sampling were performed every 5 min after starting the exercise up to and including 60 min after using indirect calorimetry measurements (Oxycon α, Mijnhardt, Breda, The Netherlands). Substrate oxidation rates were calculated from the respiratory exchange ratio (RER).[26] Energy expenditure and substrate oxidation were calculated every 5 min from expiratory gas measurements. Total energy expenditure and substrate oxidation were defined as the sum of every 5 min of energy expenditure and substrate oxidation. Gas analyzers were calibrated before each test. Blood samples were collected every 10 min (18 ml per sample) and were put into 8 ml tubes containing thrombin and heparin neutralizing agent, 7-ml tubes containing ethylenediaminetetraacetic acid (EDTA)-2Na plus heparin-Na and sodium fluoride, and 2- and 1-ml tubes containing EDTA-2Na. The tubes were immediately centrifuged at 3000 r.p.m. for 10 min at 4°C. Blood in the 8-ml tubes was used for analyses of plasma concentrations of FFA, glycerol and insulin. Blood in the 7-ml tubes was used for analysis of the plasma concentration of epinephrine and norepinephrine. HR was recorded continuously throughout the exercise bout with an electrocardiograph (Dyna Scope, Fukudadenshi, Tokyo, Japan).

The total fat availability to exercise was determined as the area under the concentrations of FFA and glycerol curves calculated using the trapezium rule.[27]

Blood analysis

Blood samples were collected to measure plasma concentrations of FFA, glycerol, glucose, epinephrine, norepinephrine and insulin. Plasma concentrations of FFA were analyzed by enzymatic colorimetric method (ACS-ACOD). Plasma concentrations of insulin were measured by radioimmunoassay. Plasma concentrations of glycerol were determined by an enzymatic colorimetric method (GPO-DAOS). Plasma concentrations of epinephrine and norepinephrine were quantified by high-performance liquid chromatography.

Statistical analysis

Data were expressed as means ± s.e. A one-way analysis of variance (ANOVA) was used to test for significance of differences in anthropometric variables, area values under the curve, total energy expenditure and fat oxidation during endurance exercise between groups. A repeated measures two-way ANOVA, with group and time as factors, was used to determine differences in metabolite concentration and RER between VF-Ob and SF-Ob men during the endurance exercise. A Tukey's *post hoc* comparison test was used to locate significant difference. Statistical significance was set at $P<0.05$.

Results

Subject characteristics

The physical characteristics of the two groups are presented in Table 1. No difference was found in any variables between VF-Ob and SF-Ob men, with the exception of VFA. Mean VFA was significantly higher in VF-Ob (193.0 ± 13.6 cm^2) than SF-Ob (139.5 ± 7.5 cm^2) men. There was no difference in the means peak $\dot{V}O_2$ between the two groups.

Exercise characteristics

There was no difference in absolute ($\dot{V}O_2$ relative to FFM) (21.6 ± 1.5 and 20.5 ± 1.7 ml/FFM/min, respectively) and relative intensity (% peak $\dot{V}O_2$) (51.6 ± 1.7 and 48.5 ± 2.4%, respectively) between VF-Ob and SF-Ob men during the exercise.

Plasma hormonal responses during endurance exercise

Plasma hormonal responses during endurance exercise are shown in Figure 1. Plasma concentrations of epinephrine and norepinephrine increased progressively in both groups during endurance exercise, but there was not a significant group × time interaction. By contrast, plasma insulin concentrations decreased progressively in both groups during endurance exercise. There were similar responses in plasma insulin between the two groups.

Plasma free fatty acid and glycerol responses to endurance exercise

Plasma concentrations of FFA and glycerol responses to endurance exercise are shown in Figure 2. A significant group × time interaction was observed in the plasma concentration of FFA during the exercise bout. Total FFA availability, defined as the total area under the curve of

Table 1 Characteristics of participants in the VF-Ob and SF-Ob groups

	VF-Ob (n = 7)	*SF-Ob (n = 7)*
Age (years)	52.0 ± 2.5	57.3 ± 2.8
Height (cm)	168.6 ± 1.8	167.5 ± 2.1
Body weight (kg)	81.0 ± 3.2	75.4 ± 1.5
BMI (kg/m^2)	28.4 ± 0.7	26.9 ± 0.2
Waist circumference (cm)	99.1 ± 6.9	93.0 ± 1.7
Body fat (%)	27.3 ± 1.0	28.9 ± 1.3
Body fat mass (kg)	22.4 ± 1.2	21.5 ± 1.3
Body fat-free mass (kg)	58.6 ± 2.5	53.9 ± 1.8
$\dot{V}O_2$ max (ml/kg/min)	31.1 ± 1.7	30.5 ± 1.6
Abdominal fat area		
Total fat area (cm^2)	380.8 ± 16.7	332.7 ± 21.1
Visceral fat area (cm^2)	193.0 ± 13.6*	133.5 ± 5.1
Subcutaneous fat area (cm^2)	187.7 ± 13.3	199.3 ± 18.9

Abbreviations: BMI = body mass index; SF-Ob = subcutaneous fat obese; VF-Ob = visceral fat obese. Values are mean ± s.e. *Significantly different from SF-Ob ($P<0.05$).

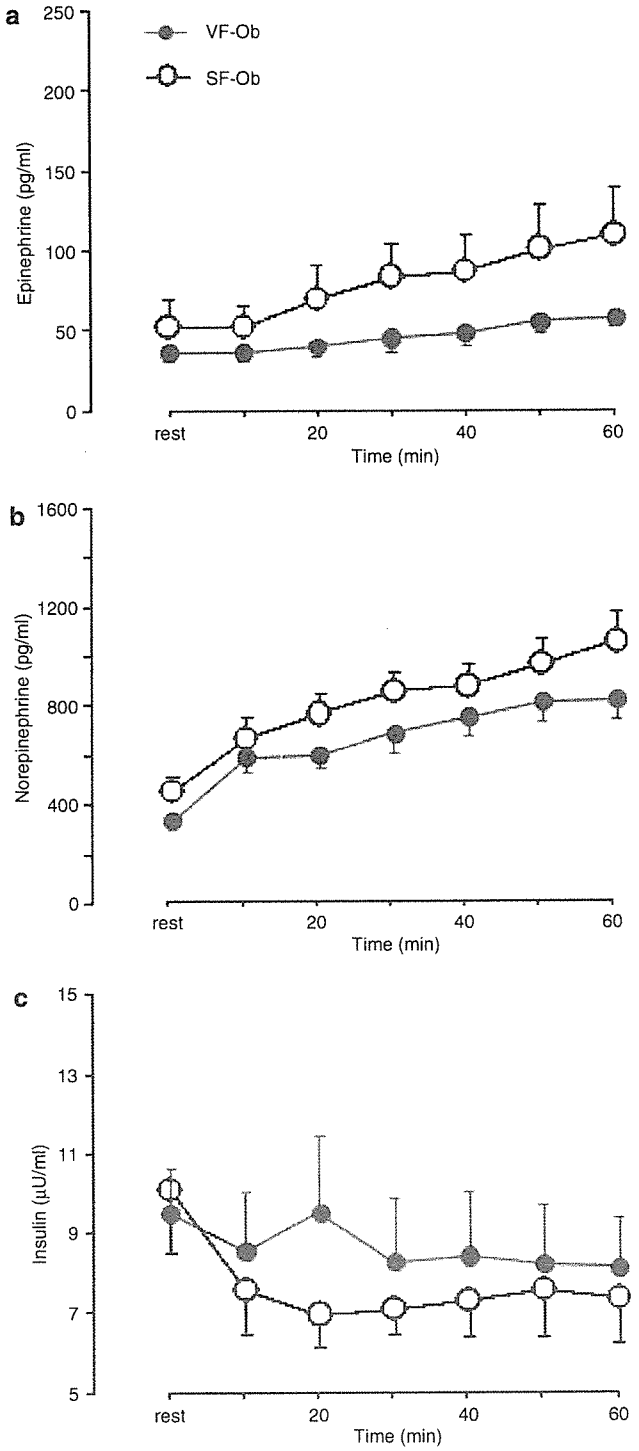

Figure 1 Plasma epinephrine (**a**), norepinephrine (**b**) and insulin (**c**) concentrations in visceral fat obese (VF-Ob) and subcutaneous fat obese (SF-Ob) men during 60 min endurance exercise. There was no significant group × time interaction ($P > 0.05$).

FFA, was significantly higher in VF-Ob than SF-Ob men (Table 2). At rest, no difference was found in plasma glycerol levels between VF-Ob and SF-Ob men. Plasma glycerol levels

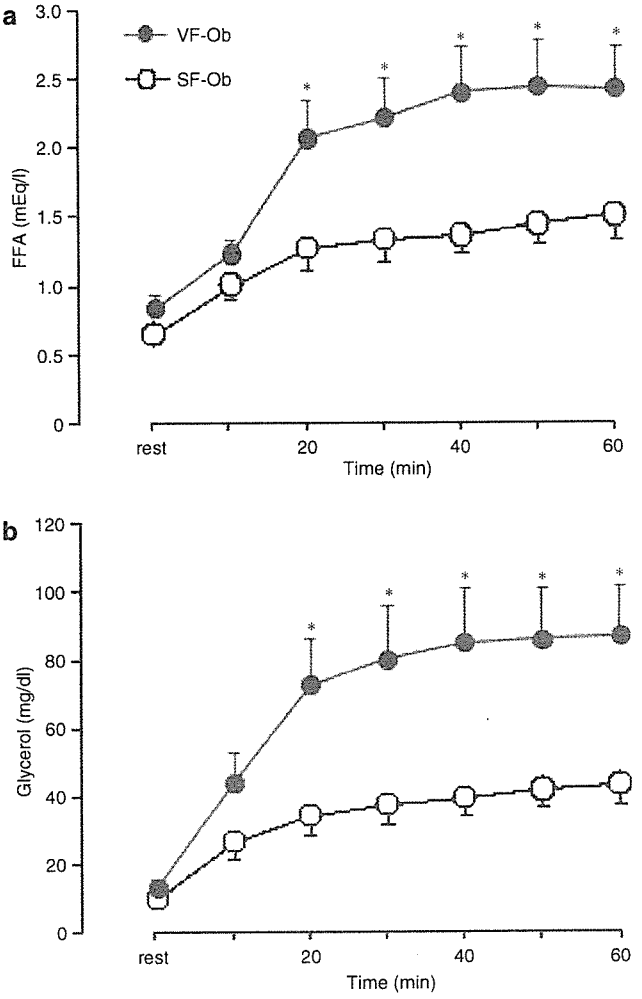

Figure 2 Plasma concentration of free fatty acid (FFA) (**a**) and glycerol (**b**) in visceral fat obese (VF-Ob) and subcutaneous fat obese (SF-Ob) men during 60-min endurance exercise. There was a significant group × time interaction ($P < 0.05$). *Significantly different from SF-Ob ($P < 0.05$).

Table 2 Area under the plasma concentration of fat curve on the 60-min endurance exercise

	VF-Ob (n = 7)	*SF-Ob (n = 7)*
Free fatty acid (mEq/l/60 min)	1.99 ± 0.24*	1.25 ± 0.13
Glycerol (mg/dl/60 min)	69.6 ± 12.5*	34.4 ± 5.1

Abbreviations: BMI = body mass index; SF-Ob = subcutaneous fat obese; VF-Ob = visceral fat obese. Values are mean ± s.e. *Significantly different from SF-Ob ($P < 0.05$).

increased progressively until 20 min of exercise in both groups. There was a significant group × time interaction in plasma concentration of glycerol during the exercise bout. A significant difference was found in the area under the curve of glycerol between VF-Ob and SF-Ob men (69.6 ± 12.5 and 34.4 ± 5.1 mg/dl/60 min, respectively) (Table 2).

Total energy expenditure and substrate oxidation during exercise
Changes in RER during endurance exercise are shown in Figure 3. At rest, there was no difference in RER between VF-Ob and SF-Ob men (0.81±0.06 and 0.76±0.01, respectively). RER increased at 10 min of the exercise, and then progressively decreased until the end of the exercise. The changes in RER during exercise were not significantly different between the two groups. The amount of total energy expenditure and fat oxidation during endurance exercise is shown in Figure 4; there was no difference in total energy expenditure between VF-Ob and SF-Ob men (389.6±35.4 and 330.6±27.5 kcal/60 min, respectively). Also, no difference was found in fat oxidation during exercise between VF-Ob and SF-Ob men (176.5±25.7 and 183.0±12.8 kcal/60 min, respectively).

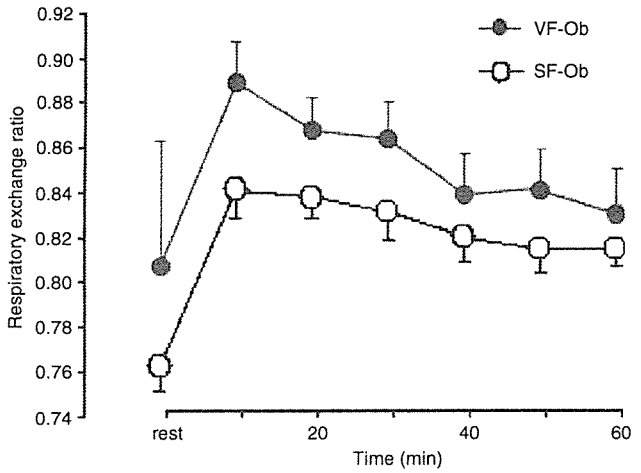

Figure 3 Respiratory exchange ratio (RER) in visceral fat obese (VF-Ob) and subcutaneous fat obese (SF-Ob) men during 60-min endurance exercise. There was no significant group × time interaction ($P > 0.05$).

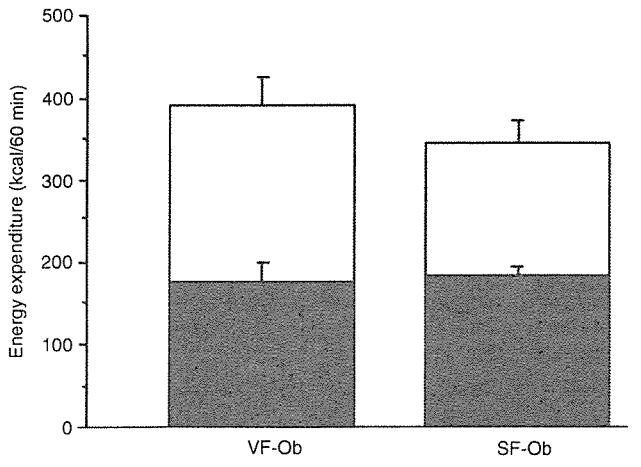

Figure 4 Total energy expenditure consisted of fat (filled bar) and carbohydrate (open bar) in visceral fat obese (VF-Ob) men during 60-min endurance exercise. There was no difference in total, carbohydrate and fat energy expenditure between VF-Ob and subcutaneous fat obese (SF-Ob) men.

Discussion

In this study, we investigated the influence in obese men of obesity phenotype on fat metabolism during endurance exercise. Fat availability was evaluated by plasma hormones and FFA and glycerol concentrations, and substrate oxidation was evaluated by indirect calorimetry at rest and during endurance exercise in VF-Ob and SF-Ob men. The groups were matched on whole-body FM and aerobic capacity with the exception of VFA. We found that the response of fat availability was different between the groups. Also, total fat availability during endurance exercise was higher in VF-Ob than SF-Ob men. Furthermore, fat oxidation was similar between the two groups during endurance exercise, despite the fact that fat availability was different.

Our study found that the total plasma concentrations of FFA and glycerol were higher in VF-Ob than SF-Ob men during the exercise bout. The balance of several hormones, for example, catecholamines and insulin, has been reported to play a critical role in fat availability both at rest and during exercise.[12,13] However, in our study no difference was observed in the responses of plasma epinephrine, norepinephrine and insulin throughout the endurance exercise between the two obesity groups. Therefore, it is inferred that in this study the differences of fat availability between the two obese groups could not always be explained simply by plasma concentrations of epinephrine, norepinephrine and insulin during exercise.

Whole-body fat volume may affect fat availability and oxidation during endurance exercise.[28] As the whole-body FM was the same in the VF-Ob and SF-Ob men, the reasons for the difference of fat availability might be the differences in regional adipose tissue lipolytic sensitivity to catecholamines and antilipolytic sensitivity to insulin. Lipolytic responses to catecholamines are higher in visceral adipocytes than in subcutaneous ones,[29–32] and also the antilipolytic and activation of FFA re-esterification response to insulin was lower in visceral compared with subcutaneous adipocytes.[33,34] These earlier studies suggested that the lipolysis of visceral adipocytes might be increased compared with that of subcutaneous adipocytes during endurance exercise. Plasma concentrations of catecholamines and insulin during endurance exercise were similar between the two groups in our study. This in turn indicates that the high plasma concentrations of FFA and glycerol in VF-Ob men would partly reflect the difference in lipolytic characteristic between visceral and subcutaneous adipocytes.

The fat availability in VF-Ob and SF-Ob men during endurance exercise observed in our study was inconsistent with data from previous studies that have investigated fat metabolism between two obesity phenotypes in obese women during endurance exercise.[18,19] The reasons for the discrepancy between the present study and previous studies[18,19] can be accounted for by several other factors. The first factor is the baseline of the plasma insulin concentration. The second factor is the difference of indices to classify

the obesity phenotype (WHR vs CT).[35,36] In addition, the strongest factor on our results is the difference in the subject's gender between the present study and earlier ones.[18,19] Lipolysis of visceral and subcutaneous adipocytes has been reported to differ between obese men and women. Lonnqvist *et al.*[37] observed obese men were 12 times higher in the lipolytic β_3-adrenoreceptor sensitivity of visceral adipocytes and 17 times lower in the antilipolytic α_2-adrenoreceptor sensitivity of visceral adipocytes compared with obese women. Furthermore, in obese women, no difference was observed in the rate of norepinephrine-stimulated lipolysis between VF and SF cells, whereas in obese men, it was higher in VF than in SF cells.[6] These results suggest that the lipolysis of VF is considerably higher in obese men compared with obese women and that the FFA and glycerol release from VF into the systemic circulation would be greater than those from SF in obese men. Therefore, it is possible that high availability of FFA and glycerol occur in the VF-Ob men, not in obese women,[18,19] during endurance exercise.

Although fat availability was higher in VF-Ob than in SF-Ob men, total fat oxidation was similar in the two groups during endurance exercise. This finding suggests that total fat oxidation during endurance exercise is not entirely regulated by plasma fat availability. Previous studies showed evidence that the rate of fat oxidation during endurance exercise is primarily influenced by energy requirements,[38] exercise intensity,[14,15] aerobic fitness,[39] muscle oxidative capacity,[40] glucose availability[41] and body FM.[28] Exercise training, which increases muscle oxidative capacity,[42] enhances fat oxidation during exercise without increasing plasma FFA availability.[38,39] In addition, the increasing plasma FFA availability by the administration of lipids and the infusion of heparin during endurance exercise is not accompanied by a corresponding increase in fat oxidation.[43–45] Thus, total fat oxidation during endurance exercise was similar in the VF and SF-Ob men, because the participants were matched for whole-body fat and fitness level, thereby indicating that they performed exercise at nearly the same relative intensity.

Our finding that total fat oxidation during endurance exercise was similar between the VF-Ob and SF-Ob men is consistent with data from previous studies.[18,19,46,47] In addition, Deriaz *et al.*[48] indicated no correlation between abdominal VFA and fat oxidation during submaximal exercise, when the correlation was adjusted for age and FM. Therefore, it seems logical to state that total fat oxidation during endurance exercise would not be influenced by obesity phenotype. Although the source of FFA oxidized is different in obesity phenotype,[49,50] this was not measured in the present study.

Limitations: To examine gases ($\dot{V}O_2$, $\dot{V}CO_2$, RER) in each participant breath, we used an indirect calorimetry. There is a limitation attached to this approach. Although the assumption of indirect calorimetry is that the RER adequately reflects the respiratory quotient (RQ), the RQ values are usually slightly higher than the RER measured simultaneously at rest and during exercise.[51] Furthermore, we utilized the equation of Peronnet and Massicotte[26] to estimate the total energy expenditure and substrate oxidation during endurance exercise. Carbohydrate used as fuel during exercise is not only glucose but also glycogen. The stoichiometry for glucose and glycogen differs slightly and carbohydrate oxidation rate are ~10% lower when calculated using the stoichiometry of glycogen compared to that of glucose.[52] The discrepancy is not taken into consideration in the equation of Peronnet and Massicotte.[26] Therefore, there is the possibility that we could not exactly estimate the total energy expenditure, carbohydrate oxidation and fat oxidation in this study. However, even if the total energy expenditure, carbohydrate oxidation and fat oxidation are overestimated and/or underestimated, it is unlikely that our results (no difference in the total energy expenditure and fat oxidation between VF-Ob and SF-Ob) would be affected. As we applied the same equation to all participants to calculate the total energy expenditure and fat oxidation, the errors containing the estimated values of each participant was similar.

In summary, the results of the present study demonstrated that obesity phenotype affects fat metabolism during endurance exercise. We found fat availability during endurance exercise was higher in VF-Ob men than in SF-Ob men. The difference of plasma fat availability may be due partly to the differences of lipolytic sensitivity to catecholamines and antilipolytic sensitivity to insulin between visceral and subcutaneous adipocytes. These differences may partly indicate the existence of a response peculiar to visceral adipocytes during endurance exercise. However, regardless of the difference in plasma fat availability, total fat oxidation during endurance exercise seems to be similar in VF and the SF of obese men.

Acknowledgements

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Cutoff and Target Values for Intra-Abdominal Fat Area for Prevention of Metabolic Disorders in Pre- and Post-Menopausal Obese Women Before and After Weight Reduction

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Background The Japan Society for the Study of Obesity originally proposed a cutoff value of >100 cm^2 for the intra-abdominal fat area (IFA) as a definition for "visceral fat obesity" in Japanese adults. There are no studies on the cutoff or target values after weight reduction in pre- and post-menopausal women.

Methods and Results In the present study 149 pre-menopausal obese women (PreM, 43.3 years, 27.3 kg/m^2) and 58 post-menopausal women (PostM, 53.9 years, 27.7 kg/m^2) participated in a 14-week weight reduction program. The IFA was measured by computed tomography. The program induced significant reductions in body weight (8.6 kg in PreM and 7.8 kg in PostM). The IFA decreased significantly from 80.4±41.3 to 50.7±23.8 (PreM) and from 115.4±38.0 to 75.7±30.5 (PostM).

Conclusions The receiver-operating characteristic curve analyses revealed that the appropriate cutoff values were 80 cm^2 (PreM) and 110 cm^2 (PostM) before the program, and after the program the appropriate target values were determined as 60 and 70 cm^2, respectively. (*Circ J* 2006; **70:** 110–114)

Key Words: Diet; Exercise; Fat body; Menopause; Metabolic syndrome

The "visceral fat obesity" refers to the condition of excess intra-abdominal fat (IF), which places people having this type of excess fat at high risk for obesity-related metabolic disorders, such as hyperglycemia and dyslipidemia. The Japan Society for the Study of Obesity (JASSO)[1] originally defined visceral fat obesity in Japanese as having an IF area (IFA) >100 cm^2 and indicated that such people tend to have 1 or more metabolic disorders! Nakamura et al reported that approximately 62% of patients with coronary artery disease have an IFA =100 cm^2 or more[2] and Banno et al found that sleep-disordered breathing was closely associated with obesity[3]

JASSO used a cross-sectional study design to validate the cutoff value for IFA of 100 cm^2 for the diagnosis of visceral fat obesity! but intervention studies for assessing an appropriate target value that can be used for people who reduce their IF significantly have been lacking, and it is unclear whether, or at what point, decreasing IF improves metabolic disorders.

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There are several studies of the effects of menopause on the relationship of IF with metabolic diseases. Excess IF deposition is more prevalent in post-menopausal women than in pre-menopausal women[4] although it occurs more frequently in males of all ages[5] Hunter et al[6] and Gower et al[7] showed that the IFA and the risk of coronary heart disease (CHD) were positively correlated and that each average in post-menopausal women was higher than that in pre-menopausal women. The results of the study by Rebuffe-Scrive et al[8] suggest that one of the reasons for this phenomenon is the more pronounced activation of lipoprotein lipase in the omental adipose tissue of post-menopausal women than in that of pre-menopausal women. The cutoff value for the IFA derived by JASSO[1] was defined using a combination of pre- and post-menopausal women; the standards were, therefore, not established while considering the presence of menopause.

Based on these results, the current study assesses JASSO's visceral fat obesity IFA cutoff value of 100 cm^2 in pre- and post-menopausal women and also assesses the IFA target value after a weight reduction program. We tested 2 related hypotheses: (1) the cutoff value would be valid when applied to a group consisting of only pre- or post-menopausal women and (2) it would remain valid in each group after reducing the IFA.

Methods

Participants

Advertisements were placed in local newspapers and on bulletin boards in Toride City in Ibaraki Prefecture and Abiko City in Chiba Prefecture in Japan to locate potential

participants with a desire to lose weight. Those who responded to the advertisements were interviewed by telephone. The participants supplied information on demographics, menstrual status, and medical history. They were excluded from the study if their weight had been unstable for the past 6 months, if they had attended any weight reduction programs in the past year, or if they were breast feeding or pregnant. A study physician confirmed if participants were possibly pregnant. Further, the study staff and radiologic technologist explained to all participants that computed tomography (CT) can have deleterious effects. After applying the exclusion criteria to potential participants, the selected participants (n=220) received the details of the study's purpose and protocol. Oral informed consent, following the Helsinki Declaration principles and approved by the Higashi Toride Hospital Review Board, was obtained from each person. We defined "menopause" as the status of no menses for 1 year prior to the study. "Pre-menopause" was used define individuals who were not experiencing menopause. Therefore, the pre-menopausal group consisted of women who declared having menses in the year prior to the study (PreM). The post-menopausal group included those women who had not had menses for more than 1 year prior to the beginning of the study (PostM).

IFA

We measured the IFA and subcutaneous fat area (SFA) at the level of the umbilicus using cross-sectional CT (SCT-6800TX; Shimadzu, Japan). Scans were performed with the participants in the supine position. Details of the scanning have been reported by Tokunaga et al[9] and Yoshizumi et al![10] Measurements taken before and after the program were conducted at the same time of day by the same technician to minimize technical error. The IFA and SFA were calculated using a computer-software program (FatScan; N2system, Japan)![10] The intra-class correlation for repeated IFA determinations in the laboratory (Institute of Health and Sport Sciences, University of Tsukuba) is 0.99 (n=30).

Obesity-Related Metabolic Disorders

The obesity-related metabolic disorders were defined as follows: accumulation of IF (waist circumference ≥90 cm in female) plus 2 or more co-morbidities consisting of (i) triacylglycerol (TG) ≥150 mg/dl and/or high-density lipoprotein cholesterol (HDLC) <40 mg/dl, (ii) systolic blood pressure (SBP) ≥130 mmHg or diastolic blood pressure (DBP) ≥85 mmHg, or (iii) fasting plasma glucose ≥110 mg/dl![11,12] These biochemical assays were performed on approximately 10 ml of blood drawn from each participant after an overnight fast. The blood assays were analyzed by technicians at the Koto Biken Research Institute in Tsukuba, Japan. Total body composition was assessed by bioelectrical impedance methods![13] We used the Tanaka formula[13] to estimate the total body density (Db) and the Brozek formula[14] to determine the percentage of body fat. The Tanaka formula accurately predicts the total Db in obese Japanese women (R=0.903, SEE=0.0061 g/cm^3, with the hydrodensitometrically determined Db). SBP and DBP were taken from the right arm using a mercury manometer after at least a 20-min rest while seated. Cuff sizes were selected based on upper arm girth and length.

Weight Reduction Program

A 14-week weight reduction program was monitored by a physician, dietician, exercise instructors, and graduate school students majoring in exercise intervention. After the baseline assessment, participants received instruction on the diet program, which comprised weekly 90-min diet consultations, at which a diet-recording notebook and several handouts were given to participants to help them adhere to the principles of the daily diet. They were asked to take a well-balanced supplemental food product (MicroDiet; Sunny Health Co, Ltd, Japan) daily as 1 of their meals, preferably as lunch or dinner. The MicroDiet, which includes various amino acids, vitamins, and minerals, was developed for very low-energy diets. To prevent boredom, the MicroDiet was served in 7 flavors: coffee, milk tea, cocoa, yogurt, banana, strawberry, and apple. Participants received packages consisting of 7 meals (each flavor) once a week. The nutritional values for each flavor were slightly different (ie, there was a range for protein (20.6–21.5 g), carbohydrate (15.0–18.1 g), fat (1.6–3.0 g), and energy (169–173 kcal) for each meal). The diet records were obtained from 86 participants (60 in the PreM group, 26 in the PostM group), who were randomly selected. One week before the study, the participants were asked to record everything they had eaten for the 3 days prior to the study. Furthermore, they were asked to record their diets for 3 days during week 7, the midpoint of the intervention.

The exercise program included 3 weekly 45-min sessions. During the first and second weeks of the 14-week program, exercise sessions consisted mainly of walking and stretching, with the gradual addition of a bench-stepping exercise[15] as the main element. Thereafter, the exercise session consisted of a 10-min warm-up, 25-min bench stepping, and a 10-min cool-down. The bench stepping targeted an exercise intensity in which the participant's heart rate reached a level 10–15% higher than the level corresponding to her lactate threshold (LT). The LT was defined as the point at which blood lactate concentration maintained a non-linear increase above the level at rest![16] To determine LT, a series of venous blood samples (1 ml each) was drawn from the antecubital vein every minute during a maximal cycling exercise test, which was done with an accompanying electrocardiogram as a baseline assessment. All blood samples were analyzed by the electrochemical enzymatic method using a lactate analyzer (model 23L, YSI Inc, OH, USA). For establishing LT, the log (oxygen uptake)−log (lactate) transformation method was used![16]

Exercise was consistently performed for 45 min throughout the 14 weeks, but the intensity was progressively increased. In the first 2 weeks, the bench-stepping instructor targeted the intensity as described. After the 3rd week, the instructor progressively increased the intensity by increasing the cadence of the step and adding more dynamic movements. Ratings of the perceived exertion (RPE)[17] by all participants were also monitored during the bench stepping. Based on their RPE, the instructor moderated the intensity as "somewhat hard" to "hard," which corresponded to LT or a little above LT![18]

Statistical Analysis

Differences in variables between the beginning and end of the program were tested in each group by using Student's paired t-tests. Data were analyzed with the SPSS 11.01J statistical software package (SPSS, Chicago, IL, USA), and P-values less than 0.05 were considered statistically significant.

To assess the cutoff value (before weight reduction) and

Table 1 Baseline Characteristics of Participants

	PreM + PostM (n=207)	PreM (n=149)	PostM (n=58)
Age (years)	46.2±8.1	43.3±6.7 (24–57)	53.9±6.0 (45–62)
Height (cm)	157.0±5.2	157.9±5.1 (146.1–171.8)	154.6±4.9 (145.6–165.4)
Weight (kg)	67.6±8.2	68.1±7.6 (53.6–87.6)	66.3±9.7 (50.0–111.3)
Body mass index (kg/m^2)	27.4±3.0	27.3±2.9 (21.8–37.3)	27.7±3.3 (20.9–40.7)
Percent body fat (%)	34.6±4.9	34.1±4.2 (24.9–46.7)	35.9±6.2 (24.1–51.9)
Intra-abdominal fat area (cm^2)	90.2±43.3	80.4±41.3 (12.2–222.9)	115.4±38.0 (32.3–191.2)
Subcutaneous fat area (cm^2)	252.2±82.1	250.9±75.4 (103.5–548.0)	255.5±97.9 (90.5–684.0)
Abdominal circumstance (cm)	95.7±8.6	95.1±8.4 (73.8–118.0)	97±8.9 (80.5–131.5)

Values are means±standard deviations (minimum–maximum).
PreM, pre-menopausal obese group; PostM, post-menopausal obese group.

Table 2 Effects of a 14-Week Weight Reduction Program on Anthropometric Variables, Abdominal Fat Area, Metabolic Variables, and Blood Pressures

	PreM + PostM (n=207)		PreM (n=149)		PostM (n=58)	
	Before	After	Before	After	Before	After
Weight (kg)	67.6±8.2	59.3±7.4* (–12%)	68.1±7.6	59.6±6.9* (–12%)	66.3±9.7	58.5±8.5* (–12%)
Body mass index (kg/m^2)	27.4±3.0	24.0±2.7* (–12%)	27.3±2.9	23.9±2.6* (–12%)	27.7±3.3	24.4±2.9* (–12%)
Percent body fat (%)	34.6±4.9	29.4±4.6* (–15%)	34.1±4.2	28.8±4.0* (–15%)	35.9±6.2	31.1±5.5* (–13%)
Intra-abdominal fat area (cm^2)	90.2±43.3	57.7±28.1* (–32%)	80.4±41.3	50.7±23.8* (–31%)	115.4±38.0	75.7±30.5* (–34%)
Subcutaneous fat area (cm^2)	252.2±82.1	181.6±76.9* (–29%)	250.9±75.4	176.2±73.2* (–31%)	255.5±97.9	195.5±84.6* (–24%)
Abdominal circumference (cm)	95.7±8.6	85.2±8.6* (–6%)	95.1±8.4	84.9±8.0* (–5%)	97.3±8.7	86.2±10.0* (–7%)
Fasting plasma glucose (mmol/L)	5.41±1.13	4.94±0.68* (–7%)	5.25±0.86	4.88±0.68* (–6%)	5.84±1.57	5.10±0.67* (–10%)
Total cholesterol (mmol/L)	5.71±0.95	5.17±0.89* (–9%)	5.59±0.95	5.02±0.83* (–9%)	6.00±0.89	5.57±0.92* (–7%)
Triacylglycerol (mmol/L)	1.18±0.59	0.80±0.41* (–23%)	1.13±0.59	0.76±0.38* (–24%)	1.30±0.58	0.91±0.48* (–21%)
HDLC (mmol/L)	1.70±0.38	1.65±0.33* (–1%)	1.72±0.36	1.65±0.32* (–2%)	1.66±0.44	1.65±0.35 (+2%)
SBP (mmHg)	132.4±18.8	120.6±16.5* (–8%)	129.9±17.9	118.6±15.5* (–8%)	138.7±19.7	125.8±17.7* (–9%)
DBP (mmHg)	82.1±11.7	74.4±11.0* (–9%)	81.0±11.5	74.2±10.5* (–8%)	84.9±11.9	74.8±12.4* (–12%)

Values are means±standard deviations (relative change, %).
PreM, pre-menopausal obese group; PostM, post-menopausal obese group; HDLC, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.
**Significant intra-group difference (P<0.05).*

Table 3 Number and Percentage of Participants That Exceeded Each Criterion of the Metabolic Disorders Before and After Weight Reduction Program

	PreM (n=149)		PostM (n=58)	
	Before	After	Before	After
High abdominal circumference	112 (75%)	37 (25%)	50 (86%)	18 (31%)
High triacylglycerol and/or low HDLC	27 (18%)	6 (4%)	11 (19%)	6 (10%)
High triacylglycerol	26 (17%)	5 (3%)	9 (16%)	5 (9%)
Low HDLC	3 (2%)	3 (2%)	4 (7%)	2 (3%)
High systolic and/or diastolic blood pressure	80 (54%)	36 (24%)	41 (71%)	23 (40%)
High systolic blood pressure	74 (50%)	33 (22%)	39 (67%)	23 (40%)
High diastolic blood pressure	52 (35%)	22 (15%)	28 (48%)	8 (14%)
High fasting plasma glucose	11 (7%)	6 (4%)	14 (24%)	6 (10%)

Abbreviations see in Table 2.

the target value (after weight reduction) for IFA, receiver-operating characteristic (ROC) curve analysis was applied to the data derived from the IFA and the number of metabolic disorders. By provisionally varying the cutoff/target values of IFA, we calculated the sensitivities and specificities for each value. Sensitivity was defined as the proportion of participants having a given disorder who also had an IFA equal to or greater than the provisional value to all participants having a given disorder. Specificity was defined as the proportion of participants having no disorders who had an IFA that fell below the provisional value to all participants having no disorders. The sensitivities and specificities were calculated for every 10 cm^2 of IFA from 30 to 140 cm^2. At each 10 cm^2 provisional value, the sensitivity was multiplied by the specificity, and the point having the maximum product of sensitivity×specificity was considered to be the most valid cutoff/target value.

Results

Of the 220 women originally enrolled in this study, 13 dropped out because they moved out of the area, needed to care for a family member, or felt fatigued. Consequently, 207 women completed the study (Table 1), and attendance averaged 92% (range 83–100%).

There were significant decreases in the anthropometric variables, IFA, SFA, metabolic variables, and blood pressures in each group (Table 2). Total body composition analysis revealed that the reduction in body weight was mostly from loss of body fat. The reduction in fat-free mass

Table 4 Sensitivities and Specificities From Each Provisional Cutoff/Target Value of Intra-Abdominal Fat Area (IFA)

Cutoff/target value (IFA, cm^2)	PreM				PostM			
	Before		After		Before		After	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
30	0.96	0.04	0.93	0.22	1.00	0.00	1.00	0.06
40	0.91	0.23	0.87	0.42	1.00	0.06	1.00	0.11
50	0.89	0.37	0.73	0.57	0.98	0.12	1.00	0.3
60	0.80	0.47	0.67	0.71	0.95	0.18	0.91	0.40
70	0.72	0.55	0.33	0.82	0.93	0.29	0.91	0.63
80	0.63	0.74	0.27	0.89	0.90	0.41	0.82	0.66
90	0.53	0.81	0.07	0.96	0.88	0.59	0.64	0.72
100	0.45	0.89	0.07	0.98	0.85	0.59	0.64	0.79
110	0.40	0.95	0.07	0.99	0.73	0.71	0.45	0.96
120	0.27	0.96	0.07	0.99	0.56	0.82	0.36	0.98
130	0.21	0.97	0.07	1.00	0.41	0.94	0.18	1.00
140	0.16	0.97	0.00	1.00	0.29	0.94	0.09	1.00

Abbreviations see in Table 1.
Underlined values indicate the most valid cutoff/target values.

was significant, but the absolute change was less than the change in fat mass.

The daily average energy intake in the PreM group was 2,100±354 kcal at 1 week before the study and it decreased significantly to 1,163±242 kcal. The PostM group significantly reduced their energy intake from 1,870±394 kcal to 1,029±152 kcal. The daily protein intake in the PreM group was 78.1±15.1 g, and it decreased significantly to 70.3±14.2 g. In the PostM group, it decreased significantly from 86.1±33.5 g to 65.1±9.0 g. The daily fat intake decreased significantly from 66.3±14.9 g to 33.1±9.9 g in the PreM group and from 56.6±20.4 g to 27.4±6.2 g in the PostM group. The daily carbohydrate intake also decreased significantly from 285.5±61.2 g to 147.5±31.3 g in the PreM group and from 272.3±94.1 g to 136.1±23.8 g in the PostM group.

The percentage of participants that exceeded each criterion of the metabolic disorders is shown in Table 3. More than 50% of the participants had a high abdominal circumference before the program (PreM, 75%; PostM, 86%). The most frequent disorder in both groups was hypertension, with hyper-SBP (PreM, 50%; PostM, 67%) and hyper DBP (PreM, 35%; PostM, 48%). After the program, the percentages of all disorders, except for hypo-HDLC in the PreM group, decreased.

The characteristics of the 12 provisional cutoff/target values for IFA from 30 cm^2 to 140 cm^2 are presented in Table 4. Sensitivities before the program ranged from 0.16 to 0.96 in the PreM group and from 0.29 to 1.00 in the PostM group. Specificities ranged from 0.04 to 0.97 for the PreM group and from 0.00 to 0.94 for the PostM group. The products obtained by multiplying the sensitivity by the specificity at each provisional value ranged from 0.04 to 0.47 in the PreM group and from 0.00 to 0.52 in the PostM group. The largest products of sensitivity and specificity were found at 80 cm^2 (0.47) for the PreM group and 110 cm^2 (0.52) for the PostM group. Therefore, the cutoff values with the best equilibrium between sensitivity and specificity approached 80 cm^2 in the PreM group and 110 cm^2 in the PostM group before weight reduction. Using the same method of analysis, the most valid target values after the weight reduction program were determined to be 60 cm^2 for the PreM group and 70 cm^2 for the PostM group.

Discussion

In only a few studies, attempts have been made to determine the cutoff or target value for obesity-related metabolic disorders[1,19,20] In the present study the cutoff values of IFA were 80 cm^2 for pre-menopausal women and 110 cm^2 for post-menopausal women before weight reduction, which are similar to the 100 cm^2 value considered appropriate by JASSO[1] in a study that did not differentiate between pre- and post-menopausal women. Williams et al, in a combined study of both pre- (n=133) and post-menopausal women (n=87), concluded that 110 cm^2 was the cutoff value for IFA above which the risk of metabolic disorders increases[20] Despres and Lamarche indicated that 130 cm^2 of IFA was the point at which the metabolic risks increase significantly, derived from a sample of 115 males and 72 females[19] Considering those findings, the cutoff values in the current study seem to be reasonable.

A difference of 30 cm^2 in the cutoff values was noted between the PreM women (80 cm^2) and the PostM women (110 cm^2) before weight reduction. Williams et al reported that menopause has little effect on the risks of metabolic disorders, such as HDLC, TG, SBP, DBP, and TC:HDLC ratio;[20] although in a review by Knopp;[21] post-menopausal women were found to have elevated risks because of decreased estrogen contributing to increased low-density lipoprotein cholesterol (LDLC) and decreased HDLC concentrations. Hunter et al[6] have also reported that post-menopausal women showed a greater IFA than pre-menopausal women and that menopausal status was significantly related to an increased risk for CHD risk factors (ie, LDLC, TC:HDLC ratio). Therefore, in the current study, the cutoff values were expected to differ according to the menopausal status of the participants. Because estrogen decreases the risk of CHD during the pre-menopausal period, perhaps counterbalancing some of the CHD risks brought on by excess IF;[21] further assumptions were made that the cutoff value for PreM women would be the same or even greater than that of PostM women. The ROC analyses revealed a difference of 30 cm^2 between the cutoff values in each group, but the value of the PreM group was lower than that of the PostM group. The study from the Women's Health Initiative also showed that estrogen would not confer benefits for preventing CHD among women with estrogen plus progestin therapy relative to women given a placebo;[22]

There seem to be other factors in addition to estrogen affecting the risk of metabolic disease; for example, aging, which correlates to an increase in IFA[2,6,23] and adiponectin[24] may be a factor.

In previous studies, a cross-sectional design was used to determine an IFA cutoff value[1,19,20] but because it is also important to determine a target IFA value for reducing the risk of metabolic disease, an intervention design was used in the current study. The IFA relates to the risk of obesity-related metabolic disorders; therefore, we assumed that the target values after weight reduction would remain the same as before the program, but they were lower. Although the reasons for this are unclear, we speculate that once a person is suffering from a metabolic disorder, a significant reduction in IFA may not be enough in itself to ameliorate the situation.

Study Limitations

The reasons for the relatively low sensitivities and specificities derived from IFA and metabolic disorders are unclear. Some unmeasured factors, such as diet and the genetic effect of metabolic disorders, may play a part. Furthermore, homeostasis was not maintained during and just after the weight loss. Another limitation is that the number of participants was small and that the mean body mass index or IFA was not very high, although most participants were obese. Future studies should include a larger number of extremely obese participants to verify the target values for risk of IFA after weight reduction. A significant decrease in HDLC in the PreM group was found after weight reduction, which may have been caused by the diet. Hagan et al[25] reported that HDLC decreased as middle-aged women lost body weight during a 12-week diet program. The significant decrease in TC could be attributed to the fact that TC includes HDLC.

In conclusion, this study presents the cutoff values for IFA in both pre- and post-menopausal obese women, as well as the target values after weight reduction, which are useful for the diagnosis of obesity-related metabolic disorders. Before weight reduction, the cutoff values with the best equilibrium were 80 cm^2 for pre-menopausal women and 110 cm^2 for post-menopausal women. After weight reduction, the target values shifted to 60 cm^2 and 70 cm^2, respectively. Using these values, persons diagnosed with visceral fat obesity can clearly see the benefits of engaging in a diet and exercise program. Furthermore, awareness of a target value makes adherence to the program more likely.

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Evaluation of blood rheology in patients with cyanotic congenital heart disease using a microchannel array flow analyzer

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Abstract. Blood hyperviscosity due to secondary erythrocytosis is a common pathologic feature of cyanotic congenital heart disease (CCHD). In CCHD, it is possible that hematological parameters other than red blood cells influence blood rheology. We measured blood passage time to evaluate the blood rheology in patients with CCHD ($n = 18$, age: 15.3 ± 11.9 years, mean ± SD) and age-matched control subjects ($n = 27$) using the microchannel array flow analyzer (MC-FAN), and the results [several hematological parameters, including hematocrit (Hct)] were compared. Blood passage time in the CCHD group was prolonged, compared with the control group (67.6 ± 27.2 s *vs.* 44.6 ± 6.7 s). For the CCHD group, blood passage time correlated significantly with red blood cell (RBC) count, hemoglobin (Hb) concentration, Hct, mean corpuscular hemoglobin concentration (MCHC), platelet (Plt) count, high-density lipoprotein cholesterol (HDL-C) level, and triglycerides (TG) level (RBC, $r = 0.77$; Hb, $r = 0.69$; Hct, $r = 0.73$; MCHC, $r = -0.64$; Plt, $r = -0.49$; TG, $r = 0.53$; HDL-C, $r = -0.49$, $p < 0.05$ for each variable). For all 45 subjects, blood passage time correlated significantly with HbA1c level ($r = 0.45$, $p < 0.01$) and tissue-type plasminogen activator (t-PA) antigen level ($r = 0.46$, $p < 0.01$). Our results indicated that blood rheology is reduced in patients with CCHD as expressed by prolonged blood passage time, and it may be defined by several blood parameters in addition to erythrocytosis.

Keywords: Blood rheology, cyanotic congenital heart disease (CCHD), erythrocytosis, fibrinolysis, lipid

1. Introduction

In patients with cyanotic congenital heart disease (CCHD), particularly when the disease is unsuitable for radical surgery, hypoxemia with long duration induces significant erythrocytosis, altering blood rheology. Derangement of coagulation and fibrinolysis associated with reduced rheology may provoke cerebrovascular events, one of the most serious complications in CCHD [9,25].

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Blood rheology has been assessed as blood viscosity, which is strongly influenced by hematocrit (Hct), increasing exponentially when the Hct increases [18,24]. However, blood consists of various elements, such as white blood cells, platelets, lipids and proteins, and thus, Hct is probably not the only factor that defines blood rheology [6]. Although it is well known that patients with CCHD tend to develop secondary erythrocytosis; resulting in thrombosis and abnormal hemostasis [10,11], factors that affect blood rheology have not been fully investigated.

Recently, Kikuchi et al. [16,17] developed a microchannel array flow analyzer (MC-FAN) to comprehensively measure blood rheology. The principle of the MC-FAN is filtration, and the method has been applied for screening of life-style related diseases, since it can easily detect abnormal blood rheology in patients with fatty liver disease [19] and hypercholesterolemia [20]. In this study, we determined the blood rheology as reflected by blood passage time in patients with CCHD using MC-FAN, and investigated the relationship between the blood passage time and hematological variables.

2. Materials and methods

2.1. Subjects

We studied 18 patients with CCHD complicated by secondary erythrocytosis (Hct > 45%) (mean age 15.3 ± 11.9 years; ±SD, 13 men and 5 women, CCHD group) and 27 age-matched subjects (age 15.3 ± 9.0 years, 12 men and 15 women, control group) free of hematological or cardiopulmonary disease. Patients with diabetes were also excluded from the study. Before the commencement of the study, informed consent was obtained from each patient or from his/her parents when the patient was under 18 years of age. The study was approved in advance by the ethics committee for human research of Institute of Clinical Medicine, University of Tsukuba.

2.2. Blood sampling

Blood samples were collected from the antecubital vein after at least 15 minutes rest. The first sample was drawn into two polypropylene tubes, one for serum collection and one containing ethylenediamine tetra-acetic acid (EDTA) for whole blood cell count. Whole blood cell count, including red blood cell (RBC) count, hemoglobin (Hb) concentration, Hct, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count and platelet (Plt) count were measured using Cell-Dyn (model 4000, Dinabot Inc., Tokyo, Japan). With serum samples, total cholesterol (TC) level, high-density lipoprotein cholesterol (HDL-C) level, low-density lipoprotein cholesterol (LDL-C) level, and triglycerides (TG) level were quantified.

The second sample was collected into a polypropylene tube containing sodium fluoride, heparin sodium, and EDTA for measurement of hemoglobin A1c (HbA1c) level.

The third sample was gently collected into a polypropylene tube containing 1/10 volume of 3.13% sodium citrate, one for platelet-poor plasma (PPP), which was obtained by centrifugation at 1000 rpm for 10 min at 4°C, and stored in plastic tubes at −80°C until analysis. With PPP samples, the plasma levels of tissue-type plasminogen activator (t-PA) antigen and plasminogen activator inhibitor-1 (PAI-1) activity were determined using commercially available kits (Hyphen BioMed for PAI-1, Hyphen BioMed for t-PA; France). The plasma level of fibrinogen (Fbg) was quantified using routine laboratory techniques.

2.3. Measurement of blood passage time using MC-FAN

Blood passage time was measured using the MC-FAN system (Hitachi Haramachi Electronic Industrial Company, Ibaraki, Japan) as shown in Fig. 1. The blood sample (0.9 ml) was stored in a polypropylene tube containing 2 ml of EDTA and 0.1 ml of heparin, and then 0.1 ml of the whole sample was introduced into a glass cylinder and passed through the siliconized chip with 8736 slits of 7 μm width

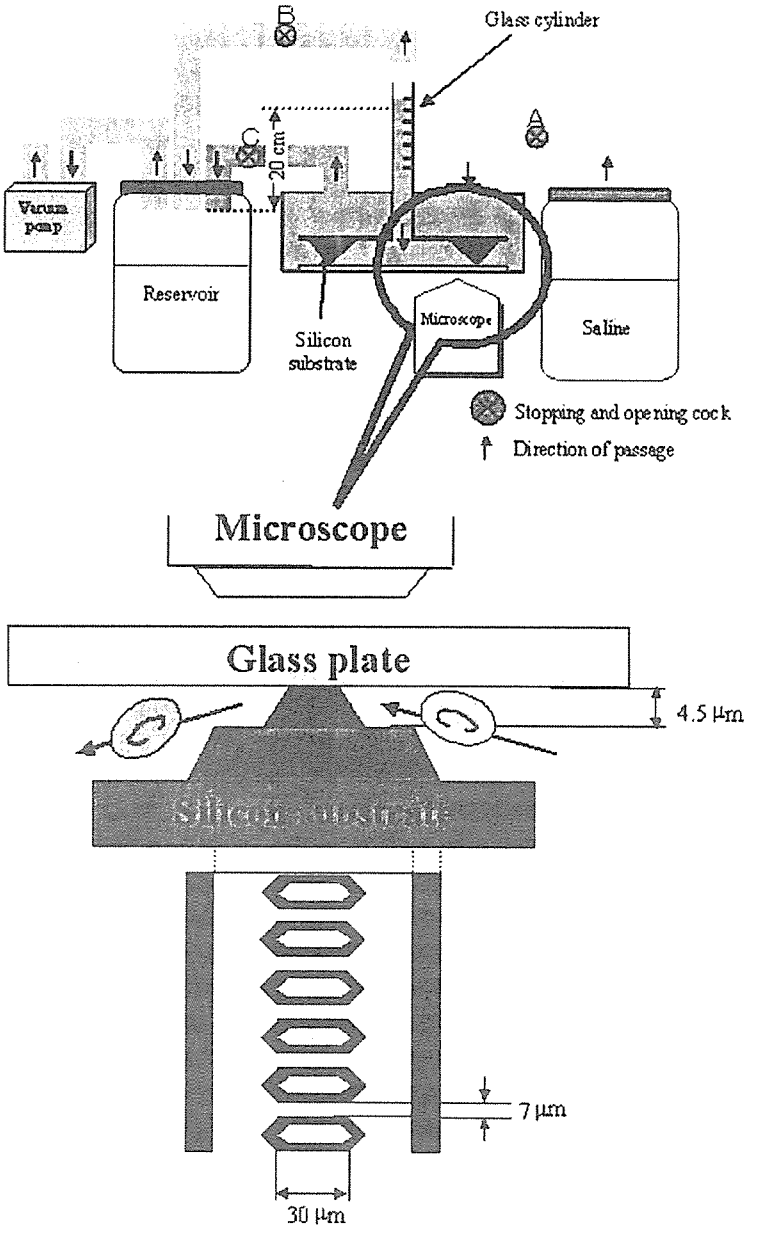

Fig. 1. Schematic representation of the microchannel array flow analyzer (MC-FAN) and microchannel array (filters). The whole sample is introduced into a glass cylinder and is passed through a siliconized chip with 8736 slits of 7 μm width and 30 μm length under negative pressure of 20 cm H_2O.

and 30 μm length under a negative pressure of 20 cm H_2O (Fig. 1). The time for the blood sample of 0.1 ml to pass through the filters was measured as the blood passage time. Calibration with saline (0.1 ml) was repeated immediately before each new measurement. Reproducibility of the blood passage time using MC-FAN was verified, as reported in our previous study [15].

2.4. Statistical analysis

Results were expressed as mean ± SD. The unpaired t-test was used to compare the mean values of the CCHD group and control group. Pearson's product moment correlation coefficient was used to estimate the relationship between blood passage time and each hematological variable. A p-value of <0.05 was considered significant.

3. Results

3.1. Blood passage time

Blood passage time was significantly longer in the CCHD group than in the control group (67.6±27.2 s *vs.* 44.6 ± 6.7 s; $p < 0.01$, Table 1). The blood passage time was >50 s in 14 (78%) subjects of the CCHD group. In comparison, the values were <50 s in 23 (85%) subjects of the control group. The patient with the longest value of blood passage time (158.6 s) had multiple cerebrovascular infarctions

Table 1
Blood passage time and hematological variables

		Control	CCHD	p-value
n		27	18	
Blood passage time	(s)	44.6 ± 6.7	67.6 ± 27.2	<0.001
WBC	(/μl)	6944 ± 2689	7433 ± 2663	0.552
RBC	(10^4/μl)	465 ± 44	621 ± 112	<0.001
Hb	(g/dl)	13.3 ± 1.7	18.4 ± 2.6	<0.001
Hct	(%)	40.2 ± 4.6	56.1 ± 9.1	<0.001
MCV	(fl)	86.6 ± 8.1	90.9 ± 8.1	0.089
MCH	(pg)	28.7 ± 3.1	29.9 ± 2.9	0.202
MCHC	(g/dl)	33.1 ± 1.1	32.8 ± 1.0	0.380
Plt	(10^3/μl)	206 ± 145	219 ± 80	0.737
TC	(mg/dl)	163 ± 29	148 ± 34	0.127
HDL-C	(mg/dl)	58.1 ± 13.6	47.1 ± 13.6	0.014
LDL-C	(mg/dl)	88.9 ± 23.9	84.3 ± 25.7	0.560
TG	(mg/dl)	90.2 ± 59.1	88.2 ± 60.9	0.913
HbA1c	(%)	4.7 ± 0.3	5.3 ± 0.5	<0.001
Fbg	(mg/dl)	262 ± 56	285 ± 57	0.242
PAI-1	(ng/ml)	0.27 ± 0.12	0.50 ± 0.59	0.115
t-PA	(ng/ml)	2.29 ± 0.72	4.71 ± 3.16	0.004

Values are mean ± SD. Fbg: fibrinogen; Hb: hemoglobin; HbA1c: hemoglobin A1c; Hct: hematocrit; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; PAI-1: plasminogen activator inhibitor 1 activity; Plt: platelet; RBC, red blood cells; TC: total cholesterol; TG, triglyceride; t-PA: tissue-type plasminogen activator antigen; WBC: white blood cells.

on MR imaging of the brain. The patient with second longest blood passage time (90.7 s) developed pulmonary hemorrhage, and ultimately died one year after the study.

3.2. Hematological variables

The mean values of RBC, Hb, Hct, MCV, HbA1c and t-PA antigen of the CCHD group were significantly higher than the corresponding values of the control group (Table 1). The mean value of HDL-C was significantly lower in the CCHD group than in the control group.

3.3. Relationship between blood passage time and hematological variables

For the CCHD group, blood passage time correlated significantly with RBC count, Hb concentration, Hct, MCHC, Plt count, HDL-C level, and TG level (RBC, $r = 0.77$; Hb, $r = 0.69$; Hct, $r = 0.73$; MCHC, $r = -0.64$; Plt, $r = -0.49$; TG, $r = 0.53$; HDL-C, $r = -0.49$, $p < 0.05$ for each variable).

For the control group, blood passage time correlated significantly with HDL-C level and t-PA antigen level (HDL-C, $r = -0.44$; t-PA, $r = 0.49$; $p < 0.05$ for each variable, Fig. 2). None of the values of WBC count, MCV, MCH, TC level, LDL-C level, HbA1c level, Fbg level and PAI-1 activity level correlated significantly with blood passage time for each group and for all 45 subjects.

For all subjects, blood passage time correlated significantly with RBC count, Hb concentration, Hct, MCHC, HDL-C level, HbA1c level, and t-PA antigen level (RBC, $r = 0.77$, $y = 0.1x - 2.91$; Hb, $r = 0.69$, $y = 4.16x - 9.39$; Hct, $r = 0.73$, $y = 1.24x - 1.98$; HbA1c, $r = 0.45$, $y = 19.62x - 42.96$; t-PA, $r = 0.46$, $y = 4.18x - 41.39$; HDL-C, $r = -0.51$, $y = -0.75x + 95.297$, $p < 0.01$; MCHC, $r = -0.37$, $y = -7.35x + 296.55$, $p < 0.05$ for each variable).

4. Discussion

4.1. Relationships between RBC and blood rheology

The present study demonstrated that blood passage time was significantly longer in the CCHD group (67.6 ± 27.2 s) than in the control group (44.6 ± 6.7 s, Table 1), and that RBC count, Hb concentration and Hct correlated significantly with blood passage time, indicating that RBC mass influences blood rheology. Furthermore, it is intriguing that MCHC correlated negatively with blood passage time in this study although no difference was found in MCHC values between the CCHD and control groups. MCV is another index of deformability of RBC. When the MCV decreases, RBC deformability decreases, that is, the blood rheology decreases [5,27]. However, MCV values in the CCHD group were higher than those in the control group in our present study. Thus, the effects of decreased RBC deformability had little impact on the prolonged blood passage time in patients with CCHD. This suggests that the mass effects of RBC rather than individual RBC properties define the prolonged blood passage time in CCHD.

4.2. Relationships between blood biochemical variables and blood rheology

The present study suggests that altered blood rheology is not necessarily explained only by the RBC mass. Firstly, HDL-C level correlated negatively with blood passage time. Lee et al. [20] used the MC-FAN to demonstrate that three parameters influence blood passage time: TC level, LDL-C level and HDL-C level, and that HDL-C level correlates positively with blood passage time, which is contrary

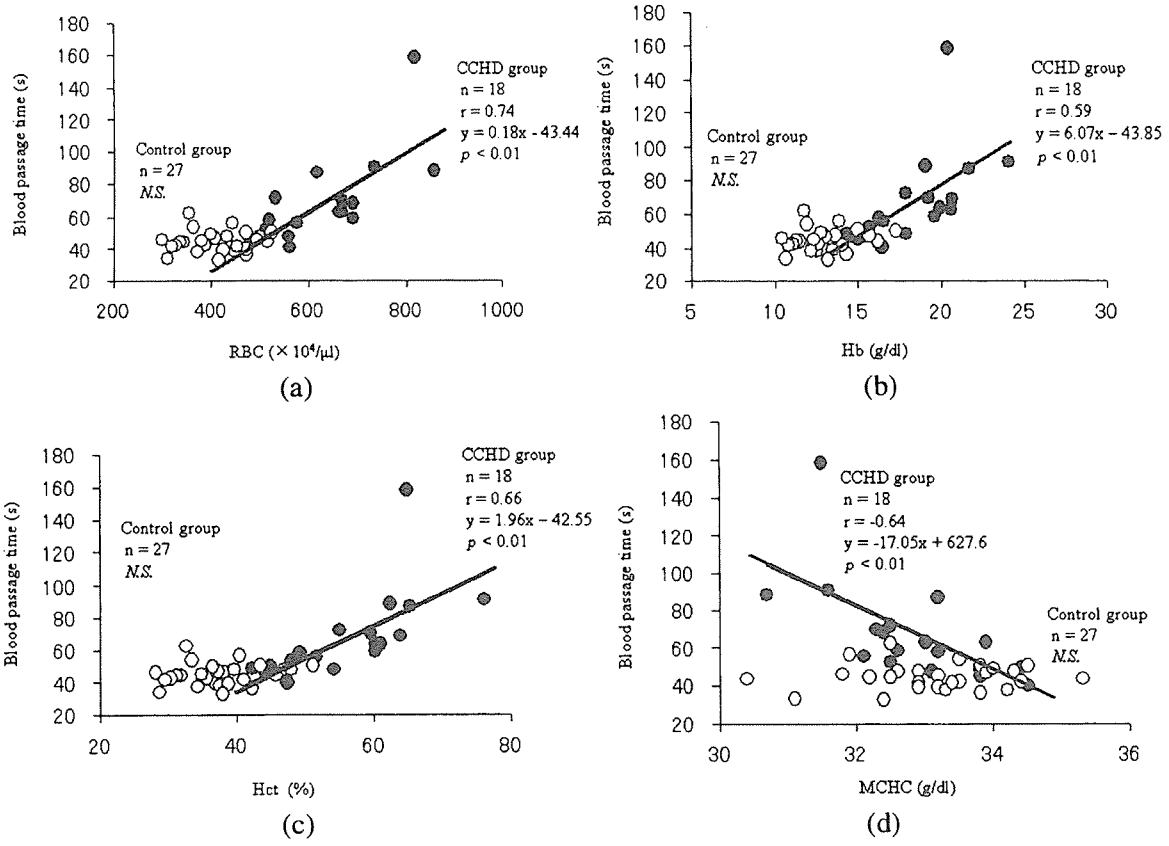

Fig. 2. Blood passage time correlated with hematological variables. Open circles: control subjects, closed circles: patients with CCHD, solid lines: regression lines for CCHD group, dotted lines: regression line for control group, double solid lines: regression line for all 45 subjects. (a) Relationship between red blood cell (RBC) count and blood passage time. For CCHD group, RBC count correlated significantly with blood passage time ($r = 0.74$, $y = 0.18x - 43.44$, $p = 0.001$). For the control group, RBC count did not correlate with blood passage time ($p = 0.621$). (b) Relationship between hemoglobin (Hb) concentration and blood passage time. For CCHD group, Hb concentration correlated significantly with blood passage time ($r = 0.59$, $y = 6.07x - 43.85$, $p = 0.010$). For the control group, Hb concentration did not correlate with blood passage time ($p = 0.109$). (c) Relationship between hematocrit (Hct) and blood passage time. For CCHD group, Hct correlated significantly with blood passage time ($r = 0.66$, $y = 1.96x - 42.55$, $p = 0.003$). For the control group, Hct did not correlate with blood passage time ($p = 0.079$). (d) Relationship between mean corpuscular hemoglobin concentration (MCHC) and blood passage time. For CCHD group, MCHC correlated significantly with blood passage time ($r = -0.64$, $y = -17.05x + 627.6$, $p = 0.004$). For the control group, MCHC did not correlate with blood passage time ($p = 0.790$). (e) Relationship between platelet (Plt) count and blood passage time. For CCHD group, Plt count correlated significantly with blood passage time ($r = -0.49$, $y = -0.16x + 103.68$, $p = 0.040$). For the control group, Plt count did not correlate with blood passage time ($p = 0.373$). (f) Relationship between triglyceride (TG) level and blood passage time. For CCHD group, TG level correlated significantly with blood passage time ($r = 0.53$, $y = 0.24x + 46.75$, $p = 0.023$). For the control group, TG level did not correlate with blood passage time ($r = 0.11$, $p = 0.596$). (g) Relationship between tissue-type plasminogen activator (t-PA) antigen level and blood passage time is significant for whole subjects ($r = 0.46$, $y = 4.18x + 41.39$, $p < 0.01$) as well as for control group ($r = 0.49$, $y = 4.86x + 32.75$, $p = 0.045$). (h) Relationship between high-density lipoprotein cholesterol (HDL-C) level and blood passage time. For CCHD group, HDL-C level correlated significantly with blood passage time ($r = -0.49$, $y = -0.97x + 115.09$, $p = 0.046$). For the control group, HDL-C level also correlated significantly with blood passage time ($r = -0.44$, $y = -0.21x + 57.51$, $p = 0.027$). (i) Relationship between hemoglobin A1c (HbA1c) level and blood passage time is significant not for each group but only for the entire data of the two groups ($r = 0.46$, $y = 19.62x - 42.96$, $p < 0.01$).

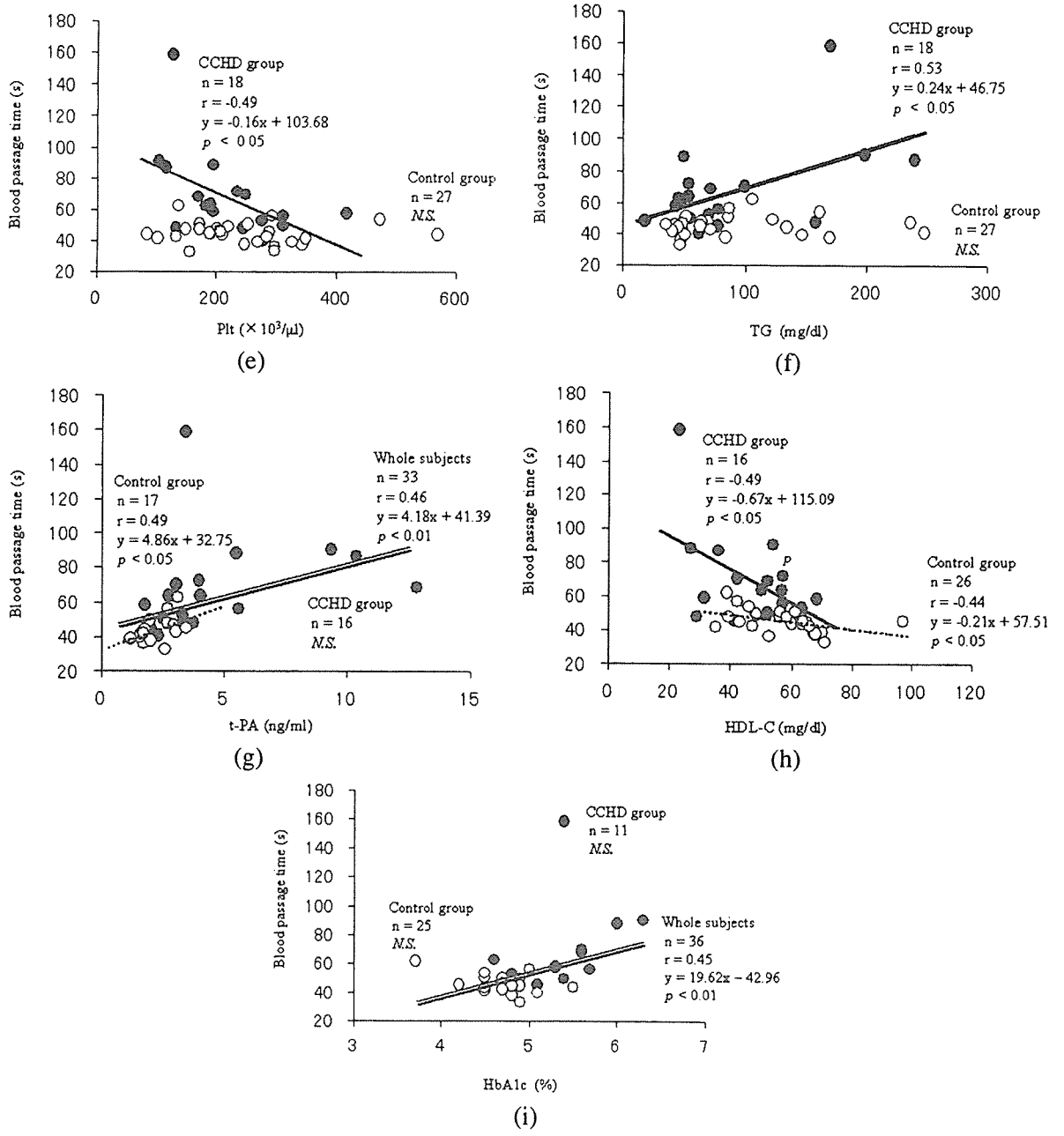

Fig. 2. (Continued.)

to the present findings. They studied subjects with hypercholesterolemia (serum TC, 220 ± 21 mg/dl; HDL-C, 50.3 ± 11.5 mg/dl) and control subjects (serum TC, 133 ± 14 mg/dl; HDL-C, 32.4 ± 14.8 mg/dl). HDL-C level in their control group was paradoxically lower than those in patients with hypercholesterolemia, possibly leading to distortion of the results. Other previous reports indicated that LDL-C and TG augmented blood viscosity with an increase in RBC concentration/aggregation, and that HDL-C had the opposite effect, in accordance with our results [6,26]. It is also intriguing that HDL-C level was

significantly lower in patients with CCHD than in the control in our study, although the reason for the decrease is not clear at present. One possible explanation for this is the restricted physical activity in CCHD patients, as it is known that exercise may increase the HDL-C level.

4.3. Relationship between hemoglobin A1c and blood rheology

Previous studies showed that blood rheology is reduced in diabetic individuals [7,12], and that patients with diabetes have high levels of blood viscosity [12] and RBC aggregation [7]. In the present study, HbA1c level was significantly higher in patients with CCHD and correlated with blood passage time, although none of the patients in the CCHD group had high level of fasting blood glucose or had glucosuria. It is necessary to observe whether apparent diabetes develops later among these patients.

4.4. Relationships between fibrinolytic factors and blood rheology

Plasma levels of fibrinogen and PAI-1 activity are also known to correlate with blood rheology, in addition to the risk factors for cerebrovascular disease and coronary heart disease [3,13,23,30]. However, in the present study, these parameters did not correlate with blood passage time (Fbg, $r = 0.20$; PAI-1, $r = 0.20$; $p > 0.05$). In addition, we found no difference in levels of Fbg and PAI-1 activity between the CCHD and control groups. Ben-Ami et al. [2] demonstrated that, in patients with acute myocardial infarction, a decrease in plasma Fbg level brought about by fibrinolysis contributed to RBC aggregation, and that high level of plasma Fbg degradation products (FDP) further interfere with RBC aggregation. Thus, FDP is possibly more informative when evaluating blood rheology in relation to RBC aggregation. Unlike their study, the subjects in the present investigation had chronic hematological disorder and no fibrinolytic intervention. Furthermore, we did not measure the plasma level of FDP this time. Investigation of fluctuation of fibrinogen and FDP in a larger trial would be useful to establish the management strategy for hematological complications in CCHD patients.

On the other hand, our results showed that plasma levels of t-PA antigen correlated positively with blood passage time when data of whole subjects were included in the analysis ($r = 0.46$, $p < 0.01$). Using a viscometer, Wei et al. [29] also showed that t-PA antigen level correlated positively with whole blood viscosity. The MC-FAN used in the present study does not necessarily reflect *in vivo* microcirculation, but the filters of the system are designed to match the human capillary diameter. Baskurt et al. [1] demonstrated that enhanced RBC aggregation leads to suppressed production of endothelial nitric oxide (NO), thereby altering vasomotor tone, through decreased wall shear stress derived from axial accumulation of RBCs in microcirculation. Considering that t-PA antigen is a sensitive marker of endothelial dysfunction, a significant correlation between t-PA antigen level and blood passage time, though only for whole subjects, is an intriguing result. The latter could be a useful predictor for the development of cerebrovascular complications in CCHD. In fact, two of our patients with prolonged blood passage time subsequently developed multiple cerebrovascular infarctions or fatal pulmonary hemorrhage.

In conclusion, our study demonstrated prolonged blood passage time in patients with CCHD using a microvessel model. The reduced blood rheology correlated not only with erythrocytosis but to other hematological parameters, including decreased HDL-C level and increased HbA1c and t-PA antigen levels. Surveillance for development of cerebrovascular complications is needed to confirm the usefulness of MC-FAN for management of CCHD patients.

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