine examinations, with the consent of all subjects. Blood samples were obtained from subjects in a fasting state at 8:30 AM. Serum concentrations of total cholesterol (TC), triglyceride (TG), HDL cholesterol (HDL-C), fasting blood sugar (FBS), glycosylated hemoglobin (HbA_{1c}), uric acid (UA), and high-sensitivity C-reactive protein (hCRP) were measured. Serum concentrations of LDL-cholesterol (LDL-C) were calculated by the Friedewald formula.[12] The Framingham Risk Score (FRS)[13] and the Prospective Cardiovascular Munster study (PROCAM) score,[14] both useful markers in predicting CHD within the next 10 years, were also evaluated. The PROCAM score is different from the FRS in that the PROCAM score has two more scores regarding the presence or absence of diabetes mellitus and family history of myocardial infarction.

Ultrasound Protocol

The IMT was measured by ultrasonic diagnosis equipment (Shimadzu SDU-2200) that was programmed by the IMT measurement software, Intimascope (Media Cross Co. Ltd.). In a manual method, the IMT value was conventionally measured by the eye-measurement method without this software. Intimascope is an innovative software

Table 1. Subject profile by decade of age

Characteristic	≤39 y	40–49 y	50–59 y	60–69 y	70–79 y
Number	91	172	132	30	2
Age (y)	35.5 (3.5)	44.4 (3.0)	53.8 (2.7)	63.1 (2.3)	73.0 (0.0)
Men/women	45/46	96/76	81/51	20/10	1/1
Smoker/nonsmoker	25/66	44/128	33/99	3/27	0/2
BMI	21.6 (3.5)	22.8 (3.5)	23.0 (2.8)	23.9 (2.5)	20.5 (2.2)
% Body fat	23.6 (5.8)	24.6 (5.6)	24.4 (4.9)	24.8 (4.9)	20.0 (0.1)
SBP (mm Hg)	107.5 (13.2)	112.0 (13.7)	116.6 (15.5)	123.1 (14.8)	115.0 (7.1)
DBP (mm Hg)	64.4 (9.4)	67.1 (10.3)	70.4 (11.0)	72.5 (10.0)	66.0 (5.7)
FBS (mg/dL)	91.1 (8.0)	95.5 (14.8)	100.7 (26.3)	100.4 (21.2)	99.0 (4.2)
HbA_{1c} (%)	4.67 (0.33)	4.88 (0.61)	5.04 (0.81)	5.07 (0.69)	5.05 (0.07)
TC (mg/dL)	192.1 (32.0)	207.4 (30.5)	213.0 (33.4)	196.4 (33.9)	253.0 (1.4)
TG (mg/dL)	103.2 (76.4)	132.0 (137.4)	123.0 (81.1)	111.7 (80.3)	95.0 (43.8)
HDL-C (mg/dL)	60.1 (14.4)	59.2 (14.8)	56.5 (15.0)	53.6 (10.9)	65.0 (21.2)
LDL-C (mg/dL)	111.3 (25.1)	121.9 (28.9)	131.9 (28.5)	120.5 (35.1)	169.0 (28.6)
UA (mg/dL)	5.28 (1.49)	5.32 (1.58)	5.72 (1.52)	5.61 (1.35)	4.90 (0.85)
Smoking index	87.8* (155.5)	169.8† (325.4)	229.3‡ (389.9)	151.2§ (421.2)	0.0 (0.0)

BMI = body mass index; DBP = diastolic blood pressure; FBS = fasting blood sugar; HDL-C = HDL-cholesterol; LDL-C = LDL-cholesterol; SBP = systolic blood pressure; TC = total cholesterol; TG = triglyceride; UA = uric acid.

Values are given as mean (SD).

* N = 81.

† N = 150.

‡ N = 103.

§ N = 21.

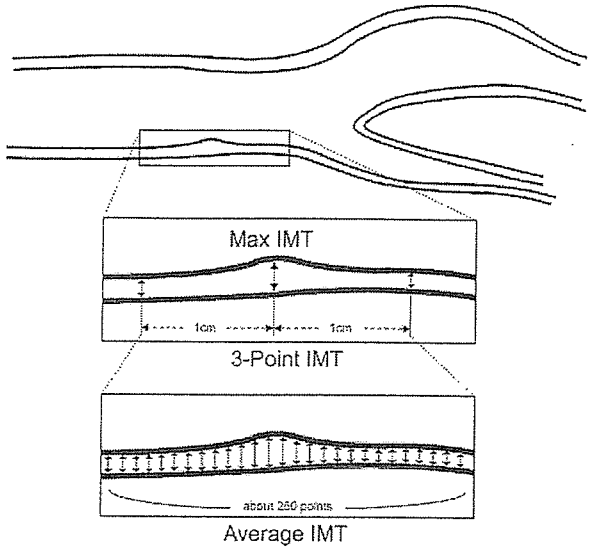

FIG. 1. Schematic representation of the carotid artery segment as measured in this study and the three methods used for measuring intima-media thickness (IMT): 3-point, maximal, and computer-based average (aver-IMT).

developed for IMT measurement to minimize measurement errors, which are usually caused by the meandering of blood vessels and differences in the skill levels of measurers. This software was devised to determine the perpendicular distance to the external membrane of the blood vessel. The software makes it possible to recognize automatically the edges of the internal and the external membranes of the blood vessels and to measure automatically the distance at a sub-pixel level (estimated to be 0.01 mm), using a three-dimensional polynomial measurement formula.

Carotid ultrasonography was performed using a 10-MHz scanning frequency in B mode. One skilled observer, blinded to the clinical data, scanned the vessel in transverse planes. Subjects were examined in a supine position. Images were obtained in the 20 mm proximal to the origin of the bulb at the far wall of the right common carotid artery (CCA) (Fig. 1). It is easy for examiners to access the origin of the bulb with echography, thus facilitating the correct positioning of the segment to be measured. In all subjects examined in this study, no plaque was observed in this segment. Thus, in this plaque-free region, computer-based IMT was evaluated by three methods: 3-point, maximal, and average evaluations (Fig. 1). Three-point evaluation refers to the average value of 3-point IMT, including two end points and the middle point in the >2 cm region. Maximal (max) evaluation was obtained by the IMT value at a maximal point of the region. Average IMT (aver-IMT) is the average value of 250 computer-based points in the region. In a manual method, IMT was measured by eye-measurement based on the average value of 3-point IMT.

Statistical Analysis

Agreement between different IMT measurement methods was assessed by a simple plot of the difference between the methods against their mean, as previously described by Bland and Altman.[15] The Kolmogorov-Smirnov test was used to examine whether respective IMT values of the 427 subjects measured by the different methods show normal distribution pattern. As a result, all IMT values by four different methods including computer-based methods (max IMT, 3-point IMT, and aver-IMT methods) and manual 3-point IMT did not show a normal distribution pattern ($P < .001$). The difference among the methods against their mean was then statistically examined using a nonparametric method, the Mann-Whitney test. Correlations between IMT values and various parameters including BMI, body fat ratio, systolic blood pressure (SBP), diastolic blood pressure (DBP), and smoking index were evaluated. Linear univariate regression and linear multiple regression analyses were performed using the SPSS version 11.5 for Windows software package (SPSS Inc., Chicago, IL), with significance for both set at $P < .05$. In the linear multivariate regression analysis, aver-IMT was adjusted as a dependent (response) variable whereas age, FBS, TC, HDL-C, TG, LDL-C, UA, HbA_{1c}, BMI, percentage of body fat, SBP, DBP, hCRP, smoking index, FRS, and PROCAM score were adjusted as independent variables and extracted by stepwise method using forward–backward selection. In this selection, F values as inclusion criteria were ≤ 0.05, whereas F values as exclusion criteria were ≥ 0.10. To compare the effectiveness of variables on the aver-IMT, a standardized regression coefficient was also calculated.

Results

Comparison of IMT Values Between Computer-Automated and Manual Methods

The intraobserver variation for IMT measurements (10 different examiners for three subjects) was calculated as mean (±SD) of coefficient of variation (CV; SD/mean × 100%) of the IMT. Mean (± SD) values of such CV values for manual 3-point IMT, computer-based 3-point IMT, and aver-IMT were 7.7% ±1.0%, 7.0% ±1.2%, and 5.6% ± 0.8%, respectively. The interobserver variation for IMT by 3-point IMT and aver-IMT methods were evaluated using the computer-based methods and these values were compared with those by the 3-point IMT using the manual method. The interobserver CV computer-automated 3-point IMT method findings ranged from 2.2% to 13.5%, and the average value of the 11 subjects was 8.5% In comparison the findings by the aver-IMT method ranged from 2.5% to 10.9%, and the average value of the 11 subjects was 5.9 %. On the other hand, the interobserver variation by 3-point IMT based on the manual method ranged from 13.5% to 28.4%, and the average value of the 11 subjects was 20.6%. From these findings, the computer-automated aver-IMT evaluations by 3-point or aver-IMT methods were shown to be more reliable than manual 3-point method.

Table 2. Mean ± SD (mm) and coefficient of variation (CV, %) of intima-media thickness (IMT) values of 427 healthy subjects

Value	Mean (mm)	SD (mm)	CV (%)
Max IMT	0.7331	0.1565	21.3
Aver IMT	0.5700	0.1053	18.5
3-Point IMT	0.5852	0.1180	20.2
Eye-M IMT	0.5832	0.1467	25.2

Maximal (Max) IMT, average (Aver) IMT, and 3-point IMT are computer-generated values. Eye-M IMT indicates conventional eye-measurement 3-point IMT.

Next we also compared the values of the computer-automated IMT values by the three methods (max-IMT, 3-point IMT, aver-IMT and eye-measurement 3-point methods) and the IMT values by conventional method in 427 asymptomatic subjects presenting for a regular medical check-up (Table 2). By mean value, the maximal IMT value was the highest, followed by the 3-point IMT value, the aver-IMT value, and manual 3-point IMT value. Agreement between different IMT measurement methods based on a simple plot of the difference between the computer-based method and manual method against the mean[15] revealed that only the difference between max IMT and manual IMT (0.1499 ± 0.132 mm, mean ± SD) was quite large compared with those between aver-IMT and manual IMT (−0.0135 ± 0.1049) and between 3-point IMT and manual IMT (0.0020 ± 0.1145). Figure 2 provides an example of the agreement between aver-IMT and manual IMT against the mean, indicating the methods to be comparable with each other. Agreement between different IMT measurement methods was also tested by a statistical analysis using a nonparametric method, the Mann-Whitney *U* test. The mean value of computer-based aver-IMT and 3-point IMT matched the value of the eye-measurement IMT, respectively, but the max IMT did not. With regard to SD and CV values of IMT in the 427 subjects by the four methods mentioned above, the values of computer-based aver-IMT and 3-point IMT were much smaller than those of the eye-measurement methods (Table 2).

Correlation Between aver-IMT and Other Parameters

In linear multivariate regression analyses using the stepwise method (forward–backward selection), age, BMI, and LDL-C were factors significantly correlated with aver-IMT in men ($P < .05$). In men, standardized regression coefficient values of age, BMI, and LDL-C were 0.502, 0.182, and 0.109, respectively, indicating that age had the strongest effect on IMT. Similarly age, SBP, and LDL-C were factors significantly correlated with aver-IMT in women ($P < .01$). In women, standardized regression coefficient values of age, LDL-C, and SBP were 0.448, 0.155, and 0.142, respectively. In addition, in univariate analysis, the aver-IMT values showed a positive correlation with FRS both in men ($n = 243$, $r = 0.389$, $P < .001$) and in women ($n = 184$, $r = 0.470$, $P < .001$). Similarly the IMT values showed a similar significant correlation with the PROCAM risk score both in men ($r = 0.387$, $P < 0.001$) and in women ($r = 0.406$, $P < 0.001$). As the results of multivariate analyses, the FRS and PROCAM score were quite similar for both sexes, univariate regression analyses between IMT and each parameter were performed in a combined group of both men and women.

We evaluated the correlations between the IMT values and various parameters, which are closely associated with the metabolic syndrome and atherosclerosis (Table 3). Parameters that positively correlated with aver-IMT values in the total group of subjects ($n = 427$) with a significance of $P < .05$, were age, FBS, TC, LDL-C, UA, HbA_{1c}, BMI, SBP, DBP, smoking index, FRS, and PROCAM risk scores. As previously reported in a number of studies,[16] aver-IMT was largely age dependent in normal subjects. The actual aver-IMT values with aging are shown in Fig. 3, and the rate of increase of aver-IMT with aging was 0.007 mm/year. On the other hand, HDL-C was negatively correlated with aver-IMT values. Importantly, computer-based IMT values by aver-IMT and 3-point IMT always showed higher coefficient values with the above parameters except TC than did conventional eye-measurement IMT. However, it was difficult to conclude which was superior between computer-based aver-IMT and 3-point IMT, because the difference of coefficient values between these two methods were not particularly evident apart from the parameter age.

Discussion

Carotid IMT is a very useful marker of coronary atherosclerosis in terms of the presence and extent of atherosclerosis as well as of coronary events.[3–10] However there

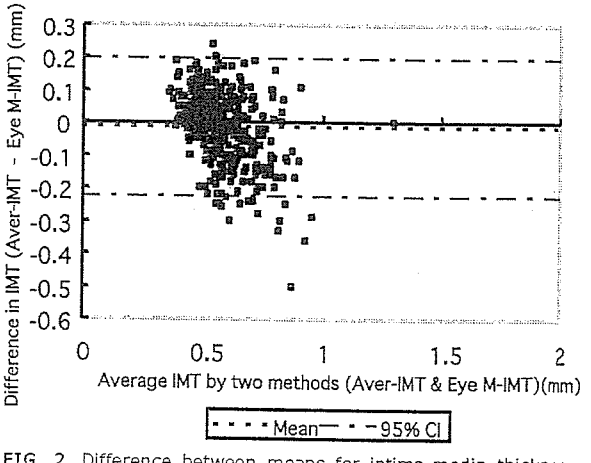

FIG. 2. Difference between means for intima-media thickness; Aver-IMT - Eye-M-IMT). The 95% confidence intervals (CI) range from −0.2233 to 0.1963. Aver-IMT = computer-based average intima-media thickness; Eye-M = eye-measurement method (3-point value).

Table 3. Coefficient values between carotid intima-media thickness (IMT) and various parameters in total group of subjects (n = 427)

Risk factor	Computer-based values		Eye-M IMT
	Aver IMT	3-Point IMT	
Age	0.544*	0.501*	0.433*
FBS	0.207*	0.215*	0.187*
TC	0.182*	0.174*	0.187*
HDL-C	−0.187*	−0.172*	−0.106*
TG	0.076	0.046	0.137†
LDL-C	0.241*	0.246*	0.162*
UA	0.167*	0.163*	0.152*
HbA_{1c}	0.248*	0.264*	0.195*
BMI	0.280*	0.262*	0.224*
% Body fat	0.090	0.066	0.040
SBP	0.293*	0.289*	0.254*
DBP	0.274*	0.272*	0.217*
hCRP	0.085	0.067	0.069
Smoking index	0.120†	0.117†	0.109†
FRS	0.444*	0.418*	0.372*
PROCAM score	0.386*	0.357*	0.317*

hCRP = high-sensitivity C-reactive protein; other abbreviations as in Table 1.

Only the numbers of examinees for smoking index are 357 (see Table 1).

* $P < .01$.

† $P < .05$.

remains a problem in that eye-measurement methods using devices such as slide calipers do not always allow precision and reproducibility of the IMT value, especially when measured by inexperienced observers. Many studies have adopted max IMT as a predictor of CHD[5,6,9]; however, the measurement of max IMT does not necessarily typify the whole character of carotid atherosclerosis. A reasonable approach to increase the accuracy and reproducibility would be to extend measurements of IMT in respect of numbers of points and locations to as many as possible. For example, average IMT measurements by 10 points in both carotid arteries including common and internal arteries[10] or even at 80 points in common carotid artery[3] have been reported to be very useful for such evaluations. However there is a physical limitation to the numbers of the points that can be measured manually, because the measurements consume much time and require a large expenditure of resources.

For these reasons, a more sensitive and handy method for such measurements has long been sought. In the present study, we evaluated a newly developed B-scope carotid artery ultrasonography device programmed by an innovative measurement software, Intimascope, which enables measurement of IMT with an axial resolution 10 times higher than previously possible, at an estimated scale of 0.01 mm. The IMT values based on this software were highly reliable in that the average interobserver variation was much superior to values obtained by the manual method. The difference analysis between the methods against their mean IMT values in 427 healthy subjects revealed that the computer-based aver-IMT and 3-point IMT values were statistically equivalent to the manual IMT value, respectively. Under these conditions, the computer-based aver-IMT evaluations were much more reliable than the 3-point IMT by either computer-based or manual methods, in respect to precision from the findings of the smallest values of SD and CV in the 427 subjects and from the highest positive correlation with various risk factors. This is reasonable because aver-IMT measurement takes advantages of the power of computer software, thus making it possible to measure an average value from 250 computer-based points of IMT in a 2-cm segment. The system also saves an examiner's time because the aver-IMT values can be automatically measured and calculated by the software. The total time for getting a result including measurement is usually <5 min per single subject. This software also produces easy to read reports of the IMT measurements. Thus the quickness and the easy applicability of IMT measurements using this software provides many advantages, especially when used in multiple locations and on different vessels or when used in a large numbers of patients. However a limitation of this software is that it is sometimes difficult to trace correctly the edges of the internal and the external membranes of blood vessels in some images containing many plaques or reverberation.

It is noteworthy that aver-IMT values in the healthy subjects in our study were positively correlated with age, BMI, SBP, DBP, FBS, HbA_{1c} TC, LDL-C, and UA, and were negatively correlated with HDL-C in univariate regression analysis, and also positively correlated with age and LDL-C in multivariate regression analysis. Similar

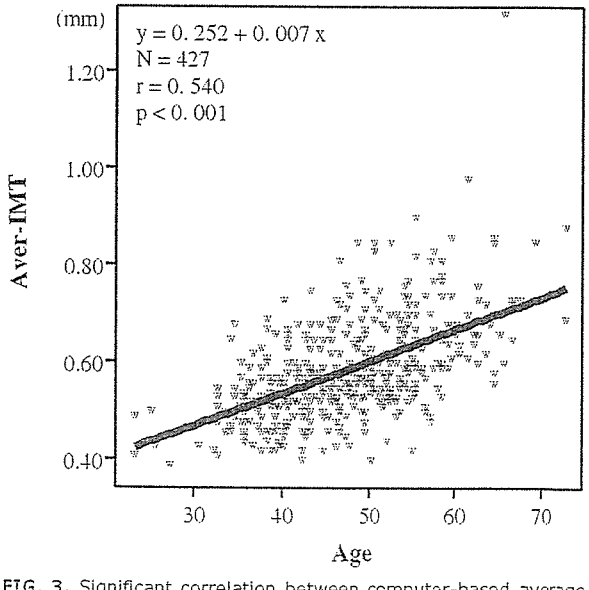

FIG. 3. Significant correlation between computer-based average intima-media thickness (aver-IMT) and age in the total population of subjects (n = 427).

results have been reported in a number of other IMT studies,[16–21] but most of these were reported in populations that included patients with CAD, hypercholesterolemia, cerebrovascular disease, diabetes mellitus, and hypertension. Reasonable correlations between aver-IMT values and several risk factors for CAD, even in our asymptomatic and healthy subjects undergoing routine health examination, suggest not only the validity of our study but also the high sensitivity of our method using this computer-automated measurement system.

The Framingham study stresses the importance of a multivariate risk profile in the prediction and prevention of CAD.[22] Indeed the traditional risk factors for CAD are associated with carotid artery IMT.[3,19–24] The utility of the FRS for predicting CHD in an person within the next 10 years has been demonstrated in individuals of white and nonwhite ethnicity, and in middle-aged and older populations.[21,22–25] PROCAM score is another type of multivariate risk score, and has also been shown to be useful in predictions of probability of CAD within the next 10 years.[26] We observed a positive correlation between aver-IMT values and those scores, indicating the impact of multiple risk factors on atherosclerosis even in a healthy, asymptomatic population. Similarly it is reported that a positive correlation between IMT and FRS in a young (age 20 to 38 years), population[27] supported the usefulness of FRS for guiding intervention in CAD risk factors from an early age.

In conclusion, our method for measurement of carotid IMT using the Intimascope software was found to be useful for evaluations of atherosclerosis, showing accuracy and reproducibility measurements in healthy subjects. This system is a powerful tool for the assessments of IMT in either the progression or regression of atherosclerosis in patients with risk factors for CHD as well as in studies evaluating various drug therapies.

References

1. Pignoli P: Ultrasound B-mode imaging for arterial wall thickness measurement. Atheroscler Rev 1984;12:177–184.
2. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R; Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation 1986;74:1399–1406.
3. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, Azen SP: The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann Intern Med 1998;128:262–269.
4. Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G: Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults: the Atherosclerosis (ARIC) Study Investigators. Stroke 1995;26:386–391.
5. Wofford JL, Kahl FR, Howard GR, McKinney WM, Toole JF, Crouse JR 3rd: Relation of extent of extracranial carotid artery atherosclerosis as measured by B-mode ultrasound to the extent of coronary atherosclerosis. Arterioscler Thromb 1991;11:1786–1794.
6. Salonen JT, Salonen R: Ultrasound B-mode imaging in observational studies of atherosclerotic progression. Circulation 1993;87: 56–65.
7. Hulthe J, Wikstrand J, Emanuelsson H, Wiklund O, de Feyter PJ, Wendelhag I: Atherosclerotic changes in the carotid artery bulb as measured by B-mode ultrasound are associated with the extent of coronary atherosclerosis. Stroke 1997;28:1189–1194.
8. Wendelhag I, Wiklund O, Wikstrand J: On quantifying plaque size and intima-media thickness in carotid and femoral arteries. Comments on results from a prospective ultrasound study in patients with familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 1996;16:843–850.
9. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr: Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340:14–22.
10. Kotsis VT, Pitriga VC, Stabouli SV, Papamichael CM, Toumanidis ST, Rokas SG, Zakopoulos NA: Carotid artery intima-media thickness could predict the presence of coronary artery lesions. Am J Hypertens 2005;18:601–606.
11. Girerd X, Boutouyrie P, Pannier B, Mourad J-J, Safar M, Laurent S: Noninvasive ultrasound methods for the measurement of arterial wall thickness, in Touboul P-J (ed): Intima-Media Thickness and Atherosclerosis Predicting the Risk? Parthenon Publishing, New York, USA, 1997 pp 45–57.
12. Friedewald WT, Levy RI, Fredrickson DS: Estimation of plasma low density lipoprotein cholesterol concentration without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
13. Anderson KM, Wilson PW, Odell PM, Kannel WB: An updated coronary risk profile. A statement for health professionals. Circulation 1991;83:356–362.
14. Assmann G, Cullen P, Schulte HL: Simple scoring scheme for calculating the risk of acute coronary events based on the 10 year follow-up of the Prospective Cardiovascular Munster (PROCAM) study. Circulation 2002;105:310–315.
15. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–310.
16. Bonithon-Kopp C: Prevalence of and risk factors for intima-media thickening: a literature review, in Touboul P-J (ed): Intima-Media Thickness and Atherosclerosis: Predicting the Risk? Parthenon Publishing, New York, USA, 1997 pp 27–44.
17. Crouse JR, Goldbourt U, Evans G, Pinsky J, Sharrett AR, Sorlie P, Riley W, Heiss G: Risk factors and segment-specific carotid arterial enlargement in the Atherosclerosis Risk in Communities (ARIC) cohort. Stroke 1996;27:69–75.
18. Sharrett AR, Patsch W, Sorlie PD, Heiss G, Bond MG, Davis CE: Associations of lipoprotein cholesterols, apolipoproteins A-I and B, and triglycerides with carotid atherosclerosis and coronary heart disease. The Atherosclerosis Risk in Communities (ARIC) study. Arterioscler Thromb 1994;14:1098–1104.
19. Folsom AR, Pankow JS, Williams RR, Evans GW, Province MA, Eckfeldt JH: Fibrinogen, plasminogen activator inhibitor-1, and carotid intima-media wall thickness in the NHLBI Family Heart Study. Thromb Haemost 1998;79:400–404.
20. Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D, Bonaa KH: Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. Arterioscler. Thromb Vasc Biol 1996;16:984–991.
21. Davis PH, Dawson JD, Riley WA, Lauer RM: Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age. Circulation 2001;104:2815–2819.
22. Kannel WB: Contributions of the Framingham Study to the conquest of coronary artery disease. Am J Cardiol 1988;62:1109–1112.
23. Ramachandran S, French JM, Vanderpump MP, Croft P, Neary RH: Using the Framingham model to predict heart disease in the United Kingdom: retrospective study. Br Med J 2000;320:676–677.

24. D'Agostino RB Sr, Grundy S, Sullivan LM, Wilson P, CHD Risk Prediction Group: Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic group investigation. J Am Med Assoc 2001;286:180–187.
25. Mahoney LT, Burns TL, Stanford W, Thompson BH, Witt JD, Rost CA, Lauer RM: Usefulness of the Framingham risk score and body mass index to predict early coronary artery calcium in young adults (Muscatine Study). Am J Cardiol 2001;88:509–515.
26. Athyros VG, Papageorgiou AA, Athyrou VV, Demitriadis DS, Kontopoulos AG: Atrovastatin and micronized fenofibrate alone and in combination in type 2 diabetes with combined hyperlipidemia. Diabetes Care 2002;25:1198–1202.
27. Kieltyka L, Urbina EM, Tang R, Bond G, Srinivasan SR, Berenson GS: Framingham risk score is related to carotid artery intima-media thickness in both white and black young adults: the Bogalusa Heart Study. Atherosclerosis 2003;170:125–130.