International Journal of Obesity (2003) 27, 1325–1331
 0307-0565/03 $25.00
www.nature.com/ijo

PAPER

Serum bFGF levels are reduced in Japanese overweight men and restored by a 6-month exercise education

A Seida[1], J Wada[1]*, M Kunitomi[1], Y Tsuchiyama[1], N Miyatake[2], M Fujii[2], S Kira[3], K Takahashi[4], K Shikata[1] and H Makino[1]

[1]*Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine and Dentistry, Shikata-cho, Okayama, Japan;* [2]*Okayama Southern Institute of Health, Okayama, Japan;* [3]*Department of Public Health, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan; and* [4]*Faculty of Education, Okayama University, Okayama, Japan*

OBJECTIVE: To investigate whether the changes in vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) concentrations before and after weight reduction in Japanese overweight men are associated with changes in body mass index (BMI), visceral, subcutaneous fat, VO_2 and work rate (WR) at ventilatory threshold (VT).
DESIGN: Cross-sectional and longitudinal clinical intervention study with exercise education.
SUBJECTS: In total, 30 Japanese overweight men (BMI, $29.0 \pm 2.2\,kg/m^2$) and 31 normal-weight men (BMI, $22.5 \pm 1.6\,kg/m^2$) at baseline were enrolled: 30 overweight men (BMI, $29.0 \pm 2.2\,kg/m^2$) were further enrolled into a 6-month exercise program.
MEASUREMENTS: Fat distribution evaluated by visceral fat (V) and subcutaneous fat (S) areas measured with computed tomography scanning at umbilical levels, angiogenic peptides including VEGF and bFGF, exercise tests at baseline and after 6 months.
RESULTS: In normal-weight and overweight subjects at baseline, VEGF positively correlated with S area ($r = 0.350$, $P = 0.007$) but not with V area. In contrast, bFGF negatively correlated with BMI ($r = -0.619$, $P < 0.001$), S ($r = -0.457$, $P < 0.001$) and V areas ($r = -0.466$, $P < 0.001$). By intervention with exercise education, 30 overweight subjects showed reduction in BMI (29.0 ± 2.2 to 28.0 ± 2.0, $P < 0.001$), V and S areas, increase in VO_2 and WR at VT, increase in bFGF (9.21 ± 5.82–21.2 ± 7.04 ng/ml, $P < 0.001$), and no change in VEGF (1.45 ± 0.72–1.88 ± 0.52 ng/ml, $P = 0.016$). The stepwise multiple regression analysis revealed that ΔBMI ($\beta = -6.052$) and ΔVO_2 ($\beta = 2.806$) were independently related to ΔbFGF ($P < 0.001$) and all other variables including ΔS area, and ΔV area, and ΔWR did not enter the equation at significant levels.
CONCLUSION: The present study indicated a negative correlation between serum bFGF levels and BMI at baseline as well as an association of ΔBMI and ΔVO_2 with ΔbFGF after exercise intervention. The exercise-induced elevation of bFGF may be beneficial in the prevention of the atherosclerosis in overweight subjects.
International Journal of Obesity (2003) **27,** 1325–1331. doi:10.1038/sj.ijo.0802408

Keywords: angiogenesis; vascular endothelial growth factor; basic fibroblast growth factor; exercise; atherosclerosis

Introduction

Excess of body mass increases the risk of death from cardiovascular diseases in adults up to 75 y of age, and the relative risk associated with greater body weight is higher among younger subjects.[1] The regional difference of body fat is one of the critical determinants for vascular complications and the accumulation of abdominal fat is the major risk factor of cardiovascular diseases both in men and women.[2,3] In its molecular basis, it has been postulated that secreted bioactive substances derived from white adipose tissues, adipocytokines, would play a role in the development of insulin resistance, dyslipidemia, hypertension, and vascular diseases.[4]

In such secreted proteins or peptides, white adipose tissues are known to secrete angiogenic peptides including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Originally, VEGF was identified as an endothelial and vascular smooth muscle cells-derived hormone which stimulates local angiogenesis in response to hypoxia. Regarding adipose tissues, the role of VEGF in new vessel growth in brown fat has been demonstrated.[5–7] In white

*Correspondence: Dr J Wada, Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan.
E-mail: junwada@md.okayama-u.ac.jp
Received 28 November 2002; revised 3 April 2003; accepted 29 May 2003

adipose tissues, it has been also described that physiological concentration of insulin stimulates VEGF secretion, that is, insulin-stimulated VEGF formation.[8] Basic FGF is another potent endothelial cell mitogen and stimulates synthesis of proteases, including plasminogen activator and metalloproteinases that are important for extracellular matrix digestion in the process of angiogenesis.[9] In preadipocytes, bFGF stimulates the replication and inhibits their differentiation,[10] and the expression of bFGF decreases during the adipocyte differentiation.[11] In fully matured adipocytes, the addition of high concentration of bFGF into culture media induces the reversal of adipocyte differentiation[12] and marked suppression of activity of glycerol-3-phosphate dehydrogenase (GPDH), a marker of adipose differentiation.[13] In addition to VEGF and bFGF, leptin is also a white adipose-tissue-derived hormone which induces angiopoietin-2 expression,[14] and synergistically stimulates angiogenesis with VEGF and bFGF.

In line with these experimental evidences, one can speculate that the status of angiogenesis activities is dynamically changed during the accumulation and decrease of white adipose tissues in overweight and obese subjects. Angiogenesis factors directly derived from adipose tissues or indirectly simulated at the vasculatures may affect the vascular function of overweight subjects and may be related to the development of the atherosclerosis. To investigate such relations, we investigated Japanese overweight men aged 30–59 y who volunteered for the study. We measured leptin, VEGF, bFGF, and endostatin concentrations and compared with the visceral and subcutaneous fat areas estimated by computed tomography (CT) at baseline and after a 6-month exercise intervention.

Subjects and methods

Subjects

Japanese overweight men ($n=30$, 46.3 ± 7.4 y) and normal-weight men ($n=31$, 33.7 ± 2.7 y) were enrolled into this study with written informed consent. Overweight was diagnosed according to the criteria of WHO[15] and the average body mass index (BMI) of overweight and normal-weight subjects was 29.0 ± 2.2 and 22.5 ± 1.6 kg/m^2, respectively. All subjects were not taking any medications for diabetes, hypertension, and/or dyslipidemia throughout the observation period.

In a first cross-sectional analysis, we used baseline data on 30 overweight and 31 normal-weight subjects, and investigated the relations among body fat distribution, leptin, VEGF, bFGF, and endostatin serum concentrations.

In a second longitudinal analysis, we used follow-up data of 30 overweight subjects, who joined the exercise program at Okayama Southern Institute of Health. They visited the Okayama Southern Institute of Health every week and were monitored for 6 months. Daily steps were measured by pedometer (WZ100A, SEIKO Corporation, Japan) and the average of 7 days was monitored every week throughout the follow-up period. They were instructed to carry and check the pedometer every day and to walk 1000 steps more besides their daily walk at baseline. In addition, trained nutritionists determined total calorie intake using food diaries before and after the 6-month follow-up. Exercise tests were performed before and after the 6-month follow-up, and body fat distribution, percentage body fat, leptin, VEGF, bFGF, and endostatin serum concentrations were also measured.

Blood sampling and assays

We measured overnight fasting serum levels of total cholesterol and high-density lipoprotein (HDL) cholesterol,[16] triglycerides (L Type Wako Triglyceride.H, Wako Chemical, Osaka, Japan), insulin, leptin, and plasma glucose. Homeostasis model assessment of insulin resistance (HOMA-IR = [fasting insulin (μU/ml) $\times$ fasting glucose (mmol/l)/22.5]) was calculated as an indicator of insulin resistance.[17] Insulin levels were measured by immunoradiometric assay (IRMA) using INSULIN RIABEAD®II (DAINABOT, Tokyo, Japan). Serum leptin was measured by the human leptin radioimmunoassay (RIA) kit (Linco Research, St Charles, MO, USA). Serum VEGF, bFGF, and endostatin levels were determined using the ACCUCYTE EIA kit (CYTIMMUNE SCIENCES, College Park, ML, USA).

Visceral and subcutaneous fat areas and percentage body fat

The intra-abdominal visceral fat and the subcutaneous fat areas were measured by CT scan at the umbilical levels. CT films were converted into digital images and both visceral and subcutaneous fat areas were measured with image analysis software OPTIMAS version 6.5 (Media Cybernetics, Silver Spring, MD, USA). The intraperitoneal area with same density as the subcutaneous fat layer was defined as visceral fat area.[18] The relative percentage body fat was measured by a new air displacement plethysmograph, called the BOD POD Body Composition System (Life Measurement Instruments, Concord, CA, USA) as previously described.[19]

Exercise testing

A graded ergometer exercise protocol[20] was performed after an overnight fast. At 2 h after breakfast, an electrocardiogram was recorded and blood pressure measured. Then, all subjects were given graded exercise after 3 min of pedaling on an unloaded bicycle ergometer (Excalibur V2.0, Lode BV, Groningen, Netherlands). The profile of incremental workloads was defined by the methods of Jones *et al*,[20] in which the workloads reach the predicted VO_{2max} within 10 min. A pedaling cycle of 60 rpm was maintained. Loading was terminated when the appearance of symptoms forced the subject to stop. During the test, ECG was monitored

continuously together with the recording of heart rate (HR) and blood pressure using an auscultator with a pressure cuff around the upper arm connected to a mercury sphygmomanometer. Expired gas was collected and rates of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured breath-by-breath using the cardiopulmonary gas exchange system (Oxycon Alpha, Mijnhrdt b.v., Netherlands). Ventilatory threshold (VT) was determined by the standard of Wasserman *et al*,[21] Davis *et al*,[22] and the V-slope method of Beaver *et al*.[23] At VT, VO_2 (ml/kg/min), work rate (WR), and HR (beats/min) were measured and recorded.

Statistical analysis

All data are expressed as mean ± standard deviation (s.d.) values. Since some of the parameters did not show normal distribution, nonparametric tests were employed. Differences between the normal-weight and overweight subjects were compared by the Mann-Whitney *U*-test. Spearman correlation coefficients were used to evaluate whether bFGF and VEGF concentrations were correlated with BMI, subcutaneous (S) and visceral (V) areas. Baseline assessments of overweight subjects were compared with those after 6-month exercise education by means of Wilcoxon's signed-rank test. To determine the variables independently associated with changes in bFGF levels, a stepwise multiple regression analysis was performed. Normality of distribution of the variables was assessed through the use of the Shapiro–Wilks test and they were used as dependent and independent variables. *P*-values less than 0.01 were considered statistically significant. The data were analyzed with Dr. SPSS II for Windows release 11.0.1J.

Results

Simple correlations of BMI and fat distribution with insulin, HOMA-IR, and leptin

The fasting serum insulin ($P<0.001$), HOMA-IR ($P<0.001$), and leptin ($P<0.001$) were significantly higher in overweight subjects compared with normal-weight subjects (Table 1). The data indicated that Japanese men with BMI more than 25 kg/m^2 had hyperinsulinemia, insulin resistance, and hyperleptinemia. The insulin levels positively correlated with both S area (Spearman $r=0.582$, $P<0.001$) and V area (Spearman $r=0.483$, $P<0.001$) and the fasting serum leptin concentration also correlated with both S area (Spearman $r=0.613$, $P<0.0001$) and V area (Spearman $r=0.573$, $P<0.001$).

Simple correlations of BMI and fat distribution with VEGF, bFGF, and endostatin

Serum VEGF levels were significantly higher in overweight subjects (1.45 ± 0.72 ng/ml) compared with normal-weight subjects (0.88 ± 0.45 ng/ml) (Table 1). The VEGF levels positively and significantly correlated with S area (Spearman $r=0.350$, $P=0.007$) but not with BMI (Spearman $r=0.311$, $P=0.017$) and V area (Spearman $r=0.183$, $P=0.166$) (Table 2 and Figure 1).

Serum concentration of bFGF levels were significantly lower in overweight subjects (9.2 ± 5.82 ng/ml) compared with normal-weight subjects (23.1 ± 11.7 ng/ml) (Table 1). The bFGF levels negatively correlated with BMI ($r=-0.619$, $P<0.001$), S area ($r=-0.457$, $P<0.001$) and V area ($r=-0.466$, $P<0.001$) (Table 2 and Figure 2).

Table 1 Clinical characteristics of Japanese normal-weight and overweight subjects

	Total subjects (n = *61*)	*Normal-weight subjects* (n = *31*)	*Overweight subjects* (n = *30*)	*Normal-weight* vs *overweight*
Height (m)	1.70 ± 0.05	1.71 ± 0.05	1.68 ± 0.06	0.052
Weight (kg)	74.3 ± 10.9	66.0 ± 6.5	82.7 ± 7.5	<0.001*
BMI (kg/m^2)	25.7 ± 3.8	22.5 ± 1.6	29.0 ± 2.2	<0.001*
V area (cm^2)	81.3 ± 60.4	42.3 ± 29.2	121.7 ± 57.9	<0.001*
S area (cm^2)	113.6 ± 56.2	76.6 ± 41.3	151.8 ± 42.4	<0.001*
T-cho (mmol/l)	5.34 ± 1.00	5.00 ± 1.09	5.69 ± 0.84	0.005*
HDL-cho (mmol/l)	1.42 ± 0.40	1.55 ± 0.42	1.28 ± 0.33	0.003*
TG (mmol/l)	1.94 ± 1.89	1.62 ± 2.19	2.28 ± 1.51	<0.001*
LDL (mmol/l)	3.07 ± 1.18	3.95 ± 0.72	3.95 ± 0.81	<0.001*
FPG (mmol/l)	5.60 ± 0.18	4.97 ± 0.96	5.92 ± 1.16	<0.001*
Insulin (μU/ml)	8.95 ± 4.90	6.07 ± 2.86	11.9 ± 4.81	<0.001*
HOMA-IR	2.19 ± 1.33	1.33 ± 0.65	3.08 ± 1.26	<0.001*
Leptin (ng/ml)	5.30 ± 3.41	3.68 ± 1.50	6.97 ± 4.00	<0.001*
VEGF (ng/ml)	1.32 ± 0.68	0.88 ± 0.45	1.45 ± 0.72	0.002*
bFGF (ng/ml)	16.3 ± 11.6	23.1 ± 11.7	9.2 ± 5.82	<0.001*
Endostatin (ng/ml)	5.23 ± 2.27	5.61 ± 2.50	4.71 ± 1.49	0.689

*$P<0.01$ Mann–Whitney *U*-test is used.
BMI, body mass index; BP, blood pressure; V area, visceral fat area; S area, subcutaneous fat area; T-cho, total cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglyceride; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor.

Serum endostatin levels did not show difference between normal-weight and overweight subjects (Table 1) and did not reveal correlation with BMI, S and V areas.

Elevation of serum bFGF levels associated with elevation of VO_2 at VT

At a 6-month follow-up, 30 overweight subjects repeated evaluation of fat distribution and exercise tests (Table 3). After exercise education by instructors, daily steps significantly increased and were maintained throughout the observation period. Their BMI decreased from 29.0 ± 2.2 to $28.0 \pm 2.0\,kg/m^2$, V area 121.7 ± 57.9 to 99.4 ± 57.3, and S area

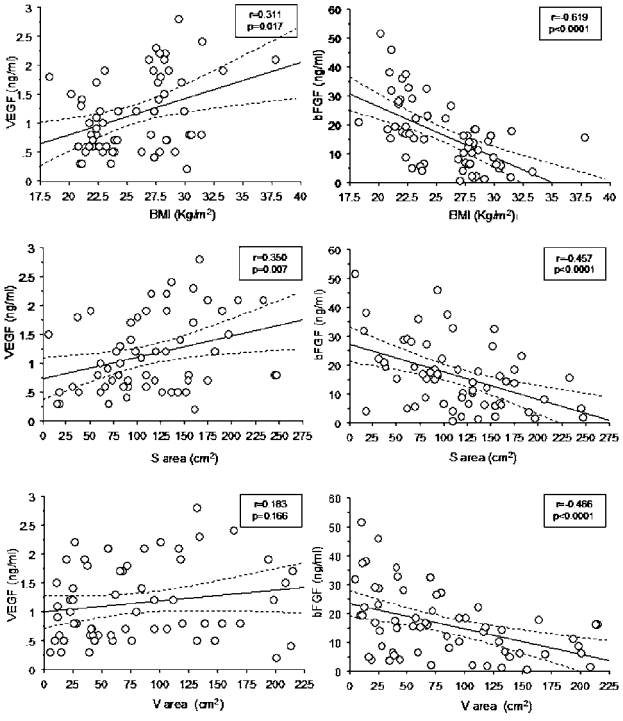

Figure 1 Correlation of VEGF and bFGF with BMI and fat distribution in normal and overweight subjects ($n = 61$). The serum VEGF levels significantly correlate with subcutaneous fat (S) area ($r = 0.311$, $P = 0.007$), but not with BMI ($r = 0.311$, $P = 0.017$) and visceral fat (V) area ($r = 0.183$, $P = 0.166$). Basic fibroblast growth factor (bFGF) negatively correlates with BMI ($r = -0.619$, $P < 0.001$), S area ($r = -0.457$, $P < 0.001$) and V area ($r = -0.466$, $P = 0.002$). Spearman correlation coefficients are used.

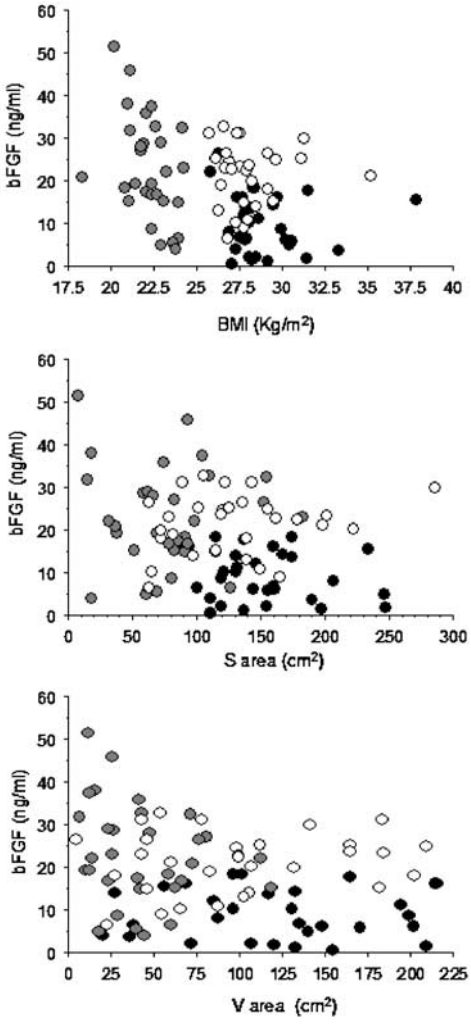

Figure 2 Basic fibroblast growth factor (bFGF) levels of overweight and normal-weight Japanese men. bFGF levels of normal-weight subjects ($n = 31$, gray circle), overweight subjects ($n = 30$) before (closed circle) and after the exercise program (open circle).

Table 2 Simple correlations of body fat distribution with insulin, leptin, VEGF, and bFGF levels of Japanese normal-weight and overweight subjects

	BMI		*S area*		*V area*		*V/S ratio*	
	r	P	r	P	r	P	r	P
HOMA-IR	0.649	<0.001*	0.626	<0.001*	0.499	<0.001*	0.159	0.222
Insulin	0.602	<0.001*	0.582	<0.001*	0.483	<0.001*	0.142	0.276
Leptin	0.616	<0.001*	0.613	<0.001*	0.573	<0.001*	0.173	0.181
VEGF	0.311	0.017	0.350	0.007*	0.183	0.166	−0.001	0.995
bFGF	−0.619	<0.001*	−0.457	<0.001*	−0.466	<0.001*	−0.195	0.131

*$P < 0.01$ Spearman correlation coefficients are used.
BMI, body mass index; V area, visceral fat area; S area, subcutaneous fat area; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor.

Table 3 Data on the intervention of 30 overweight subjects with a complete 6-month follow-up

Overweight subjects (n = *30)*	*Baseline*	*After 6 months*	P
BMI (kg/m^2)	29.0±2.2	28.0±2.0	<0.001*
Body fat percentage (%)	30.2±4.1	26.8±5.3	<0.001*
V area (cm^2)	121.7±57.9	99.4±57.3	0.019
S area (cm^2)	151.8±42.4	129.1±52.1	0.003*
Energy intake (kcal/day)	2000±460	1823±380	0.025
Steps/day	6182±2415	8584±3512	0.001*
VO_2 (ml/kg/min) at VT	13.9±2.0	15.6±2.4	<0.001*
Work rate (WR) at VT (W)	87.3±15.8	96.6±17.6	<0.001*
HR at VT (beats/min)	101.3±8.8	99.6±10.2	0.280
T-cho (mmol/l)	5.69±0.79	5.26±0.70	0.001*
HDL-cho (mmol/l)	1.28±0.32	1.32±0.30	0.355
LDL (mmol/l)	3.95±0.80	3.62±0.70	0.001*
TG (mmol/l)	2.28±1.50	1.53±0.81	0.001*
FPG (mmol/l)	5.82±0.67	5.42±0.57	<0.001*
Insulin (μU/ml)	11.9±4.81	8.45±5.14	<0.001*
HOMA-IR	3.08±1.26	2.06±1.29	0.001*
Leptin (ng/ml)	6.97±4.00	5.15±3.16	0.001*
VEGF (ng/ml)	1.45±0.72	1.88±0.52	0.016
bFGF (ng/ml)	9.21±5.82	21.2±7.04	<0.001*
Endostatin (ng/ml)	4.71±1.49	3.93±1.61	0.1213

*$P<0.01$ Wilcoxon's signed-rank test is used.
BMI, body mass index; V area, visceral fat area; S area, subcutaneous fat area; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; VT, ventilatory threshold.

151.8±42.4 to 129.1±52.1 cm^2. Total energy intake evaluated by nutritionists decreased from 2000±460 to 1823±380 kcal with no statistical differences. By exercise tests, VO_2 at VT significantly increased from 13.9±2.0 to 15.6±2.4 ml/kg/min and WR at VT also increased from 87.3±15.8 to 96.6±17.6 W ($P<0.001$), suggesting that aerobic exercise capacity of overweight subjects significantly increased by the 6-month exercise education. In addition, insulin resistance was ameliorated and HOMA-IR significantly decreased after the 6-month exercise program. Similarly, leptin and insulin levels significantly decreased during the exercise program. Serum VEGF levels revealed slight elevation but did not change significantly, 1.45±0.72 to 1.88±0.52 ng/ml ($P=0.016$), while bFGF levels were significantly elevated, 9.21±5.82 to 21.2±7.04 ng/ml ($P<0.001$) (Table 3). As shown in Figure 2, it is interesting to note that BMI levels of overweight subjects did not enter normal-weight range but bFGF levels elevated to the range comparable to normal-weight subjects. In contrast, VEGF of overweight subjects remained at high levels and the reduction of BMI from 29.0 to 28.0 was not linked to the reduction of VEGF levels.

The stepwise multiple regression analysis using the change in bFGF levels, ΔbFGF, as the dependent variable was performed to analyze the significant predictors. The changes of fat distribution and exercise capacity before and after exercise education, ΔBMI, ΔV and ΔS area, ΔVO_2, ΔWR, were used as independent variables. All variables showed normal distributions assessed by the Shapiro–Wilks test. As a result,

Table 4 Stepwise multiple linear regression analyses of overweight subjects before and after the exercise program

Dependent variables	*Independent variables*	β	β_{SE}	*Standardized* β	*P-values*	*Model* r^2
ΔbFGF	ΔBMI	−6.052	1.543	−0.545	<0.001	0.681
	ΔVO_2 at VT	2.806	0.697	0.471	<0.001	

The stepwise multiple regression analysis using the change in bFGF levels, ΔbFGF, as the dependent variable is performed to analyze the significant predictors. ΔBMI, ΔV and ΔS area, ΔVO_2 at VT, ΔWR at VT, are used as independent variables. All variables show normal distribution revealed by the Shapiro–Wilks test.
HOMA-IR, homeostasis model assessment of insulin resistance; bFGF, basic fibroblast growth factor; VT, ventilatory threshold.

ΔBMI and ΔVO_2 at VT were independently related to ΔbFGF ($P<0.001$) and all other variables including ΔS area, and ΔV area, ΔWR at VT did not enter the equation at significant levels (Table 4).

Discussion

Previous clinical studies indicated that serum VEGF and bFGF levels are elevated in patients with a broad range of human tumors, and the development of new microvessels in the surrounding stroma is a prerequisite for tumor progression. Similarly, white adipose tissue microcirculation is potentially critical for fat tissue expansion with a high degree of plasticity and unusual angiogenic activity of fat tissue has long been recognized. White adipose tissue is one of the important sources of various angiogenic factors including VEGF, bFGF, and leptin. However, the evaluation of serum concentrations of angiogenic stimulators, such as VEGF and bFGF, in a large number of overweight and obese subjects has not been well investigated.

Recently, the clinical trials of therapeutic angiogenesis using VEGF and bFGF protein administrations or gene therapy in patients with end-stage coronary artery disease have shown increases in exercise time and reductions in angina symptoms.[24] There are safety concerns associated with therapeutic angiogenesis, such as hypotension by upregulation of nitric oxide synthesis, proteinuria and leg edema caused by enhanced permeability, stimulation of tumor vascularity and growth of occult neoplasmas.[24] Another potential complication is the administration of angiogenic cytokines may promote the neointimal thickening and accelerate atherosclerosis by angiogenic stimuli on vasa vasorum of the artery wall.[25–28] So far, the clinical trials refute the idea that angiogenesis therapy stimulates tumor growth; however, animal studies have demonstrated that VEGF significantly enhances intimal thickening.[25–28] Since obesity is one of the major risk factors for atherosclerosis and coronary artery disease, the angiogenic status in overweight subjects without diabetes, hypertension, hyperlipidemia, and ischemic heart disease was evaluated in this investigation.

It has been reported that serum levels of VEGF are elevated in severely hypoxic patients with obstructive sleep apnea syndrome and are related to the degree of nocturnal oxygen desaturation.[29] Theoretically, increased VEGF production in obstructive sleep apnea syndrome might contribute to new vessel formation in ischemic and atherosclerotic vascular regions. This assumption is supported by a recent study showing that, in patients with coronary artery diseases, the degree of hypoxic induction of VEGF correlates with the extent of collateral vessel formation.[30] In one animal study, omental adipocytes were shown to have significantly greater *in vitro* rates of VEGF release compared with epididymal fat[31] and another animal study indicated cultured omental stromal cells had similar rates of VEGF secretion to those of epididymal fat stromal cells.[8] However, in our study, serum levels of VEGF positively correlated with S area but not with V area. During the reduction of average BMI from 28.0 to 29.0 in overweight subjects by exercise education, VEGF levels increased from 1.45 to 1.88 ng/ml with no statistically significant differences. The changes of VEGF did not correlate with the change in BMI, as well as V and S areas. Thus, it is premature to conclude that the elevated serum VEGF is directly derived from expanded white adipose tissues. Short-term exercise induces mRNA expression in human skeletal muscle, and the elevated VEGF levels may be attributed to the increased physical activities during the 6-month exercise education.[32,33] Although VEGF is apparently elevated in overweight subjects, it remains unknown whether VEGF enhances or protects atherosclerotic plaque in overweight subjects. Indeed, in animal studies, there are still controversies whether it reduces intimal thickening by accelerating re-endothelialization[34] or enhances intimal thickening.[25–28]

In preadipocytes in culture, bFGF stimulates the replication and inhibits their differentiation[10] and the expression of bFGF decreases during adipocyte differentiation[11]. In fully matured adipocytes, the addition of a high concentration of bFGF into culture media induces the reversal of adipocyte differentiation[12] and marked suppression of activity of GPDH[13]. In animal experiments, when reconstituted basement membrane, Matrigel, supplemented with more than 1 ng/ml bFGF was injected s.c. into 6-week-old mice, the neovascularization induced within 1 week was followed by migration of endogenous adipose precursor cells, and a clearly visible fat pad was formed.[35] In omental preadipocytes from lean and massively obese subjects, the augmented expression of bFGF in massively obese subjects was noted and probably contributes to the excessive cellularity of their white adipose tissues[10]. Thus, bFGF seems to maintain preadipocytes and promote angiogenesis in the white adipose tissue in obese subjects. One can speculate that serum bFGF levels in overweight subjects, like VEGF levels, would elevate; however, serum bFGF levels negatively correlated with BMI, S, and V areas in our investigation. A 6-month exercise program dramatically increased and normalized serum bFGF levels and ΔbFGF negatively correlates with ΔBMI and positively with ΔVO_2 at VT. Since serum bFGF levels are not directly linked to total body fat mass and positively related to improvement of exercise capacity, it is unlikely that white adipose tissue is a major source of serum bFGF; rather, the most possible explanation for the recovered serum bFGF levels may be due to the exercise. After a single exercise bout, mRNA expression of VEGF, to a lesser extent, bFGF, has been found to increase in rat skeletal muscle.[36] The elevation of VO_2 and WR at VT after 6-month exercise training may be related to the upregulation of angiogenic factors, bFGF, and vascularization in skeletal muscles. Furthermore, the reduced bFGF levels in overweight subjects may be a marker of endothelial dysfunction occurring in overweight subjects, which is restored by weight reduction with increased physical activities. It has been reported that bFGF restores endothelium-dependent responses to acetylcholine and calcium ionophore in hypercholestelemic rabbit thoracic aorta and protects from plaque formation.[37] Thus, bFGF may play a role in revascularization of skeletal muscles, improvement of exercise capacity, and a protective role in the progression of atherosclerosis in overweight and obese subjects.

In conclusion, serum VEGF levels positively correlated with S area but not with BMI and V area in Japanese overweight subjects at baseline. In contrast, serum bFGF levels negatively correlated with BMI, V, and S areas. By intervention with exercise education and reduction of BMI from 29.0 to 28.0 kg/m^2, VEGF did not change significantly while bFGF increased and ΔbFGF correlated with ΔBMI and ΔVO_2 at VT. We speculated that bFGF is not mainly derived from regional fat tissue mass; however, ΔbFGF correlated with reduction of BMI and improvement of exercise capacity. Considering the protective effects of bFGF against atherosclerosis, exercise-induced elevation of bFGF may be beneficial in the prevention of the atherosclerosis in overweight subjects.

Acknowledgements

This study was approved by Ministry of Health, Labor and Welfare, Japan. This research was supported by Health Science Research Grants for 'Research on Health Services' from the Ministry of Health, Labor and Welfare, Japan. It was partly supported by Uehara Memorial Foundation, The Naito Foundation, ONO Medical Foundation to J Wada.

References

1 Stevens J, Cai J, Pamuk ER, Williamson DF, Thun MJ, Wood JL. The effect of age on the association between body-mass index and mortality. *N Engl J Med* 1998; **338**: 1–7.
2 Kannel WB, Cupples LA, Ramaswami R, Stokes III J, Kreger BE, Higgins M. Regional obesity and risk of cardiovascular disease; the Framingham Study. *J Clin Epidemiol* 1991; **44**: 183–190.
3 Rexrode KM, Carey VJ, Hennekens CH, Walters EE, Colditz GA, Stampfer MJ, Willett WC, Manson JE. Abdominal adiposity and coronary heart disease in women. *JAMA* 1998; **280**: 1843–1848.

4 Funahashi T, Nakamura T, Shimomura I, Maeda K, Kuriyama H, Takahashi M, Arita Y, Kihara S, Matsuzawa Y. Role of adipocytokines on the pathogenesis of atherosclerosis in visceral obesity. *Intern Med* 1999; **38**: 202–206.
5 Asano A, Kimura K, Saito M. Cold-induced mRNA expression of angiogenic factors in rat brown adipose tissue. *J Vet Med Sci* 1999; **61**: 403–409.
6 Tonello C, Giordano A, Cozzi V, Cinti S, Stock MJ, Carruba MO, Nisoli E. Role of sympathetic activity in controlling the expression of vascular endothelial growth factor in brown fat cells of lean and genetically obese rats. *FEBS Lett* 1999; **442**: 167–172.
7 Asano A, Irie Y, Saito M. Isoform-specific regulation of vascular endothelial growth factor (VEGF) family mRNA expression in cultured mouse brown adipocytes. *Mol Cell Endocrinol* 2001; **174**: 71–76.
8 Mick GJ, Wang X, McCormick K. White adipocyte vascular endothelial growth factor: regulation by insulin. *Endocrinology* 2002; **143**: 948–953.
9 Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2000; **6**: 389–395.
10 Teichert-Kuliszewska K, Hamilton BS, Deitel M, Roncari DA. Augmented production of heparin-binding mitogenic proteins by preadipocytes from massively obese persons. *J Clin Invest* 1992; **90**: 1226–1231.
11 Teichert-Kuliszewska K, Hamilton BS, Deitel M, Roncari DA. Decreasing expression of a gene encoding a protein related to basic fibroblast growth factor during differentiation of human preadipocytes. *Biochem Cell Biol* 1994; **72**: 54–57.
12 Navre M, Ringold GM. Differential effects of fibroblast growth factor and tumor promoters on the initiation and maintenance of adipocyte differentiation. *J Cell Biol* 1989; **109**(Part 1): 1857–1863.
13 Hauner H, Rohrig K, Petruschke T. Effects of epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) on human adipocyte development and function. *Eur J Clin Invest* 1995; **25**: 90–96.
14 Cohen B, Barkan D, Levy Y, Goldberg I, Fridman E, Kopolovic J, Rubinstein M. Leptin induces angiopoietin-2 expression in adipose tissues. *J Biol Chem* 2001; **276**: 7697–7700.
15 Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults—The Evidence Report. National Institutes of Health. *Obes Res* 1998; **6** (Suppl 2): 51S–209S.
16 Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med* 1977; **62**: 707–714.
17 Haffner SM, Kennedy E, Gonzalez C, Stern MP, Miettinen H. A prospective analysis of the HOMA model. The Mexico City Diabetes Study. *Diabetes Care* 1996; **19**: 1138–1141.
18 Kunitomi M, Wada J, Takahashi K, Tsuchiyama Y, Mimura Y, Hida K, Miyatake N, Fujii M, Kira S, Shikata K, Maknio H. Relationship between reduced serum IGF-I levels and accumulation of visceral fat in Japanese men. *Int J Obes Relat Metab Disord* 2002; **26**: 361–369.
19 Miyatake N, Nonaka K, Fujii M. A new air displacement plethysmograph for the determination of Japanese body composition. *Diabetes Obes Metab* 1999; **1**: 347–351.
20 Jones NL, Makrides L, Hitchcock C, Chypchar T, McCartney N. Normal standards for an incremental progressive cycle ergometer test. *Am Rev Respir Dis* 1985; **131**: 700–708.
21 Wasserman K, Whipp BJ, Koyl SN, Beaver WL. Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol* 1973; **35**: 236–243.
22 Davis JA, Frank MH, Whipp BJ, Wasserman K. Anaerobic threshold alterations caused by endurance training in middle-aged men. *J Appl Physiol* 1979; **46**: 1039–1046.
23 Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* 1986; **60**: 2020–2027.
24 Freedman SB, Isner JM. Therapeutic angiogenesis for coronary artery disease. *Ann Intern Med* 2002; **136**: 54–71.
25 Celletti FL, Hilfiker PR, Ghafouri P, Dake MD. Effect of human recombinant vascular endothelial growth factor165 on progression of atherosclerotic plaque. *J Am Coll Cardiol* 2001; **37**: 2126–2130.
26 Blann AD, Belgore FM, Constans J, Conri C, Lip GY. Plasma vascular endothelial growth factor and its receptor Flt-1 in patients with hyperlipidemia and atherosclerosis and the effects of fluvastatin or fenofibrate. *Am J Cardiol* 2001; **87**: 1160–1163.
27 Celletti FL, Waugh JM, Amabile PG, Brendolan A, Hilfiker PR, Dake MD. Vascular endothelial growth factor enhances atherosclerotic plaque progression. *Nat Med* 2001; **7**: 425–429.
28 Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. *Circulation* 1999; **99**: 1726–1732.
29 Schulz R, Hummel C, Heinemann S, Seeger W, Grimminger F. Serum levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea and severe nighttime hypoxia. *Am J Resp Crit Care Med* 2002; **165**: 67–70.
30 Schultz A, Lavie L, Hochberg I, Beyar R, Stone T, Skorecki K, Lavie P, Roguin A, Levy AP. Interindividual heterogeneity in the hypoxic regulation of VEGF: significance for the development of the coronary artery collateral circulation. *Circulation* 1999; **100**: 547–552.
31 Zhang QX, Magovern CJ, Mack CA, Budenbender KT, Ko W, Rosengart TK. Vascular endothelial growth factor is the major angiogenic factor in omentum: mechanism of the omentum-mediated angiogenesis. *J Surg Res* 1997; **67**: 147–154.
32 Gustafsson T, Puntschart A, Kaijser L, Jansson E, Sundberg CJ. Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. *Am J Physiol* 1999; **276**(Part 2): H679–H685.
33 Gustafsson T, Sundberg CJ. Expression of angiogenic growth factors in human skeletal muscle in response to a singular bout of exercise. *Am J Physiol Heart Circ Physiol* 2000; **279**: H3144–H3145.
34 Hiltunen MO, Laitinen M, Turunen MP, Jeltsch M, Hartikainen J, Rissanen TT, Laukkanen J, Niemi M, Kossila M, Hakkinen TP, Kivela A, Enholm B, Mansukoski H, Turunen AM, Alitalo K, Yla-Herttuala S. Intravascular adenovirus-mediated VEGF-C gene transfer reduces neointima formation in balloon-denuded rabbit aorta. *Circulation* 2000; **102**: 2262–2268.
35 Kawaguchi N, Toriyama K, Nicodemou-Lena E, Inou K, Torii S, Kitagawa Y. *De novo* adipogenesis in mice at the site of injection of basement membrane and basic fibroblast growth factor. *Proc Natl Acad Sci USA* 1998; **95**: 1062–1066.
36 Breen EC, Johnson EC, Wagner H, Tseng HM, Sung LA, Wagner PD. Angiogenic growth factor mRNA responses in muscle to a single bout of exercise. *J Appl Physiol* 1996; **81**: 355–361.
37 Meurice T, Bauters C, Vallet B *et al*. bFGF restores endothelium-dependent responses of hypercholesterolemic rabbit thoracic aorta. *Am J Physiol* 1997; **272** (Part 2): H613–H617.