Table 4 Endoscopic diagnoses using M-NBI for reddened/same-colored mucosal lesions according to grade of certainty

Grade	Cancer	Non-cancer	Total
1	0	154	154
2	2	100	102
3	5	52	57
4	5	5	10
5	6	1	7
Total	18	312	330

M-NBI magnifying endoscopy with narrow-band imaging, *Grade 1* non-cancer with high degree of confidence (no need for biopsies), *Grade 2* non-cancer with low degree of confidence (biopsies required), *Grade 3* indeterminate (biopsies required), *Grade 4* cancer with low degree of confidence (biopsies required), *Grade 5* cancer with high degree of confidence (no need for biopsies)

Initially, we intended to compare the number of biopsies using C-WLI with a historical control. However, after completing the trials, the number of enrolled patients in the historical control over a certain period were in fact quite different from this prospective study. Therefore, since such unbalanced data sets are not suitable for analysis, we could not compare data from this prospective study with that from the historical control. In a retrospective study, the number of biopsies required to diagnose one cancer using C-WLI with chromoendoscopy was reported as 76 [31]. This suggests that M-NBI may contribute to reducing the number of biopsies required to detect one cancer in screening endoscopy.

However, to provide further information for the selection of therapeutic strategy (e.g. endoscopic resection *vs* surgical resection), we need to take biopsies in Grade 5 cases, because endoscopic diagnosis using M-NBI has not been demonstrated to provide adequate diagnostic performance for predicting histological differentiation, i.e. differentiated *vs* undifferentiated type [9]. Therefore, clinicians should be aware of the necessity to take biopsies for the determination of histological type. On the other hand, we frequently encounter the situation where we are unable to take biopsies from a suspicious lesion in a patient on intensive antithrombotic therapy which can not be discontinued because of the high risk of thromboembolic events. In such cases, the proposed strategy may be applicable in deciding whether or not we should perform excisional biopsy after heparinization.

The limitations of this study are that this was an uncontrolled study, and the number of detected cancers was small. Therefore, in the near future we need to compare the diagnostic performance of M-NBI with other conventional endoscopy methods (e.g. chromoendoscopy) dealing with a substantial number of early gastric cancer cases. A system to ease the learning curve for M-NBI procedures has yet to be established. In order to overcome these problems, we are now developing a novel e-learning system for

Table 5 Diagnostic performance of M-NBI for reddened/same-colored mucosal lesions

	All lesions (95 % CI) ($n = 330$)		High confidence prediction (95 % CI) ($n = 161$)		Low confidence prediction (95 % CI) ($n = 169$)	
Accuracy	98.1	(96.6–99.6)	99.4	(98.2–100)	91.9	(87.8–96.0)
Sensitivity	69.2	(44.1–94.3)	100		41.7	(13.8–69.6)
Specificity	98.1	(98.2–100)	99.4	(98.2–100)	95.6	(94.2–98.8)

M-NBI magnifying endoscopy with narrow-band imaging, *CI* confidence interval

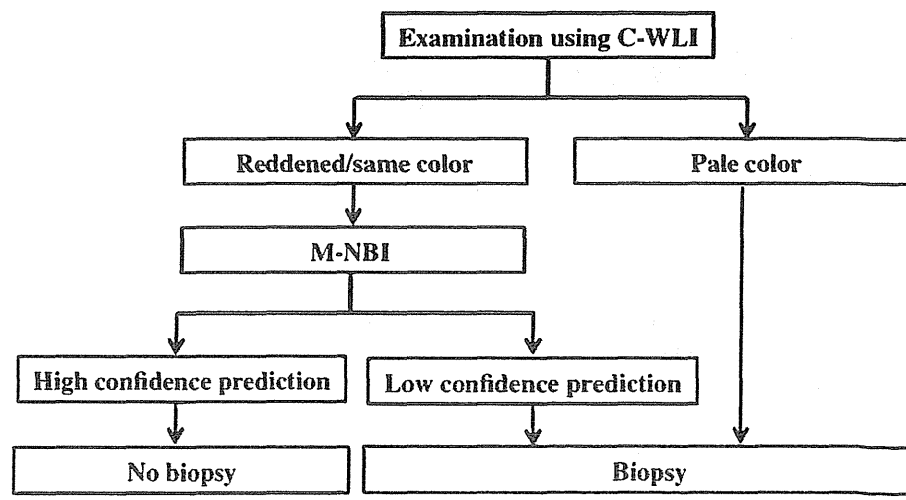

Fig. 6 A provisional strategy for M-NBI in screening gastroscopy

improving the diagnostic performance of M-NBI endoscopy (UMIN 000008569). Once it has been completed, we are planning a multicenter randomized controlled study. Once sufficient high-level evidence has been obtained that can support our provisional strategy, "optical biopsy" using M-NBI will be applied to clinical practice. The other limitations are that we have not tested the ability of NBI for detecting early gastric cancer because the image obtained by non-magnifying observation with NBI incorporated into the endoscopy system available in this study is too dark for endoscopists to detect a mucosal lesion. Recently, a new electronic endoscopy system with a bright NBI illumination (EVIS Lucera Elite, Olympus) has been launched. We are now planning a new trial to test whether NBI can detect more early gastric cancers than C-WLI. If we complete this study, it will become clear whether NBI can be helpful for detecting cancer invisible by C-WLI alone.

In conclusion, we demonstrated the high diagnostic performance and limitations of M-NBI in making a diagnosis of early gastric cancers of all macroscopic types in screening endoscopy in a multicenter prospective study, and we have proposed a provisional strategy for M-NBI in screening endoscopy for early gastric cancer that takes these limitations into consideration.

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Conflict of interest The authors have no potential conflicts of interest relevant to this article to declare.

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REGULAR ARTICLE

Changes in thioredoxin concentrations: an observation in an ultra-marathon race

Mitsuhiro Marumoto · Sadao Suzuki · Akihiro Hosono · Kazuyuki Arakawa · Kiyoshi Shibata · Mizuho Fuku · Chiho Goto · Yuko Tokudome · Hideki Hoshino · Nahomi Imaeda · Masaaki Kobayashi · Junji Yodoi · Shinkan Tokudome

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Abstract
Objectives Changes in plasma thioredoxin (TRX) concentrations before, during, and after a 130-km endurance race were measured with the aim of elucidating the relationship between exercise and oxidative stress (OS).
Methods Blood samples were taken from 18 runners participating in a 2-day-long 130-km ultra-marathon during the 2 days of the race and for 1 week thereafter. There were six sampling time points: at baseline, after the goal had been reached on the first and second day of the endurance race, respectively, and on 1, 3, and 5/6 days post-endurance race. The samples were analyzed for plasma TRX concentrations, platelet count, and blood lipid profiles.
Results Concentrations of plasma TRX increased from 17.9 ± 1.2 ng/mL (mean ± standard error of the mean) at baseline to 57.3 ± 5.0 ng/mL after the first day's goal had been reached and to 70.1 ± 6.9 ng/mL after the second day's goal had been reached; it then returned to the baseline level 1 day after the race. Platelet counts of 21.3 ± 1.2 × 10^4 cell/μL at baseline increased to 23.9 ± 1.5 × 10^4 cells/μL on Day 1 and to 26.1 ± 1.0 × 10^4 cells/μL on Day 2. On Day 7, the platelet counts had fallen to 22.1 ± 1.2 × 10^4 cell/μL. There was a significant positive correlation between plasma TRX and platelet count.
Conclusions These data suggest that plasma TRX is an OS marker during physical exercise. Further studies are needed to determine the appropriate level of exercise for the promotion of health.

Keywords Lipid profile · Marathon runner · Oxidative stress · Platelet counts · Thioredoxin

M. Marumoto · S. Suzuki · A. Hosono · K. Arakawa · S. Tokudome
Department of Public Health, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan
e-mail: Marumoto_Mitsuhiro@takeda.co.jp

K. Shibata
Kasugai City Health Care Center, Kasugai, Japan

M. Fuku
Yokohama City University Medical Center, Yokohama, Japan

C. Goto
Nagoya Bunri University, Inazawa, Japan

Y. Tokudome
Nagoya University of Arts and Sciences, Nisshin, Japan

H. Hoshino
Aichi Bunkyo Women's College, Inazawa, Japan

N. Imaeda
Nagoya Women's University, Nagoya, Japan

M. Kobayashi
Department of Bone and Orthopedics, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

J. Yodoi
Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto, Japan

S. Tokudome (✉)
National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan
e-mail: tokudome@nih.go.jp

Introduction

Moderate physical exercise is believed to be beneficial to health by reducing the risk of several diseases, including cardiovascular problems and diabetes mellitus. However, it has been suggested that extreme endurance exercise may be detrimental because reactive oxygen species (ROS) are generated by excessive oxygen consumption [1]. Oxidative stress (OS) can be defined as "an imbalance between the free radical production and antioxidant defense mechanisms of a biological organism that results, directly or indirectly, in cellular damage" [2].

The human body is protected by anti-oxidant systems, including that of thioredoxin (TRX) [3]. TRX is a ubiquitous small protein (12 kDa) that acts as an antioxidant via a cysteine thiol-disulfide exchange in which it functions as a proton donor. This function is dependent on the cyclic reduction–oxidation of a single S–S bond. When the reduced form of TRX [TRX-$(SH)_2$] captures ROS, it is transformed into oxidized-TRX (TRX-S_2) and H_2O. Oxidized-TRX is then reduced by TRX reductase to become reduced-TRX [4]. This redox reaction suggested that TRX plays an important role as an antioxidant [5].

TRX has been reported to be a sensitive OS marker, with its levels increasing in relation to hydrogen peroxide, ultraviolet irradiation, and inflammation [3, 4, 6]. It is associated with the signal transduction of cellular redox regulation and with cytoprotection against OS [3]. Elevated plasma TRX concentrations suggest not only an elevated level of OS, but also enhancement of the anti-oxidative system through the activation of TRX gene expression [7].

TRX was originally purified from *Escherichia coli* in 1964 as a proton donor to ribonucleotide reductase, and it was thought to be a regulator of cellular redox status [8]. In 1989, Yodoi's group identified the adult T-cell leukemia-derived factor as TRX [9], originally defined as an interleukin-2 receptor α-chain inducer in human T-lymphotropic virus type I-transformed cells. TRX has both extra- and intra-cellular functions, and it is one of the key signaling regulators in the cellular response to various OS. It is also associated with the redox regulation of cellular activation and redox-sensitive molecules, such as NF-kB, AP-1, and glucocorticoid receptors, indicating both anti-oxidant and effects [4, 10, 11].

Results from earlier studies suggested that extreme exercise elevates OS markers, including urinary 8-hydroxydeoxyguanosines (8-OHdG), oxidized DNA nucleosides [8, 12, 13], malondialdehydes, isoprostanes, and lipid peroxides (LPO) [2, 14]. Intense physical exercise was also found to increase the plasma concentrations of free fatty acids (FFA) and fatty acid oxidation [15]. Plasma FFA are used as an energy source and are also associated with the generation of OS through lipid peroxidation.

To the best of our knowledge, no data have been reported on the variations in plasma TRX concentrations during physical exercise. Here, we report our analysis of changes in plasma TRX concentrations in participants in an ultra-marathon, based on blood samples taken before, during, and after the race, and our comparison of these changes with the lipid profile.

Subjects and methods

Ultra-marathon race

A non-competitive ultra-marathon race was held in Gifu Prefecture, Japan, on July 24–25, 2004 [16]. The race covered a distance of 130 km and comprised running and mountaineering activities over the 2-day period. On the first day, at 11 a.m., the participants began a full-length marathon race to be completed within 6.5 h. At 3:30 a.m. on the second morning, they resumed the race, covering approximately 90 km, including climbing a mountain approximately 1100 m high, then returning to the starting point within 15.5 h. It was very hot and sultry during the race, with the temperature hovering around 35.5°C. There were no dietary restrictions during the race, and runners were free to take any food and beverage of their choice.

Subjects

Non-professional Japanese male athletes participated in our study. We asked 41 runners to participate in our analysis, of whom 24 agreed. We received written informed consent for their completion of lifestyle questionnaires, measurements of anthropometric characteristics, and blood sampling. The protocol was approved by the Institutional Review Board of Nagoya City University Graduate School of Medical Sciences and by the chairman and organizing committee of the race.

Anthropometric measurements and blood sampling

Anthropometric measurements and the sampling of venous blood were performed at six time points: i.e., before the race (baseline), immediately after completing the first stage (Day 1) and after completing the final stage (Day 2), early morning 1 day after the race (Day 3), 3 days after the race (Day 5), and 5 or 6 days after the race (Day 7) (Fig. 1). About 1–2 h before the race, we obtained spot (non-fasting) venous blood using a vacuum tube with serum separating medium (Tube 1) and with EDTA-2Na (Tube 2) and the anthropometric data. Immediately after the participants finished the race on Day 1 and Day 2, respectively, we obtained spot venous blood samples using the same

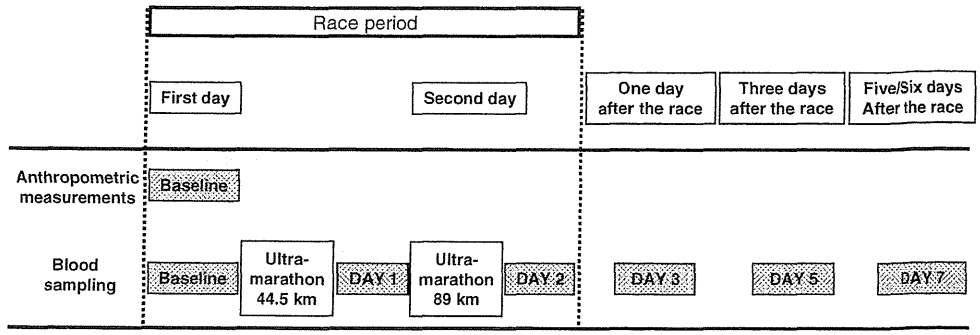

Fig. 1 Study protocol. Anthropometric measurements and blood samplings were performed at six time points: before the race (*baseline*), after completing the first stage (*DAY 1*), after completing the final stage (*DAY 2*), and 1 day (*DAY 3*), 3 days (*DAY 5*), and 5/6 days (*DAY 7*) after the race

procedure as for the baseline samples. The tubes from all three samplings (baseline, Days 1 and 2) were kept in an ice-cooled box for several hours and then transported to a commercial laboratory on the same day. Early morning (about 7:30–8:00 a.m.) fasting blood was sampled on Days 3, 5, and 7, respectively. Tube 1 was centrifuged into serum and analyzed for biochemical markers, including hematocrit, platelet, hemoglobin (Hb), total protein, FFA, triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-cholesterol) using a Hitachi 7600 machine (Hitachi High-Tech K.K., Tokyo, Japan). Tube 2 was separated into plasma, buffy coat, and red blood cell (RBC) fractions and then stored in a deep freezer (−80°C) until analysis for plasma TRX.

Measurements of TRX

All plasma TRX concentrations, including reduced- and oxidized-TRX, were determined with a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) kit (Redox Bioscience, Kyoto, Japan), as reported elsewhere [17]. Human TRX-antibody-precoated 96-microwell plates were incubated for 2 h at room temperature with 25 μL of plasma or with a standard solution (human TRX antigen; 0, 2.5, 5, 10, 20, 40, 80, 160 ng/mL) in the presence of 250 μL of 50 mmol/L sodium phosphate buffer (pH 6.0) containing 150 mmol/L NaCl, 1.0 mmol/L Mg Cl_2, 1.0% bovine serum albumin (BSA), and 0.1% NaN_3. The plates were washed five times with 10 mmol/L sodium phosphate buffer (pH 7.5) containing 0.05% Tween 20 and 150 mmol/L NaCl (Washing Solution), 200 μL horseradish peroxidase-labeled anti-human TRX antibody was then added, and the plates were incubated at room temperature for 2 h. After a further five washings with Washing Solution, we introduced 100 μL of 100 mmol/L triethanolamine-succinate buffer (pH 4.4) containing 1.5 mmol/L H_2O_2 and 0.13% ABTS [2,2′-azino-di (3-ethylbenzthiazoline sulfonic acid)]. Incubation at room temperature was continued for 30 min. The reaction was stopped by adding 100 μL of 0.25 M H_2SO_4 solution, and the optical density of the plates was measured at 450/620 nm with a microplate reader (Spectramax 340; Molecular Devices, Sunnyvale, CA). All assessments were made in triplicate, and the average value was calculated.

Statistical analysis

Data are expressed as mean with standard deviation (SD) to show the distribution of baseline values, or as the standard error of the mean (SEM) to demonstrate the difference of means. The general linear model (GLM procedure) along with the post hoc Dunnett's test was applied to detect statistical significance at $p < 0.05$ in comparison with each time point. The SAS ver. 9.1 software package (SAS Institute, Cary, NC) was used for the statistical analyses.

Results

Of the 24 runners included in the study, 18 completed the race and six dropped out during the race. All 18 were non-smokers. In terms of anthropometric and demographic measures (Table 1), the average age of the 18 runners was 54 ± 12 years (mean ± SD), and their average body mass index (BMI) was 21.4 ± 1.6 kg/m^2. These runners had a recent average monthly running distance of 247.5 ± 96.8 km. In this race, their average completion time for the 2-day ultra-marathon race was 1,106.0 ± 179.0 min (range 711.0 to 1,313.0 min).

The hematocrit rose significantly from 42.3 ± 0.7% (mean ± SEM) at baseline to 43.9 ± 0.7% on Day 1, returning to 43.0 ± 0.5% by Day 2. The Hb count was 14.4 ± 0.3 g/dL at baseline, increasing significantly to 15.1 ± 0.3 g/dL on Day 1, but returning to 14.8 ± 0.2 g/dL on Day 2. A platelet count of 21.3 ± 1.2 × 10^4 cell/μL was found at baseline; it increased to 23.9 ± 1.5 × 10^4 cell/μL on Day 1 (Fig. 2) and to 26.1 ± 1.0 × 10^4 cell/μL on Day 2. On Day 7, the platelet count had fallen to 22.1 ± 1.2 × 10^4 cell/μL. There was a significant positive correlation between plasma TRX levels and platelet counts ($r = 0.318$, $p < 0.01$) (Fig. 3). The total protein level of 7.1 ± 0.1 g/dL at baseline rose significantly to 7.6 ± 0.1 g/dL on Day 1 and

Table 1 Baseline characteristics of 18 subjects who completed the 2-day ultra-marathon

Characteristic	Mean ± SD
Age	54 ± 12
Body mass index (kg/m^2)	21.4 ± 1.6
White blood cell count (×10^3 cell/μL)	4.85 ± 1.00
Red blood cell count (×10^5 cell/μL)	4.47 ± 0.40
Hemoglobin (g/dL)	14.4 ± 1.1
Hematocrit (%)	42.3 ± 2.8
Platelet count (×10^4 cell/μL)	21.3 ± 5.1
Total protein (g/dL)	7.10 ± 0.40
BUN (mg/dL)	16.7 ± 4.6
Uric acid (mg/dL)	6.10 ± 1.10
Total cholesterol (mg/dL)	210 ± 27
HDL cholesterol (mg/dL)	81.0 ± 18.3
Free fatty acids (mEq/L)	0.120 ± 0.100
Triglycerides (mg/dL)	118 ± 83

BUN Blood urea nitrogen, *HDL* high-density lipoprotein

Data are expressed as the mean ± standard deviation (SD)

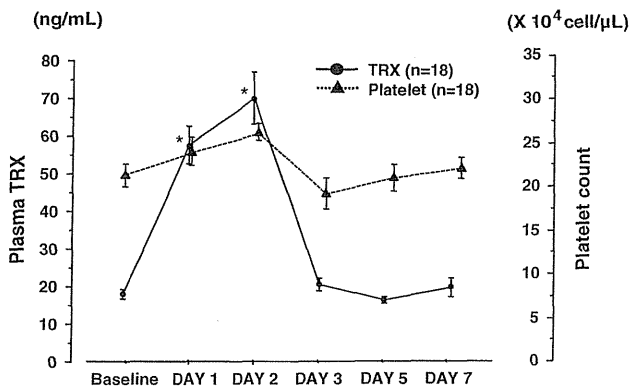

Fig. 2 Changes in plasma thioredoxin (*TRX*) and platelet counts throughout the study period. *Solid line* with *filled circles* Plasma TRX concentrations, *dotted line* with *filled triangles* platelet counts. Data are given as the mean ± standard error of the mean (SEM). *$p < 0.05$ denotes a significant difference from the baseline value

returned to 7.3 ± 0.1 g/dL on Day 2. In order to adjust the effects of dehydration [18], we multiplied the levels of TRX, FFA, platelet count, total cholesterol, HDL-cholesterol, and triglycerides by the ratio of the hematocrit at baseline/its value at five of the six time points (excluding baseline sampling).

The average baseline plasma TRX level of 17.9 ± 1.2 ng/mL increased significantly to 57.3 ± 5.0 ng/mL on Day 1 ($p < 0.05$ vs. baseline value). On Day 2 it rose to fourfold the baseline concentration (70.1 ± 6.9 ng/mL, $p < 0.05$), then decreased to 20.4 ± 1.7 ng/mL on Day 3 (not significantly different from the baseline value), stabilizing around that level on Days 5 and 7 (Figs. 2, 4).

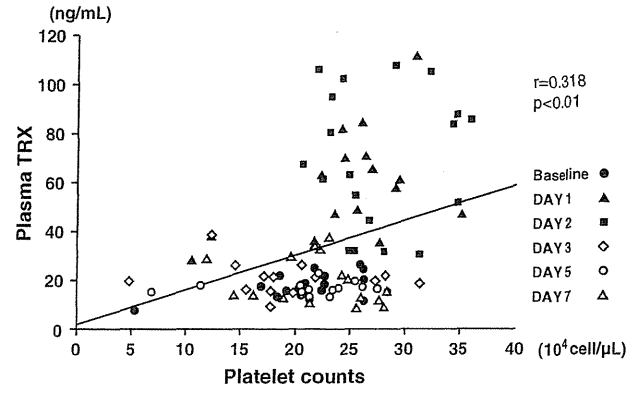

Fig. 3 Correlation between plasma TRX and platelet counts throughout the study period

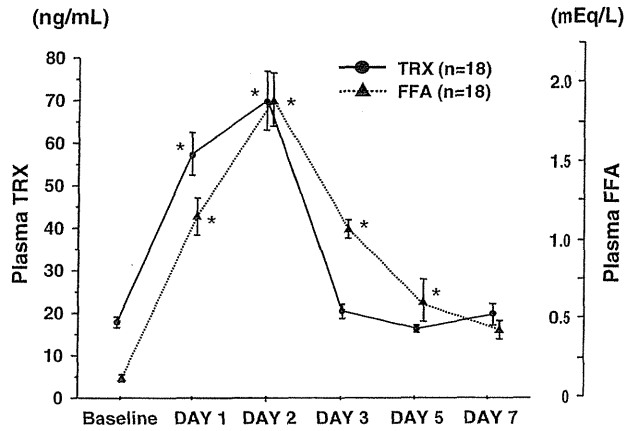

Fig. 4 Changes in plasma TRX and serum free fatty acids (*FFA*) concentrations throughout the study period. *Solid line* with *filled circles* Plasma TRX concentrations, *dotted line* with *filled triangles* plasma FFA levels. Data are expressed as the mean ± SEM. *$p < 0.05$ denotes a significant difference from the baseline value

In terms of the lipid profiles, the plasma FFA level of 0.12 ± 0.02 mEq/L at baseline increased significantly to 1.15 ± 0.12 mEq/L on Day 1 ($p < 0.05$ vs. baseline value) (Fig. 4) and 1.89 ± 0.17 mEq/L on Day 2 ($p < 0.05$) before declining on Day 3, reaching 0.42 ± 0.06 mEq/L on Day 7. There was a significant positive correlation between plasma TRX and plasma FFA concentrations ($r = 0.647$, $p < 0.0001$) (Fig. 5).

The triglyceride level was 118.3 ± 19.5 mg/dL at baseline, declining gradually to 46.0 ± 5.4 mg/dL on Day 2 ($p < 0.05$ vs. baseline value) and to 40.3 ± 4.2 mg/dL on Day 3 ($p < 0.05$) (Fig. 6) and then returning to baseline on Day 5 and Day 7. The total cholesterol level of 210.5 ± 6.4 mg/dL at baseline rose slightly to 219.8 ± 6.9 mg/dL on Day 1 and then declined to 199.1 ± 7.1 mg/dL on Day 3. There was a significant difference in total cholesterol levels on Day 7 ($p < 0.05$). The HDL-cholesterol level of 81.0 ± 4.3 mg/dL at baseline significantly

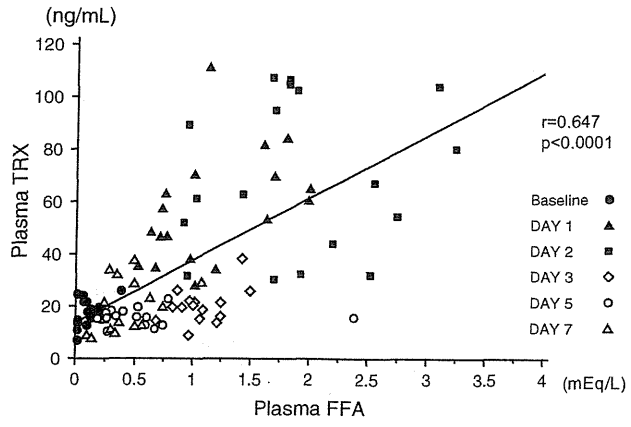

Fig. 5 Correlation between plasma TRX levels and log plasma FFA concentrations throughout the study period

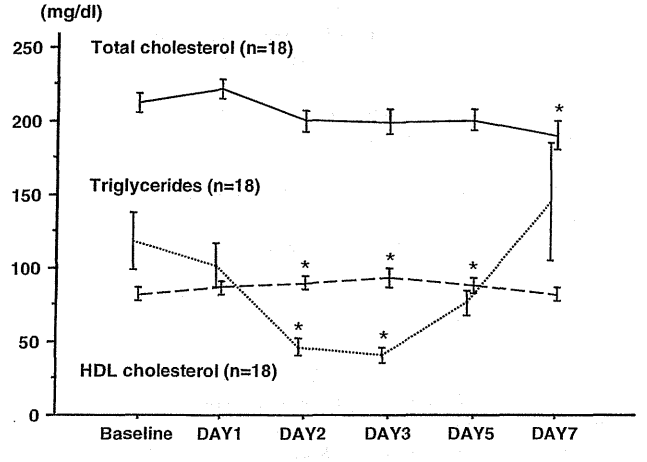

Fig. 6 Changes in lipid profiles throughout the study period. *Dotted line* Serum triglyceride concentrations, *solid line* total cholesterol concentrations, *broken line* high-density lipoprotein (*HDL*)-cholesterol. Data are expressed as the mean ± SEM. *$p < 0.05$ denotes a significant difference from the baseline value

increased to 92.5 ± 6.0 mg/dL on Day 2 ($p < 0.05$ vs. baseline value), but it had returned to the baseline value (80.9 ± 4.6 mg/dL) by Day 7.

Discussion

Our analysis of the plasma OS in marathon runners participating in a 2-day 130-km competition showed a continuous increase in plasma TRX concentrations during this extreme endurance exercise when adjustments were made for the effects of plasma volume change. The change in plasma FFA concentrations resembled that of plasma TRX levels, and there was a significant correlation between TRX and FFA levels, suggesting that plasma TRX is a useful OS marker.

We found that plasma triglycerides decreased steadily regardless of what foods/beverages were consumed during the race, but that they recovered 5/6 days after the race, as has been described earlier [19]. Most fat is stored as triglycerides in the subcutaneous and visceral fat tissues, and the latter are taken up by the skeletal muscle cells as FFA during exercise [20]. Plasma FFA subsequently become the major source of energy during exercise, indicating a reduction in plasma triglycerides. In agreement with this, we found that plasma total cholesterol gradually declined during and after the race.

A significant correlation between the plasma TRX level and platelet count has been reported earlier, with the presence of TRX in platelets confirmed by immunochemical methods [21]. In a later study, Miyamoto et al. [22] reported that the plasma TRX level relates significantly to platelet aggregability in patients with acute myocardial infarction. In our study, we found a significant positive correlation between plasma TRX level and platelet counts, suggesting that TRX is derived from the platelets. However, we did not measure platelet aggregability.

There have been many studies on OS using several markers, including urinary 8-OHdG, malondialdehydes, and plasma isoprostanes [2, 14, 22–24]. Studies on urinary 8-OHdG concentrations among healthy subjects, however, indicate that the range is narrow even in ultra-marathon runners [13, 24]. In contrast, we found that TRX is an OS marker that shows distinct changes in the blood.

ROS are generated by excess oxygen consumption during physical exercise. It is, however, difficult to detect ROS directly because they are highly labile and unstable. We analyzed TRX levels before, during, and after an ultra-marathon race and observed a significant increment in plasma TRX concentrations among ultra-marathon runners. Based on these results, it may be concluded that TRX is a useful OS marker during physical exercise. Further studies of TRX are therefore needed to investigate an appropriate level of physical exercise, including jogging, for the purpose of promoting good health.

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REGULAR ARTICLE

Abdominal circumference should not be a required criterion for the diagnosis of metabolic syndrome

Kiyoshi Shibata · Sadao Suzuki · Juichi Sato · Isao Ohsawa · Shinichi Goto · Masaru Hashiguchi · Shinkan Tokudome

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Abstract
Background Metabolic syndrome (MetS) is an established concept. However, it is characterized by a number of different definitions as well as different cut-off points (COPs) for waist circumference (WC) and different modes for incorporating WC into the diagnostic criteria.
Methods Abdominal ultrasonography was performed in 2,333 subjects who also underwent comprehensive medical examinations between April and July 2006. The odds ratios for the number of MetS components were calculated by taking central obesity status into account and considering concurrent fatty liver as an independent variable. We compared the areas under the receiver operating characteristic (ROC) curves for fatty liver and MetS using several MetS criteria.
Results Regardless of the WC criterion selected, we observed a strong linear trend for an association (trend $P < 0.0001$) between MetS and the number of components. The odds ratio (OR) of subjects without central obesity but with all three MetS components was 9.69 (95% confidence interval 3.11–30.2) in men and 55.3 (6.34–483) in women. The COP for the largest area under the curve in men and women was ≥82 cm (OR 0.701) and ≥77 cm (OR 0.699), respectively, when WC was considered as a component. When WC distribution is taken into consideration, practical and appropriate COPs should be ≥85 cm for men and ≥80 cm for women.
Conclusion We suggest that a WC of ≥85 cm for men and ≥80 cm for women would be optimal COPs for the central obesity criteria in the Japanese population. In addition, central obesity should be incorporated as a component of MetS rather than an essential requirement for the diagnosis of MetS.

Keywords Central obesity · Diagnostic criteria · Metabolic syndrome · ROC curve

K. Shibata (✉) · M. Hashiguchi
Kasugai City Medical Center, 1-1-7 Chuodai,
Kasugai, Aichi 487-0011, Japan
e-mail: s-kiyoshi@mvc.biglobe.ne.jp

K. Shibata · S. Suzuki · S. Tokudome
Department of Public Health, Nagoya City University
Graduate School of Medical Sciences, Nagoya, Japan

J. Sato
Department of General Medicine,
Nagoya University Hospital, Nagoya, Japan

I. Ohsawa
Department of Health Science,
Aichi Gakuin University, Nisshin, Aichi, Japan

S. Goto
Department of Medical Laboratory,
Kasugai Municipal Hospital, Kasugai, Japan

S. Tokudome
National Institute of Health and Nutrition, Tokyo, Japan

Introduction

The prevention of metabolic syndrome (MetS), for which visceral fat accumulation and insulin resistance are considered upstream factors, has recently attracted the attention of the medical world as a useful approach to protect against lifestyle-related diseases typified by arteriosclerotic diseases [1–8]. Visceral fat accumulates for many reasons, including hyperalimentation and inadequate exercise, among others, and causes the abnormal functioning of fat cells and excessive secretion of hormones that are involved in various pathological conditions [9, 10]. Excessive

secretion of these hormones is thought to act in combination with other factors to cause arteriosclerotic and other serious diseases, such as renal failure, blindness, lower limb amputation, cerebral apoplexy, cardiac arrest, and cerebrovascular diseases. The progression of conditions, from obesity into serious diseases, is sometimes referred as the metabolic domino effect [11, 12], and includes fatty liver disease.

Diagnostic criteria for MetS have been published by the World Health Organization [13], American National Cholesterol Education Programs, Adult Treatment Panel III (NCEP–ATP III) [14], and International Diabetes Federation (IDF) [15] for Asian countries, including Japan [16]. In Japan, the Examination Committee for Criteria of MetS introduced diagnostic criteria for Japanese metabolic syndrome (JMetS) [16], which are similar to the ones defined by IDF. The criteria essentially include central obesity and several other components, such as hypertension, hyperglycemia, and abnormal lipid metabolism. In Japan, the most prominent difference between the IDF and Examination Committee criteria for evaluating central obesity is in the cut-off point (COP) for waist circumference (WC), especially that for women: in all countries of the world, with the exception of Japan, the COP for WC is larger for men than that for women.

The relative newness of the MetS concept necessitates that the diagnostic criteria be updated as and when needed. The association between the diagnosis of MetS and downstream diseases in the metabolic domino needs to be addressed in prospective studies. In the study reported here, we applied several criteria to examine the association between metabolic status and concurrent fatty liver, which we used as a specific example of a disease in the metabolic domino. Our aim was to identify preliminary criteria and COPs for WC that can be used in diagnosing MetS.

Subjects and methods

Height, weight, and WC were measured, and abdominal ultrasonography was performed in 2,333 subjects (1,195 men and 1,138 women) of 2,428 subjects aged 40–79 years. These subjects underwent comprehensive medical examinations at the Kasugai City Medical Center during a 3-month period between April and July 2006. Patients receiving drug treatment(s) for liver diseases, hypertension, diabetes mellitus, or hyperlipidemia were excluded from the study. Height and weight were measured using an automatic scale (Tanita BF-220). The WC was measured in standing subjects with a tape measure placed horizontally at the level of the navel while the subject was gently exhaling. If the abdomen was protuberant and the navel was deviated downwards, the tape measure was placed at the midpoint level between the lower intercostal border and the anterior superior iliac spine.

Fatty liver was diagnosed after discussion with medical technologists (including ultrasound technicians), radiology technologists, and physicians and by taking fatty liver scores (as shown in Table 1) obtained at Kasugai City Medical Center into consideration. These scores were based on previous studies [17–20].

Blood pressure was measured on the right arm using a mercury sphygmomanometer; the subject was in a lying position and had rested for at least 5 min prior to the measurement. Venous blood samples were collected in the morning from subjects after a fasting period of 12 h. Triglyceride (TG) and serum high-density lipoprotein cholesterol (HDL-C) were measured by the direct enzymatic method, and fasting plasma glucose (FPG) was measured by the glucose oxidase method. Their concentrations were measured using an automated analyzer (model 7170S; Hitachi, Japan).

Current JMetS criteria require a central obesity (visceral adipose tissue area $\geq$100 cm^2 or WC $\geq$85 cm for men and $\geq$90 cm for women) and two or more of the following three components: (1) high blood pressure, based on a systolic blood pressure $\geq$130 mmHg and/or diastolic blood pressure $\geq$85 mmHg; (2) hyperglycemia, based on FPG $\geq$110 mg/dl; (3) abnormal lipid metabolism, based on TG $\geq$150 mg/dl and/or HDL-C <40 mg/dl [16]. The Examination Committee for Criteria of MetS in Japan also defined a "risk group for MetS" (yobi-gun) consisting of people who have central obesity and one of the three components listed above (high blood pressure, hyperglycemia, or abnormal lipid metabolism). In our study, as in most epidemiological studies, only WC was considered in our evaluation of central obesity; the visceral adipose tissue area was not assessed.

Our primary aim was to identify and propose new MetS criteria based on our results. Our suggested criteria (our criterion 1) considers central obesity not to be an essential requirement for MetS but as only one of the components of MetS. Accordingly, we defined our patients as having MetS when they demonstrated three or more components of

Table 1 Fatty liver score

Condition	Points
Bright echo pattern	0 or 1
Hepatorenal or hepatosplenic contrast	0 or 1 or 2
Unclear vessels	0 or 1
Deep attenuation	0 or 1 or 2
Fatty bandless sign	0 or 1
Liver swelling	0 or 1

A total score of $\geq$3 points is considered to indicate fatty liver

MetS, regardless of their central obesity status. Similarly, the risk group for MetS consisted of those individuals who demonstrated two components.

Taking the number of MetS components listed above in consideration, we first calculated the odds ratios of fatty liver according to central obesity status in men and women by logistic regression. We then constructed receiver operating characteristic (ROC) curves to assess the detecting power of MetS criteria for concurrent fatty liver and calculated the areas under the curve (AUC) for diagnostic criteria. These procedures were repeated using the IDF COP for WC in the Japanese population, i.e., ≥90 cm for men and ≥80 cm for women (our criterion 2). We also calculated the COP for the largest AUC and suggested an optimal COP for men and women based on the study results. Statistical analyses were performed using the SAS system for Windows (release 9.1.3; SAS Institute, Cary, NC), and the AUC value was obtained to refer to the c statistic in PROC LOGISTIC output. All statistical tests were two-sided, and a P value <0.05 was considered to be significant. The study was approved by the ethics committee of Nagoya City University.

Results

Table 2 shows the number of subjects diagnosed with MetS according to the JMetS criteria and our newly proposed criteria, respectively. This diagnosis was based on the number of MetS components, other than central obesity, calculated by WC status in both men and women. Only 8.4% of the women satisfied the central obesity criterion of JMetS, whereas 26.7% men satisfied the criterion. When the COP for central obesity was changed to ≥80 cm, 36.6% of women satisfied the criterion. Among the 13 men and six women who were newly diagnosed with MetS based on our criteria using the same WC COP, seven men (53.8%) and five women (83.3%) had fatty liver. The

Table 2 Criteria of metabolic syndrome and number of subjects

Number of components[a]	Criteria of JMetS	Our criteria	Number of patients diagnosed with MetS	Criteria of JMetS	Our criteria	Number of patients diagnosed with MetS
Men						
	Waist circumference <85 cm			*Waist circumference ≥85 cm*		
0	Normal	Normal	391 (32.7%)	Normal	Normal	93 (7.8%)
1	Normal	Normal	357 (29.9%)	Risk MetS	Risk MetS	152 (12.7%)
2	Normal	Risk MetS	115 (9.6%)	MetS	MetS	61 (5.1%)
3	Normal	MetS	13 (1.1%)	MetS	MetS	13 (1.1%)
Total			876 (73.3%)			319 (26.7%)
	Waist circumference <90 cm			*Waist circumference ≥90 cm*		
0	–	Normal	453 (37.9%)	–	Normal	31 (2.6%)
1	–	Normal	457 (38.2%)	–	Risk MetS	52 (4.4%)
2	–	Risk MetS	151 (12.6%)	–	MetS	25 (2.1%)
3	–	MetS	20 (1.7%)	–	MetS	6 (0.5%)
Total			1,081 (90.5%)			114 (9.5%)
Women						
	Waist circumference <90 cm			*Waist circumference ≥90 cm*		
0	Normal	Normal	603 (53.0%)	Normal	Normal	28 (2.5%)
1	Normal	Normal	357 (31.4%)	Risk MetS	Risk MetS	45 (4.0%)
2	Normal	Risk MetS	76 (6.7%)	MetS	MetS	18 (1.6%)
3	Normal	MetS	6 (0.5%)	MetS	MetS	5 (0.4%)
Total			1,042 (91.6%)			96 (8.4%)
	Waist circumference <80 cm			*Waist circumference ≥80 cm*		
0	–	Normal	458 (40.2%)	–	Normal	173 (15.2%)
1	–	Normal	211 (18.5%)	–	Risk MetS	191 (16.8%)
2	–	Risk MetS	49 (4.3%)	–	MetS	45 (4.0%)
3	–	MetS	4 (0.4%)	–	MetS	7 (0.6%)
Total			722 (63.4%)			416 (36.6%)

JMetS Japanese metabolic syndrome, *Risk MetS* individuals with central obesity and one of three components (high blood pressure, hyperglycemia, or abnormal lipid metabolism), as defined by the Examination Committee for Criteria of MetS in Japan, *MetS* individuals with MetS

[a] Number of the components of MetS other than abdominal obesity

prevalence of fatty liver was much higher than the total prevalence of fatty liver in men and women, i.e., 27.1 and 16.5%, respectively.

Table 3 shows the characteristics of the subjects diagnosed with MetS based on the application of several criteria. The prevalence of MetS using the JMetS criteria was 6.2% in men and 2.0% in women; based on our criteria using the JMetS COP for central obesity, MetS prevalence was 7.3 and 2.5%, respectively. When we applied the criterion for ≥80 cm COP for central obesity in women using our criteria, the prevalence of fatty liver increased to 4.9%. Similarly, the application of the COP increased the prevalence among the MetS risk group to 21.1%, which was close to that observed in men according to our criteria which include the ≥85 cm COP for central obesity. Since central obesity is an essential criterion for determining JMetS or the JMetS risk group, the subjects in these categories are much more obese than those falling in the normal category. The difference in WC and BMI between subjects in the MetS group and the normal group was 12.1 cm and 3.5 kg/m^2, respectively, in men and 17.6 cm and 5.7 kg/m^2 in women. When our criteria were used, these differences decreased to 10.4 cm and 3.0 kg/m^2, respectively, in men and 14.5 cm and 5.0 kg/m^2 in women.

Table 3 Characteristics of the subjects by MetS status

Characteristics	Men			Women		
	Normal	Risk MetS	MetS	Normal	Risk MetS	MetS
Criteria of JMetS (cut-off of WC)	**(85 cm)**			**(90 cm)**		
Number (row%)	969 (81.1%)	152 (12.7%)	74 (6.2%)	1,070 (94.0%)	45 (4.0%)	23 (2.0%)
Fatty liver prevalence (%)	20.6%	46.1%	73.0%	14.5%	40.0%	65.2%
Age (years)	63.0 ± 8.8	63.3 ± 8.4	63.4 ± 7.9	61.6 ± 8.0	65.8 ± 8.1	64.4 ± 6.7
BMI (kg/m^2)	22.3 ± 2.4	25.8 ± 2.4	25.8 ± 2.5	21.7 ± 2.6	27.2 ± 3.4	27.4 ± 3.1
WC (cm)	77.8 ± 6.4	89.6 ± 5.3	89.9 ± 4.9	76.5 ± 7.5	95.0 ± 5.1	94.1 ± 3.7
Systolic blood pressure (mmHg)	122.6 ± 15.1	126.5 ± 16.0	136.2 ± 12.4	122.2 ± 17.0	132.0 ± 13.8	142.3 ± 14.7
Diastolic blood pressure (mmHg)	71.9 ± 8.6	75.0 ± 8.9	79.9 ± 8.2	70.5 ± 9.3	74.6 ± 8.3	79.3 ± 7.0
Triglycerides (mg/dl)	114.3 ± 70.8	142.7 ± 71.6	196.2 ± 150.0	97.5 ± 49.8	120.7 ± 52.7	204.5 ± 101.1
HDL-cholesterol (mg/dl)	62.1 ± 16.4	53.3 ± 12.6	51.9 ± 14.8	72.2 ± 17.1	64.7 ± 14.3	54.1 ± 13.6
Fasting glucose (mg/dl)	96.0 ± 17.2	100.0 ± 18.1	122.9 ± 48.1	92.4 ± 15.8	95.1 ± 13.5	117.3 ± 30.9
Our criteria 1 (cut-off of WC)	**(85 cm)**			**(90 cm)**		
Number (row%)	841 (70.4%)	267 (22.3%)	87 (7.3%)	988 (86.8%)	121 (10.6%)	29 (2.5%)
Fatty liver prevalence (%)	17.6%	43.1%	70.1%	12.7%	35.5%	69.0%
Age (years)	62.7 ± 8.9	63.9 ± 8.1	64.2 ± 8.0	61.3 ± 8.0	64.7 ± 7.9	64.8 ± 6.8
BMI (kg/m^2)	22.2 ± 2.5	24.5 ± 2.7	25.2 ± 2.6	21.7 ± 2.6	24.0 ± 3.7	26.7 ± 3.2
WC (cm)	77.6 ± 6.6	84.9 ± 7.2	88.0 ± 6.7	76.4 ± 7.6	84.3 ± 10.1	90.9 ± 7.6
Systolic blood pressure (mmHg)	120.5 ± 14.1	130.8 ± 15.9	137.1 ± 11.9	120.7 ± 16.3	137.2 ± 14.0	143.9 ± 14.4
Diastolic blood pressure (mmHg)	71.0 ± 8.3	76.1 ± 9.0	79.4 ± 8.0	69.9 ± 9.1	76.8 ± 8.7	79.9 ± 7.5
Triglycerides (mg/dl)	103.8 ± 50.2	159.2 ± 103.1	197.4 ± 140.9	91.2 ± 40.2	152.1 ± 76.4	207.7 ± 92.4
HDL-cholesterol (mg/dl)	63.2 ± 16.0	54.4 ± 15.0	51.3 ± 14.8	73.0 ± 16.8	63.3 ± 16.7	55.2 ± 14.0
Fasting glucose (mg/dl)	93.2 ± 12.2	105.3 ± 24.5	124.5 ± 45.0	91.2 ± 13.9	100.3 ± 21.6	121.9 ± 33.2
Our criteria 2 (cut-off of WC)	**(90 cm)**			**(80 cm)**		
Number (row%)	941 (78.7%)	203 (17.0%)	51 (4.3%)	842 (74.0%)	240 (21.1%)	56 (4.9%)
Fatty liver prevalence (%)	19.6%	50.2%	74.5%	10.3%	27.9%	60.7%
Age (years)	62.8 ± 8.8	64.0 ± 8.2	63.9 ± 7.9	60.6 ± 8.0	64.9 ± 7.2	64.6 ± 7.7
BMI (kg/m^2)	22.5 ± 2.5	24.3 ± 3.0	25.6 ± 3.3	21.3 ± 2.5	23.9 ± 3.1	25.4 ± 2.9
WC (cm)	78.6 ± 6.9	84.3 ± 8.2	88.8 ± 8.6	75.0 ± 7.4	84.3 ± 7.4	87.8 ± 6.6
Systolic blood pressure (mmHg)	121.2 ± 14.4	133.4 ± 15.5	138.2 ± 11.5	118.1 ± 14.9	135.5 ± 15.2	143.1 ± 14.8
Diastolic blood pressure (mmHg)	71.5 ± 8.4	76.9 ± 9.1	80.2 ± 7.9	68.8 ± 8.6	76.1 ± 8.8	79.8 ± 8.0
Triglycerides (mg/dl)	107.6 ± 53.0	172.0 ± 112.7	211.2 ± 172.3	87.0 ± 36.5	126.3 ± 56.4	195.5 ± 102.1
HDL-cholesterol (mg/dl)	62.2 ± 15.9	54.7 ± 16.1	50.1 ± 14.9	74.6 ± 16.6	64.2 ± 15.7	56.4 ± 13.3
Fasting glucose (mg/dl)	93.9 ± 13.4	109.7 ± 28.2	131.1 ± 49.9	90.8 ± 14.4	95.4 ± 14.7	115.5 ± 30.7

Data are given as the mean ± standard deviation (SD)

WC Waist circumference, *BMI* body mass index, *HDL* high-density lipoprotein

When the COP of ≥80 cm was applied, the differences decreased to 12.8 cm and 4.1 kg/m^2, respectively.

Table 4 shows the odds ratios and 95% confidence interval (CI) for fatty liver according to the number of MetS components other than central obesity by WC status. Regardless of sex and the WC COP selected, a strong linear trend was observed for the association (trend $P < 0.0001$) with the number of components. The odds ratio for subjects without central obesity and with all three components of MetS was 9.69 (95% CI 3.1130.2) in men and 55.3 (6.34–483) in women. Using the ≥90 and ≥80 cm COP criterion for central obesity in men and women, respectively, the odds ratio was 55.3 (6.34–483) and 62.4 (6.23–626). These point estimates of odds ratios were higher than those of MetS subjects with two risk factors other than obesity among women, and even among men, they were higher than those of the risk group for MetS who satisfied the central obesity criterion.

Figure 1 shows the ROC curves for the diagnosis of fatty liver according to MetS status by the JMetS criteria and by our criteria. The AUC for the JMetS criteria and our criteria 1 and 2 in men was 0.638, 0.681, and 0.655, respectively. In women, the AUC for our criteria using ≥90 and ≥80 cm COPs for central obesity were 0.625 and 0.681, respectively, whereas that for the JMetS criteria was only 0.570. Based on the findings of our study, the largest AUC was recorded using our criterion 1 (≥85 cm) in men and our criteria 2 in women (≥80 cm). The shapes of the ROC curves of our criterion 2 for men and our criterion 1 for women were very similar, with the coordinates (false positive rate, true positive rate) for MetS and the risk group for MetS being (0.030, 0.188) and (0.204, 0.543), respectively, for men and (0.023, 0.181) and (0.205, 0.537), respectively for women. In addition, when WC was considered as a component, the COP for the largest AUC among men and women was ≥82 cm (0.701) and ≥77 cm (0.699), respectively. We therefore conclude that it would be both practical and appropriate to take WC into consideration, with WC COPs of ≥85 cm for men and ≥80 cm for women. In our study population, 26.7% of the men and 36.6% the women satisfied the criteria.

Table 4 Odds ratio and 95% confidence interval for fatty liver according to the number of the components of MetS other than obesity by waist circumstance status

Number of the components[a]	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval
Men				
	Waist circumference <85 cm		*Waist circumference ≥85 cm*	
0	1.00	Reference	5.49	3.25–9.27
1	1.99	1.32–3.01	7.09	4.51–11.1
2	5.34	3.26–8.74	18.4	9.78–34.4
3	9.69	3.11–30.2	99.7	12.6–786
P for trend	<0.0001		<0.0001	
	Waist circumference <90 cm		*Waist circumference ≥90 cm*	
0	1.00	Reference	7.66	3.59–16.32
1	1.88	1.33–2.66	11.91	6.34–22.39
2	5.17	3.40–7.85	19.96	7.67–51.93
3	14.71	5.45–39.72	31.53	3.62–274.34
P for trend	<0.0001		<0.0001	
Women				
	Waist circumference <90 cm		*Waist circumference ≥90 cm*	
0	1.00	Reference	9.59	4.32–21.3
1	2.32	1.56–3.46	7.37	3.80–14.3
2	5.42	3.10–9.48	17.4	6.45–46.8
3	55.3	6.34–483	44.2	4.85–403
P for trend	<0.0001		<0.0001	
	Waist circumference <80 cm		*Waist circumference ≥80 cm*	
0	1.00	Reference	6.67	3.82–11.7
1	2.67	1.45–4.92	8.63	5.04–14.8
2	6.02	2.70–13.4	26.0	12.5–54.1
3	62.4	6.23–626	125	14.4–1084
P for trend	<0.0001		<0.0001	

[a] Number of the components of metabolic syndrome other than abdominal obesity

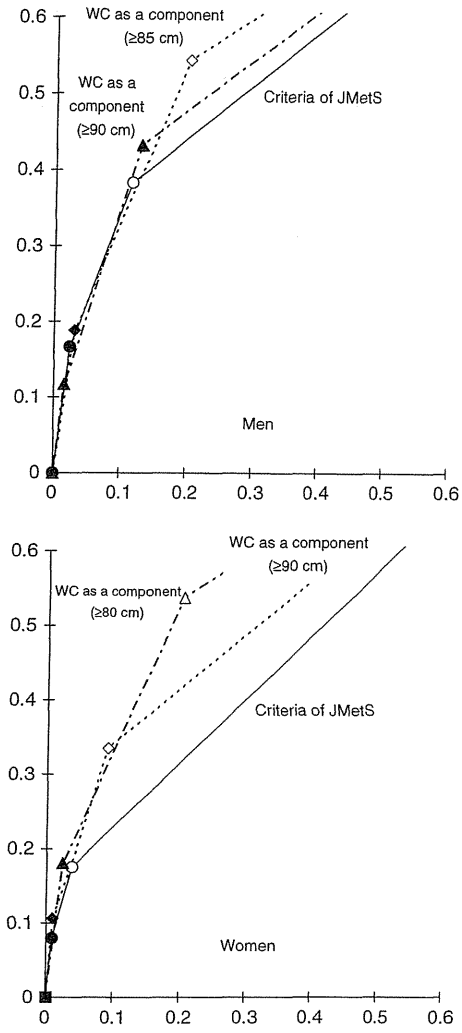

Fig. 1 Receiver operating characteristic curves for fatty liver diagnosis by metabolic syndrome status of several criteria. *JMetS* Japanese metabolic syndrom, *WC* waist circumference

Discussion

In the present study, we considered concurrent fatty liver to be a specific example of a disease in the metabolic domino of MetS and observed that the accumulation of MetS components was associated with higher odds ratios, even without the central obesity component. Taking these results as a whole, we observed stronger associations between MetS and fatty liver in men and women when we considered central obesity as a component rather than an essential requirement for the diagnosis of MetS. We therefore suggest that individuals with an accumulation of components should be regarded as having MetS even in the absence of central obesity, since fatty liver is a component of the metabolic domino. In addition, these individuals may belong to a risk group for other metabolic diseases, including cardiac arrest and cerebrovascular diseases. We also suggest that the optimal COP for WC should be ≥85 cm for men and ≥80 cm for women.

Although the main concepts of MetS are consistent, the COPs for defining central obesity for MetS are controversial, especially in Japan [21]. Several studies have been performed to elucidate the optimal COPs in which ROC analyses with obesity and two or more MetS components other than obesity [22–25] were used. The results suggested that the optimal cut-offs for men and women are 84–90 and 78–82 cm, respectively. Our results are consist with these reported values. However, these earlier studies were based on the internal consistency of obesity and MetS components other than obesity. Further ROC analyses need to be performed to establish the optimal COP for WC, and these should include certain diseases not currently included in MetS. This study is one such analysis.

An important question is whether central obesity should be considered as a requirement for the diagnosis of MetS or as a component of MetS. To answer this question, we need to examine the association between the number of MetS components and particular diseases stratified by central obesity. To date, there have been only two prospective cohort studies [26, 27] from Japan on cardiovascular diseases. Results from NIPPON DATA [26] show the existence of risk accumulation among non-obese subjects, whereas those from Hisayama-cho [27] indicate there is no risk accumulation in such subjects. Data from many studies, including those from our study, are required to facilitate further discussion on this question. However, before the absence of risk accumulation can be established among non-obese individuals, it is possible to treat central obesity as a component of MetS as a precautionary measure.

In general, if a factor is considered to be an essential requirement for the diagnosis of a certain disease, then that factor should not only be etiologically essential but also amenable to accurate measurement in practice; at the very least, the COP should be a sensitive measure. Otherwise, a considerable number of cases would not be detected by the criterion. In fact, the COPs based on the IDF criteria (≥94 cm for men and ≥80 cm for women), with central obesity as a requirement, are more sensitive than those of the NCEP-ATP III criteria (≥102 cm for men and ≥88 cm for women), wherein central obesity is considered a component. Although the JMetS definition is similar to the IDF definition, the JMetS COP for WC in women (≥90 cm) is much less sensitive than the COP of the IDF (≥80 cm). The COP for central obesity for the diagnosis of JMetS is based on the association between visceral fat area and WC [16]. The committee reported that simple correlation analysis of the regression line in women indicated that a WC corresponding to 100 cm^2 of visceral fat was 92.5 cm. However, the correlation coefficient was only 0.65, and more than half of the women with a visceral fat area

$\geq$100 cm^2 would not be found using the WC COP of $\geq$90 cm (meaning that sensitivity is <0.5). The poor sensitivity of the WC in detecting abdominal adiposity is directly linked to the poor sensitivity of the JMetS criteria, in which WC is an essential requirement.

Conclusion

Based on the findings of our study, we suggest that a WC of $\geq$85 cm for men and $\geq$80 cm for women would be optimal COPs for central obesity for the diagnosis of MetS in the Japanese population. We also suggest that central obesity should be used as a component of MetS rather than an essential requirement for the diagnosis of MetS. No definite conclusion has yet been reached regarding the most appropriate diagnostic criteria for MetS. However, within the framework of our study in which fatty liver was considered to be an independent variable, we found that defining abdominal circumference as a component of MetS was less likely to cause errors of oversight and was thus more appropriate than considering abdominal circumference to be a required criterion. The challenge for the future is to identify pathologic conditions that are responsible for MetS and to find better diagnostic criteria through further similar studies that consider factors, other than fatty liver, involved in the metabolic domino effect [11, 12] as independent variables.

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HEPATOLOGY

Fatty liver predicts impaired fasting glucose and type 2 diabetes mellitus in Japanese undergoing a health checkup

Tamaki Yamada,* Mitsuru Fukatsu,* Sadao Suzuki,† Tsuneya Wada,* Takashi Yoshida* and Takashi Joh‡

*Okazaki City Medical Association, Public Health Center, Okazaki, Departments of †Public Health and ‡Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan

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Correspondence
Dr Tamaki Yamada, Okazaki City Medical Association, Public Health Center, 1-9-1 Tatsumi-nishi, Okazaki, Aichi 444-0875, Japan.
Email: t-yamada@okazaki-med.or.jp

Abstract

Background and Aim: The question of whether fatty liver might predict impaired fasting glucose or type 2 diabetes mellitus in a longitudinal manner was assessed in Japanese subjects undergoing a health checkup.

Methods: A total of 12 375 individuals (6799 men and 5576 women) without hyperglycemia or type 2 diabetes mellitus in 2000 and participating in 2005 were included. Multiple logistic regression analyses were performed for both sexes, adjusted for age, body mass index, elevated blood pressure or hypertension, family history of diabetes mellitus, alcohol drinking and smoking.

Results: Impaired fasting glucose and type 2 diabetes mellitus were newly diagnosed in 7.6% and 1.0% of men and 3.8% and 0.5% of women, respectively, within the 5-year period. The prevalence of newly diagnosed impaired fasting glucose and type 2 diabetes mellitus was significantly higher in the participants with fatty liver than without fatty liver in both sexes. Fatty liver adjusted for the other factors was thus a risk factor for impaired fasting glucose and/or type 2 diabetes mellitus in both sexes (men odds ratio [OR] 1.91, 95% confidence interval [CI] 1.56–2.34 and women OR 2.15, 95% CI 1.53–3.01). The impact of fatty liver was stronger among the participants with a lower body mass index (men OR 0.92, 95% CI 0.86–0.99 and women OR 0.90, 95% CI 0.81–0.99, for one increment of body mass index).

Conclusion: Fatty liver is an independent risk factor for impaired fasting glucose and type 2 diabetes mellitus, having a stronger impact in those Japanese with a lower body mass index undergoing a health checkup.

Introduction

Accumulation of triglycerides in hepatocytes is increasing due to consumption of a high-fat and high-calorie diet and a sedentary lifestyle and the prevalence of fatty liver is now 20–30% in Japan and other countries.[1–7] Fatty liver is asymptomatic and the most common condition assessed by ultrasonography at health checkups.[2,4,7,8] In particular, non-alcoholic fatty liver disease (NAFLD) is considered a hepatic consequence of the metabolic syndrome, closely associated with insulin resistance.[7–11]

It is widely accepted that impaired fasting glucose (IFG), elevated systolic blood pressure, a high body mass index (BMI), a family history of diabetes mellitus (DM), and adiposity and visceral fat distribution are risk factors for type 2 diabetes mellitus (T2DM).[12–14] In addition, markers of liver injury may be associated with the metabolic syndrome and be independent predictors of T2DM.[15–19] Thus, elevation of liver enzymes caused by fatty liver appears associated with insulin resistance.[12,16,17,20] Although one study of Japanese men demonstrated that fatty liver assessed by ultrasonography was not a risk factor for T2DM,[1] the majority of investigations have revealed a link between NAFLD and impaired glucose metabolism as well as diabetes.[2,3,21,22] Recently, it was also reported that fatty liver was an independent risk factor for T2DM in participants including alcohol drinkers at a health checkup in Korea.[4] Although it thus appears likely that fatty liver is a risk factor for T2DM, one study was performed in a cross-sectional manner[2] and the others featured only small numbers of participants, only men or analysis of men and women together.[3,4,21] Because sex and weight status may modify the relationship between metabolic risk factors and NAFLD,[23] the sexes should be treated separately. Furthermore, because insulin resistance and hyperinsulinemia may be closely associated with NAFLD in the subjects with normal bodyweight and that non-obese subjects with NAFLD are prone to cardiovascular disease,[24–26] it is important to determine the interaction between fatty liver and BMI regarding the risk of IFG and/or T2DM.

The metabolic syndrome is characterized by visceral adiposity (large waist circumference), dyslipidemia, hypertension, and IFG ($\geq$ 110 mg). IFG itself is independently associated with cardiovascular risk factors such as hypertension and dyslipidemia as well as coronary artery calcification, subclinical atherosclerosis.[27,28] There is also an independent link with T2DM.[14,27,29] Therefore, it may be more beneficial to predict IFG, a prediabetic status, rather than T2DM itself in consideration of preventive measures against cardiovascular disease.

Therefore, in the present longitudinal investigation we assessed risk factors including fatty liver assessed by ultrasonography in 2000 for IFG or T2DM in both sexes of Japanese subjects undergoing a health checkup. Adjustment was made for age, BMI, elevated blood pressure or hypertension, family history of DM, alcohol drinking and smoking. A particular focus was on the relationship between fatty liver and BMI.

Methods

Design of the study

This study included retrospective longitudinal analyses to investigate whether fatty liver, assessed by ultrasonography, is associated with IFG or T2DM in apparently healthy Japanese subjects undergoing a health checkup. Informed consent was obtained from all participants.

Subjects of the study

The numbers of participants undergoing medical checkups, including ultrasonography in 2000 and 2005 were 26 247 (14 627 men and 11 620 women) and 32 548 (17 207 men and 15 341 women), respectively. A total of 14 617 (8377 men and 6240 women) underwent health checkups at both time-points. After exclusion of participants who had past and present illness of DM (551) and hepatic diseases (632), positive results for hepatitis viruses (159), fasting hyperglycemia in 2000 (1505), a total of 12 375 participants (men 6799, 49.2 $\pm$ 10.5 years old and women 5576, 50.6 $\pm$ 9.3 years old) were included.

Questionnaire

Subjects provided data for family history of DM, alcohol drinking habits and smoking status through a self-administered questionnaire which was checked during individual interview by expert nurses in the center. Alcohol drinking habits were classified into occasional and daily. Family history of DM was defined if a parent had either a past history or present illness.

Measurements

Age was categorized into four categories. Bodyweight was measured, in light clothing, to the nearest 0.1 kg and height to the nearest 0.1 cm. BMI was calculated as kg/m^2 and divided into three categories according to the criteria determined by the Japan Society for the Study of Obesity.

Blood samples were taken from each participant after overnight fasting. Fasting blood glucose (FBG) was measured with Hitachi autoanalyzer models 7600 and 7700 (Hitachi Medical, Tokyo, Japan) and the presence of IFG or T2DM was defined as values between 110 and 125 mg/dL and 126 mg/dL or more, respectively.

Blood pressure was measured to the nearest 1 mmHg by an automatic sphygmomanometry (BP-203 RV III B; Nippon COLIN, Komaki, Japan). Elevated blood pressure or hypertension was diagnosed if resting blood pressures were 130/85 mmHg or more or if the participants had either a history of hypertension or use of antihypertensive medication, respectively.

Abdominal ultrasonographic examination was performed using convex-type real-time electronic scanners (SSA 250 and 300; Toshiba Medical, Tokyo, Japan) by 10 technicians without any information about any present illness. All images were printed on the sonographic papers and reviewed by other technicians and physicians. Fatty liver was assessed according to the modified criteria reported previously.[30–33] Liver brightness (diagnosed by difference of more than 10 from the average of liver and renal cortical echo amplitudes), attenuation of echo penetration and decreased visualization of veins were included as criteria.

Statistical analyses

Logistic regression analyses were, respectively, performed to determine the risk of IFG or T2DM in both men and women separately. We evaluated two models in both sexes; an age-adjusted and a multivariate model with adjustment for age (< 40, 40–49, 50–59 and $\geq$ 60 years), BMI (< 25 kg/m^2, 25–29.9 kg/m^2 and $\geq$ 30 kg/m^2), alcohol drinking (none, occasional, daily or unknown), smoking (never, ever or unknown), family history of DM (yes, no or unknown) and fatty liver (yes or no) which were assessed in 2000.

We also determined the interaction between fatty liver and BMI in a separate study. BMI was incorporated into the models as a continuous variable. In order to simplify interpretation, BMI was transformed by subtracting 22 (centerization). Statistical differences among groups were identified using one-way ANOVA, followed by multiple comparisons using Bonferroni's method. The χ^2-test and Fisher's test were employed for comparison of prevalence of fatty liver, IFG, and T2DM. Logistic regression analyses were performed using computer software (SPSS ver. 13.0 for Windows; SPSS, Chicago, IL, USA). *P*-values less than 0.05 were considered significant.

Results

Incidences of newly diagnosed IFG and T2DM between 2000 and 2005 were, respectively, 5.9% and 0.8% overall (7.6% and 1.0% in men and 3.8% and 0.5% in women). They were 10.6% and 2.9% in men with fatty liver, and 5.2% and 0.6% in men without fatty liver. For women, the respective figures were 9.4% and 2.0% with fatty liver, and 2.6% and 0.4% without fatty liver. In both sexes, the differences were significant. The 78.0% of male and 71.3% of female participants with fatty liver in 2000 were assessed as fatty liver in 2005.

Table 1 shows the characteristics of the subjects by fatty liver status in men and women. BMI, systolic and diastolic pressures, and FBG were significantly elevated in the participants with fatty liver than those without fatty liver in both sexes. The prevalence of family history of T2DM was also significant.

Table 1 Clinical characteristics of the participants with and without fatty liver in 2000

	Overall	No fatty liver	Fatty liver
Men			
Age	49.2 ± 10.5	49.5 ± 10.7	48.1 ± 9.6*
BMI (kg/m^2)	22.9 ± 2.8	22.4 ± 2.5	25.3 ± 2.7*
Systolic blood pressure (mmHg)	118.2 ± 16.7	117.0 ± 16.6	122.8 ± 16.3*
Diastolic blood pressure (mmHg)	74.1 ± 10.9	73.4 ± 10.9	77.1 ± 10.6*
FBG (mg/dL)	95.1 ± 6.9	94.6 ± 6.9	97.1 ± 6.4*
Family history of diabetes mellitus	12.5	12.0	14.7*
Smoker (%)	45.7	47.2	40.1
Drinker (%)			
Occasional	33.6	31.7	41.4
Daily	45.9	48.5	35.5
Women			
Age	50.6 ± 9.3	50.3 ± 9.3	53.7 ± 8.8*
BMI (kg/m^2)	22.1 ± 2.8	21.8 ± 2.6	25.2 ± 3.0*
Systolic blood pressure (mmHg)	115.3 ± 17.0	114.4 ± 16.7	124.5 ± 17.2*
Diastolic blood pressure (mmHg)	71.0 ± 10.8	70.5 ± 10.6	76.2 ± 11.1*
FBG (mg/dL)	91.6 ± 7.2	91.2 ± 7.0	95.5 ± 7.1*
Family history of diabetes mellitus	15.3	14.8	19.8*
Smoker (%)	7.1	7.3	5.5
Drinker (%)			
Occasional	29.3	29.8	24.4
Daily	8.2	8.5	4.9

*$P < 0.001$ compared to no fatty liver. BMI, body mass index; FBG, fasting blood glucose.

Table 2 Multiple logistic regression analysis for impaired fasting glucose (IFG) or type 2 diabetes mellitus (T2DM)

	Age adjusted OR†	95% CI	Multivariate OR‡	95% CI
Men				
No fatty liver	1.00	Reference	1.00	Reference
Fatty liver	2.34	1.95–2.81	1.91	1.56–2.34
Women				
No fatty liver	1.00	Reference	1.00	Reference
Fatty liver	3.45	2.54–4.67	2.15	1.53–3.01

†Adjusted by age for fatty liver. ‡Adjusted for age, body mass index (BMI), elevated blood pressure or hypertension, alcohol drinking, and smoking status for fatty liver. CI, confidence interval; OR, odds ratio.

Table 2 shows the age-adjusted and multivariate odds ratios with underlying fatty liver for IFG and T2DM. After adjustment for the potential confounders, fatty liver was a significant risk factor for IFG and T2DM in both men and women. The impact did not differ with the sex. The odds ratios (OR) were significantly larger among those with lower BMI. We thus found significant decrease of OR with fatty liver for IFG and T2DM, that is 0.92 (95% confidence interval [CI] 0.86–0.99) in men and 0.90 (95% CI 0.81–0.99) in women, for one increment of BMI.

Discussion

The present study demonstrated that fatty liver as assessed by ultrasonography is an independent risk factor for IFG and T2DM in Japanese subjects undergoing health checkups. The incidence of newly diagnosed IFG or T2DM over the 5-year period was significantly higher in the participants with fatty liver than without fatty liver in both sexes. In addition, a significant interaction between fatty liver and BMI was observed and risk was higher among the leaner participants.

It has been demonstrated that fasting hyperglycemia, systolic blood pressure, BMI, family history of DM and visceral adiposity are risk factors for T2DM.[12–14] Elevation of liver enzymes, including γ-glutamyltransferase and alanine aminotransferase is associated with the metabolic syndrome and is an independent predictor of T2DM.[15–18] In most cases, elevation is due to fatty liver.[12,16,17,20] Indeed, it has been shown that NAFLD is a risk factor for impaired glucose metabolism and T2DM,[2–4,21] as confirmed for both sexes in the present study. It is well established that obesity is a strong risk factor for T2DM and a link has been found with increased BMI even within non-obese levels.[34] Insulin resistance and hyperinsulinemia appear closely associated with NAFLD in the subjects with normal bodyweight[24–26] and there may be increased risk of cardiovascular diseases.[26,35] Indeed, we demonstrated herein that the impact of fatty liver on the risk factor of IFG or T2DM was stronger in leaner participants of both sexes. Taken together with the previous reports, we conclude that non-obese participants with fatty liver should be advised to make appropriate lifestyle changes.

The mechanisms by which fatty liver might lead to IFG or T2DM could not be elucidated in the present study. However, it is widely accepted that there is a close association with insulin resistance.[7–10,20] Hepatic lipid accumulation causes impaired insulin clearance and defects in insulin suppression of glucose production which results in increased fasting serum glucose.[11,13,36,37] On the other hand, it was demonstrated that percent

bodyfat was an independent predictor of fatty liver in non-alcoholic and non-overweight subjects,[38] suggesting that increased percent bodyfat may reflect central bodyfat distribution. Thus, we speculate that increased central bodyfat distribution may be related to the mechanism of a stronger impact of fatty liver on the leaner participants.

A major limitation of the present study was the retrospective longitudinal design. The subjects were limited to the Japanese participants undergoing voluntary health checkups at our center and might not necessarily be representative of the general population. Only 55.7% of the participants in 2000 received the health checkup in 2005. Although histological diagnosis would have been more accurate, liver biopsy is not an option at a health checkup. Therefore, we had to rely on ultrasonography for the purposes of the present study. However, this approach has been widely used as a non-invasive procedure with relatively high sensitivity and specificity for screening purposes[5–7,30] and the 23.3% in men and 9.8% in women found in the present study are consistent with values in the previous reports.[2,3,7,38] Finally, it is possible that misdiagnosis of IFG or T2DM have occurred in some cases because we had to rely on a single result of FBG for assessment.

In conclusion, fatty liver as assessed by ultrasonography may predict the development of IFG and T2DM in Japanese undergoing a health checkup, having strongest impact on those with a lower BMI. We propose that irrespective of BMI, the participants with fatty liver at health checkups should be advised to take action to reduce its risk factors to avoid possible development of diabetes. Cohort studies are now necessary to confirm the present findings.

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