

Intake of a Japanese-Style Healthy Lunch Has Possibilities of Contributing to the Normalization of Serum Lipids and Adipokines: A Non-Randomized Controlled Trial Pilot Study

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To cite this article:

Hiroko Inoue, Ryosuke Sasaki, Toshiko Kuwano. Intake of a Japanese-Style Healthy Lunch Has Possibilities of Contributing to the Normalization of Serum Lipids and Adipokines: A Non-Randomized Controlled Trial Pilot Study. *International Journal of Nutrition and Food Sciences*. Vol. 8, No. 1, 2019, pp. 1-9. doi: 10.11648/j.ijnfs.20190801.11

Received: December 17, 2018; **Accepted:** January 10, 2019; **Published:** January 31, 2019

Abstract: To date, the incidence of metabolic syndrome has been increasing continuously worldwide; and recently, low vegetable consumption has become a major issue of concern in Japan. Furthermore, Japan is facing a shortfall in places offering food that can help prevent metabolic syndrome. Our study was designed to determine the influence of an on-going healthy lunch on metabolic syndrome outcomes (blood pressure, serum TNF- α , IL-6, serum lipids) in adult middle-aged office male workers, in Japan. We conducted a non-randomized controlled trial among 38 middle-aged office male workers (control group: 7 males, intervention group: 31 males) with mostly low levels of physical activity, by providing a Japanese-style healthy lunch for 3 months (intervention group) at a workplace cafeteria. The control group consumed their habitual lunches without restriction and only the nutrient contents were assessed. Furthermore, the intervention group was divided into two (non-metabolic [non-MS] and metabolic syndrome [MS] groups) for analysis. Overall 38 males with a mean age of 47.2 ± 7.9 years were included. Abdominal circumference level ($p < 0.05$), systolic and diastolic ($p < 0.05$) blood pressure ($p < 0.01$), as well as serum low-density lipoprotein (LDL-Chol), serum tumor necrosis factor (TNF- α) ($p < 0.001$) and interleukin (IL-6) ($p < 0.01$) decreased significantly after intervention compared with the baseline in the intervention/non-MS group and intervention/MS groups. Furthermore, ghrelin ($p < 0.001$) and desacyl ghrelin ($p < 0.05$) increased significantly after intervention compared with the baseline in the intervention/non-MS group and intervention/MS groups. These showed an effective outcome, demonstrated by the ongoing intake of a Japanese-style healthy lunch, of decreased blood pressure, serum TNF- α , IL-6, serum LDL-Chol and total cholesterol. This study presents new empirical data based on an original intervention program showing that the consumption of a Japanese-style healthy lunch containing many vegetables can help prevent and/or improve metabolic syndrome. The findings of this study could also lead to the opportunity for participants to practice following healthy menus at home. We therefore consider that this is valuable in promoting improvement in diet in the food environment at the workplace.

Keywords: Healthy Lunch, Metabolic Syndrome, Adipokines

1. Introduction

Metabolic syndrome (MS), which is closely associated with abdominal obesity, type 2 diabetes, and cardiovascular disease (CVD), has become a global epidemic in developed nations, including Japan [1, 2]. MS is a cluster of metabolic abnormalities characterized by concurrent hyperglycemia,

hypertension, high triacylglycerol (TG) levels, low high-density lipoprotein cholesterol (HDL-Chol) levels, and inflammation [3, 4]. These major components are often associated with decreased insulin sensitivity, proinflammatory cytokine, pro-oxidant, and prothrombotic states, as well as low levels of cardiorespiratory fitness [4]. The National Health Promotion Campaigns for the 21st Century (Healthy

Japan 21), supported by the Japanese Ministry of Health, Labour and Welfare (2000) recommends that adults ingest ≥ 350 g of vegetables per day [5]. However, all age groups in Japan are falling short of this goal [6].

Exercise and diet are important factors for the prevention and improvement of MS [7-9]. From interventional study reports, Mediterranean diet is effective for the prevention and improvement of MS and abdominal obesity [10, 11]. The Mediterranean diet derives its staple food from grains, and meals are composed mainly of vegetables, fruits, beans, and other vegetable foods such as olive oil; as well as dairy products such as cheese, and seafood. Mediterranean diet is registered as the world intangible cultural asset, similar to the Japanese food [12, 13].

According to the latest cohort study in Japan, it was reported that a dietary pattern characterized by high intake of vegetables, fruits, large and small fish, natto, and deep-fried tofu was associated with reduced prevalence of MS in a Japanese population [13]. In Japan, the Ministry of Health, Labor and Welfare; and the Ministry of Agriculture, Forestry, and Fisheries of Japan jointly developed the Japanese Food Guide Spinning Top in 2005 [14]. Kurotani *et al.* reported that a closer adherence to Japanese dietary guidelines was associated with a lower risk of total mortality and mortality from cardiovascular disease, particularly from cerebrovascular disease, in Japanese adults [15]. Although there are many reports by cohort studies in Japan, there are very few intervention studies looking at the prevention/improvement of MS [16].

We conducted a non-randomized controlled trial by providing a Japanese-style healthy lunch to middle-aged men at a workplace cafeteria in order to determine the effect on metabolic syndrome outcomes (blood pressure, serum TNF- α , IL-6, serum lipids).

2. Methods

2.1. Participants

The study participants were male office workers in the S city hall, and none partook in daily exercise; they only engage in desk work and commute to work by train or by bus. Given the office-bound nature of their jobs, even mild activities such as walking in the course of work is minimal. The study participants consisted of a group including 95% college graduates with little differences in academic background and lifestyle factors.

2.2. Sample Selection

After explaining the study aim and procedure to the entire office, we enrolled sufficient participants prior to the commencement of the study. The recruitment period for the study participants was one month. We chose not to consider the use of medications for fat reduction or for carbohydrate metabolism as exclusion criteria. We excluded data from participants who left in the middle of the study ($n = 20$).

Finally, data from 38 males (mean age, 48.0 ± 8.1 years) were included in our analysis (Figure 1).

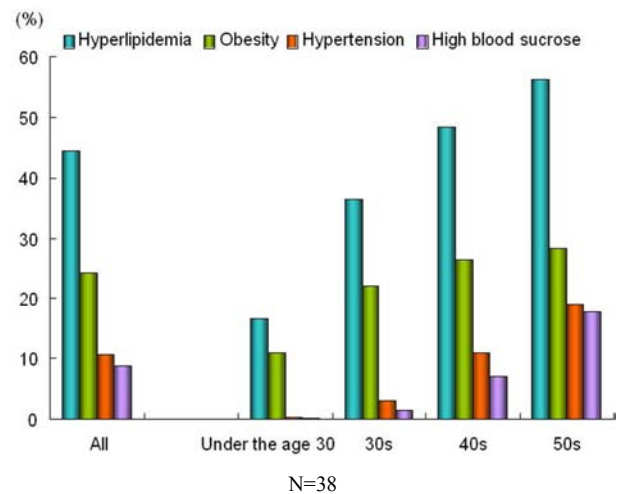


Figure 1. Lifestyle-related diseases classification among participants by age.

Emerald green bar, green bar, red bar, and purple color bar shows hyperlipidemia, obesity, hypertension, and high blood sucrose, respectively.

2.3. Ethics

The University of Shizuoka Review Board approved the study protocol, and all selected participants provided written informed consent for their participation (No. 20-5). In addition, this clinical study was registered in UMIN-CTR (Trial registration: UMIN000020171, Registered: 11 December 2015, https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000023304).

2.4. Study Design

This intervention study was a non-randomized controlled trial. Participants were able to self-select themselves into a control or intervention group. The intervention group received a Japanese-style healthy lunch at their workplace cafeteria for 3 months. The control group consumed their habitual lunches without restriction and only the nutrient contents were assessed. The weekend dietary habits of all participants were also unrestricted.

2.5. Concept of a Japanese-Style Healthy Lunch Menu

The energy and nutritional contents of the provided Japanese-style healthy lunch menu were based on the Guidelines for the Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases (Japan Atherosclerosis Society, 2007) [9]. The criteria for vegetable intake matched those recommended by Healthy Japan 21. We calculated the nutritive value of the healthy lunch using the standard body type described in the results of the 2006 National Health and Nutrition survey in Japan [6]. We designed the Japanese-style healthy lunch menu to provide balanced nutrition and sufficient vegetable consumption over

the course of three months (600 kcal \leq energy < 650 kcal, fat < 18 g, cholesterol \leq 100 mg, fiber \geq 8 g, total vegetables \geq 130 g). The menu, based on the calculated nutrients was prepared by the cook in the workers' cafeteria.

2.6. Nutritional Assessment

We assessed the participants before and after the study intervention (the consumption of Japanese healthy-style lunches for 3 months) in terms of the following items: anthropometric data, blood parameters, and dietary intake. All participants fasted after 8:00 p.m. on the day before the anthropometric measurements and the blood collection. Weight and body fat percentage (BFP) (%) were measured using a BODY FAT ANALYZER TBF-215 (Tanita Co. Ltd., Tokyo, Japan). Height and weight were measured while standing barefoot and wearing light clothing. Abdominal circumference, for all participants, was measured after exhalation at the position of the navel, by the same staff member. Blood pressure was measured using a Medinote HEM-5001 digital automatic sphygmomanometer (Omron Health Care Co. Ltd., Kyoto, Japan).

2.7. Blood Analysis

A nurse collected blood from fasting participants early in the morning. Blood sampling was done properly by a skilled nurse under the supervision of a doctor. Blood was placed in vacuum tubes containing EDTA-2Na and trasylol for the active ghrelin and desacyl ghrelin analyses, and into vacuum tubes containing heparin otherwise. Plasma was promptly collected by centrifugation at $1200 \times g$ for 15 min at 4°C for active ghrelin and desacyl ghrelin analyses, placed in microtubes, mixed with hydrochloric acid (10% v/v), and stored at -80°C. Plasma active ghrelin and desacyl ghrelin levels were measured using ELISA kits (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). We measured serum levels for T-Chol, LDL-Chol, HDL-Chol, triacylglycerol (TG), glucose, HbA1C and leptin. Serum was separated by centrifugation at $1200 \times g$ and 4°C for 15 min after blood collection. Serum T-Chol, LDL-Chol, HDL-Chol, TG, glucose, and HbA1C levels were measured at SRL Inc. (Tokyo, Shizuoka, Japan). Serum leptin levels were measured using Human Leptin Quantikine ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA). Serum tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) levels were measured using Quantikine TNF- α ELISA Kit and Human IL-6 Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN, USA).

2.8. Dietary Intake

Dietary intake was assessed using a 24-h dietary recall at baseline and after 3 months. We collected data regarding the daily total intake on the day before assessment. Participants were instructed to partake in a normal weekday diet and to avoid ceremonial occasions or events prior to the day of the diet survey. The diet was documented by RD for precision and consistency. The participants responded to questions about the typical daily food intake, frequency of eating during the day,

and variety and brand of consumed foods. Nutrition and food intake was analyzed using EXCEL Eiyokun software version 5.0 (Kenpaku-sha Co. Ltd., Tokyo, Japan).

2.9. Statistical Analysis

We divided the intervention group into two (intervention/non-MS and intervention/MS groups) for analysis. Data were analyzed using SPSS software (version 18.0, SPSS Inc., Chicago, IL, USA). Normalcy of the data was assessed using the Shapiro–Wilk test. Statistical differences in parameters were assessed by comparison either between the control, intervention/non-MS, and intervention/MS groups at baseline by independent sample t-test (parametric data) or by Mann–Whitney test (non-parametric data). Statistical differences between the baseline and after 3-month parameters were analyzed by paired t-test (parametric data) or Wilcoxon's signed rank test (non-parametric data). The significance level was set at $p < 0.05$. All data were described as means \pm standard deviation.

3. Results

3.1. Characteristics of the Participants

Table 1 shows the characteristics of the participants, overall. Body mass index (BMI), abdominal circumference, serum lipid, blood pressure was slightly higher than normal, overall, and a few people had MS at baseline. Furthermore, the group had low intake of dietary fiber.

Table 1. Characteristic of subjects.

Variables			
Age (y)	48.0	\pm	8.1
Anthropometric			
Height (cm)	168.3	\pm	5.9
Weight (kg)	72.8	\pm	11.6
Body fat percentage (%)	26.6	\pm	5.9
Body mass index (kg/m ²)	25.6	\pm	3.3
AC (cm)	89.2	\pm	9.4
SBP (mmHg)	139.0	\pm	13.6
DBP (mmHg)	88.9	\pm	10.7
Blood parameter			
TP (g/dL)	7.49	\pm	0.31
HDL-Chol (mg/dL)	55.8	\pm	9.9
LDL-Chol (mg/dL)	133.4	\pm	30.4
Alb (g/dL)	4.66	\pm	0.19
TG (mg/dL)	157.6	\pm	98.9
FFA (μ Eq/L)	491.8	\pm	165.3
T-Chol (mg/dL)	216.4	\pm	26.3
UA (mg/dL)	6.32	\pm	1.32
CRP (mg/dL)	0.10	\pm	0.12
HbA _{1c} (%)	5.39	\pm	0.64
Glu (mg/dL)	113.4	\pm	23.1
Energy and nutrition intakes			
Energy	2,217	\pm	541
Protein	76.7	\pm	23.0
Fat	66.3	\pm	20.2
Carbohydrate	318.1	\pm	92.7
Dietary fiber	16.6	\pm	7.1
Sodium chloride	12.0	\pm	4.0

N=38, Data shows the mean \pm SD.

3.2. BMI, BFP, and Blood Pressure in the Control, Intervention/Non-MS and Intervention/MS groups

Figure 2 shows the BMI, BFP, and blood pressure in the control, intervention/non-MS and intervention/MS groups, respectively. Abdominal circumference level in the intervention/MS group had significantly decreased after

intervention compared with the baseline ($p < 0.05$). Systolic blood pressure decreased significantly in the intervention/non-MS group ($p < 0.05$). Diastolic blood pressure also decreased significantly after intervention compared with the baseline in the intervention/non-MS and intervention/MS groups ($p < 0.01$).

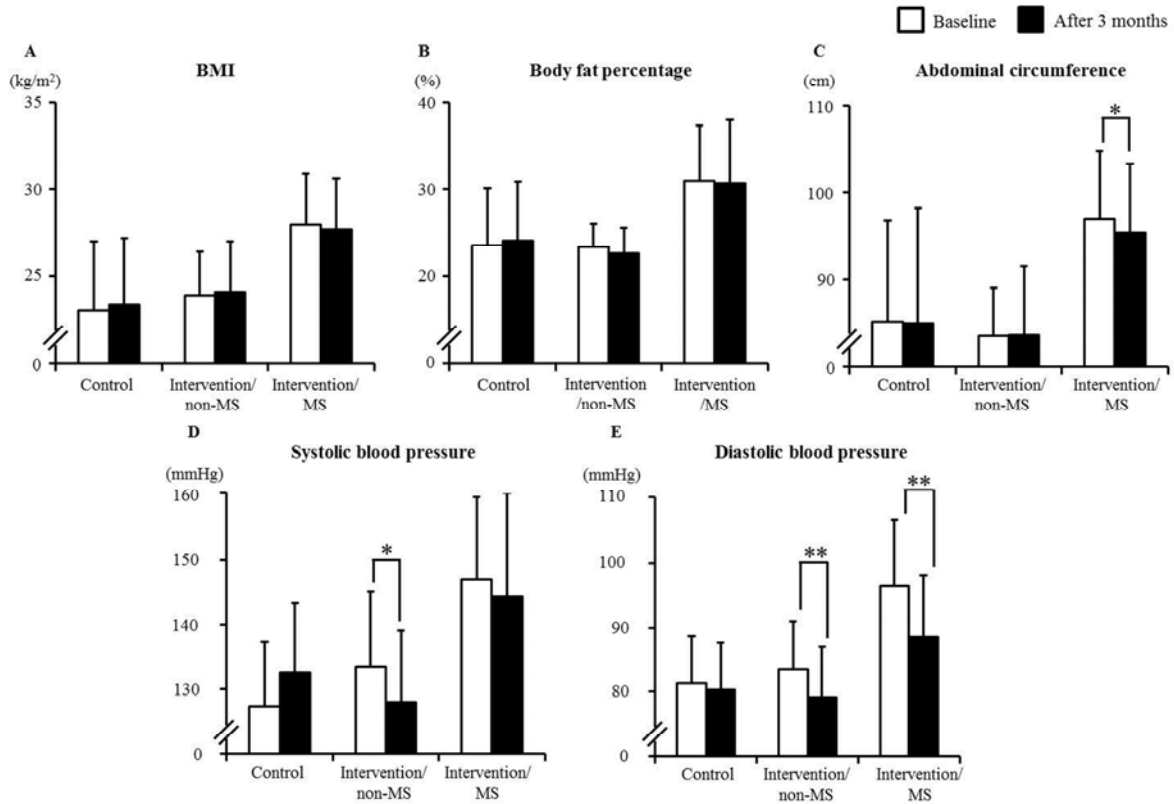


Figure 2. Comparison of body size and blood pressure at baseline and after 3 months.

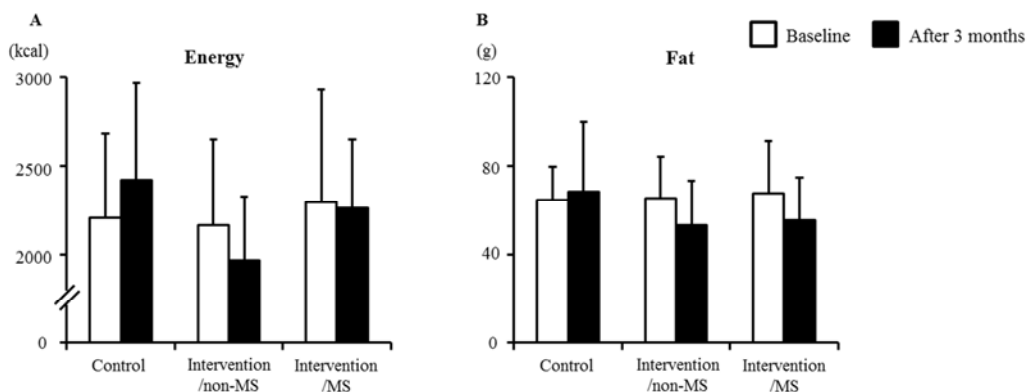
N=Control: 7; Intervention/non-MS: 18; Intervention/MS: 13

A; Body mass index (BMI), B; Body fat percentage, C; Abdominal circumference, D; Systolic blood pressure, E; Diastolic blood pressure

Unfilled bars and filled bars show the before and after intervention, respectively. Each value is mean ±SD. Means with asterisk symbols show significant differences at $p < .001$ or 0.01 or 0.05 by Wilcoxon’s signed rank test or paired t-test. * $p < .05$, ** $p < .01$.

3.3. Food Intake in the Control, Intervention/Non-MS and Intervention/MS Groups

Figure 3 shows the food intake in the control, intervention/non-MS and intervention/MS groups. No significant difference was observed between groups, but the amount of dietary fiber showed an increasing trend after intervention, in the intervention group.



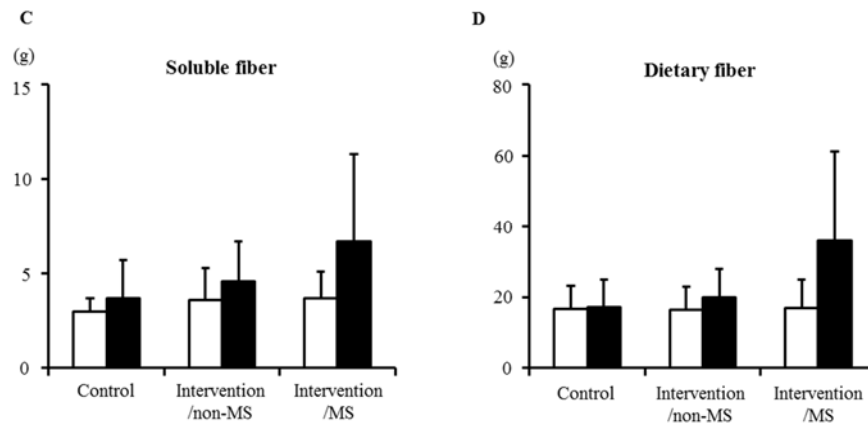


Figure 3. Comparison of energy, fat, soluble fiber, and dietary fiber intake at baseline and after 3 months by 24h dietary record.

N=Control: 7; Intervention/non-MS: 18; Intervention/MS: 13.

A; Energy, B; Fat, C; Soluble fiber, D; Dietary fiber.

Unfilled bars and filled bars show the before and after intervention, respectively. Each value is mean ±SD. These data were analyzed by paired t-test.

3.4. Serum LDL-Chol, TG, Free Fatty Acids, Total Cholesterol, Glucose, Active Ghrelin, Desacyl Ghrelin, and Leptin Level in the Control, Intervention/Non-MS, and Intervention/MS Groups

Figure 4 shows the serum LDL-Chol, TG, free fatty acids (FFA), total cholesterol (T-Chol), glucose, active ghrelin, desacyl ghrelin, and leptin level in the control,

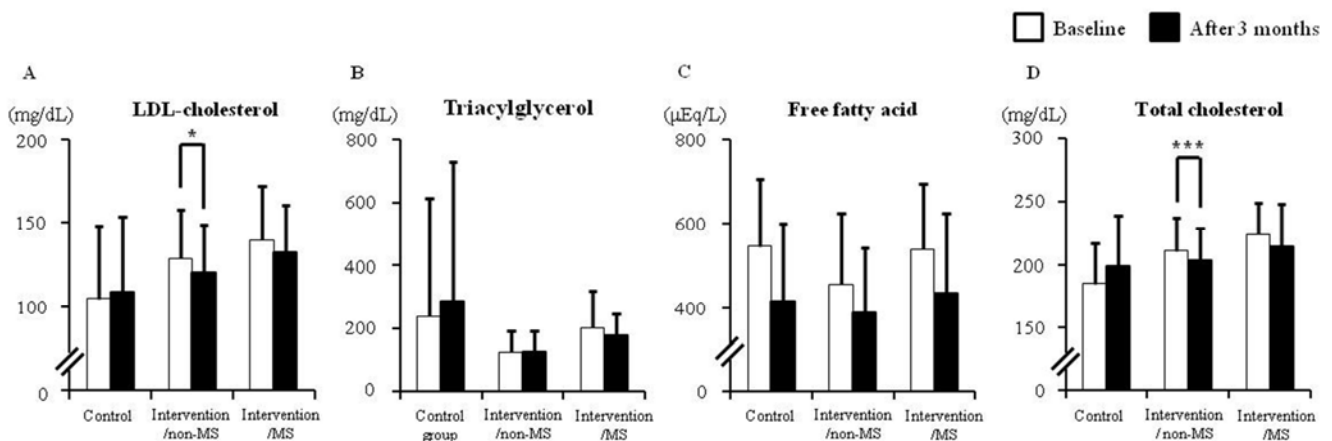
intervention/non-MS, and intervention/MS groups, respectively. Serum LDL-Chol (Figure 4-A) and T-Chol (Figure 4-D) had significantly decreased after intervention compared with the baseline in the intervention/non-MS group ($p < 0.05$, $p < 0.001$). Ghrelin (Figure 4-F) and desacyl ghrelin (Figure 4-G) had significantly increased after intervention compared with the baseline in intervention/non-MS and intervention/MS groups ($p < 0.001$, $p < 0.05$, respectively).

Table 2. Comparison of adipocytokine at baseline and after 3 months.

	Control (n=7)		Intervention/non-MS (n=18)		Intervention/MS (n=13)	
	Baseline	After 3 months	Baseline	After 3 months	Baseline	After 3 months
PAI-1 (ng/mL)	16.8±3.3	26.6±17.0	18.3±5.3	19.9±9.7	20.3±2.9	17.6±5.0
TNF-α (pg/mL)	128.5±6.8	115.9±7.2*	124.8±2.2	112.1±3.8***	129.0±5.1	114.0±4.9**
IL-6 (pg/mL)	151.6±10.6	144.5±1.4	154.5±7.8	143.3±2.8**	156.4±3.5	144.7±4.4**

Data shows the mean ± SD.

*p stands for the difference between the baseline parameters and the parameters after 3 months analyzed by the Wilcoxon’s signed rank test or paired t-test. Difference was considered significant at $p < *p < .05$, $**p < .01$, $***p < .001$.



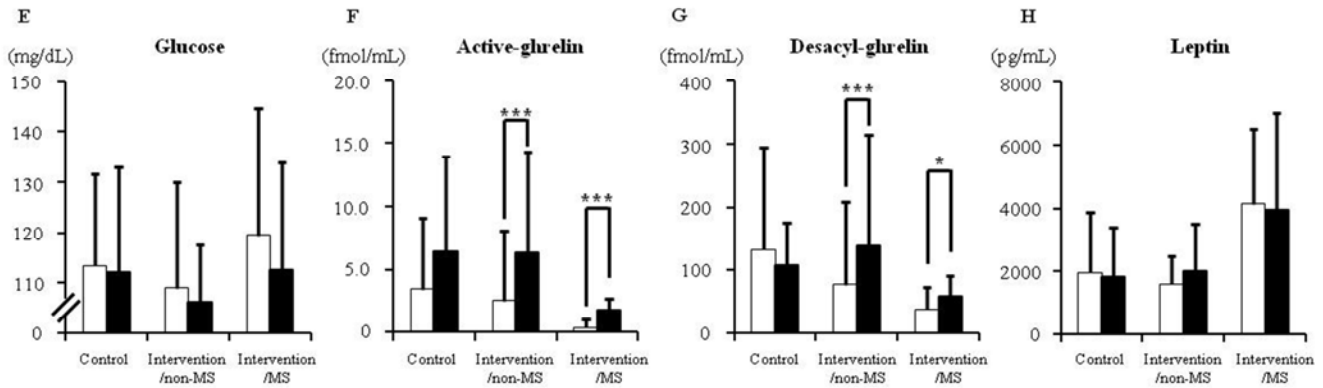


Figure 4. Comparison of serum lipids, glucose and eating related hormone in baseline and after 3 months.

N=Control: 7; Intervention/non-MS: 18; Intervention/MS: 13

A; Low-density lipoprotein (LDL)-cholesterol, B; Triacylglycerol, C; Free fatty acid, D; Total cholesterol, E; Glucose, F; Ghrelin, G; Desacyl-ghrelin, H; Leptin. Unfilled bars and filled bars show the before and after intervention. Each value is mean \pm SD. Means with asterisk symbols show significant differences at $p < .001$ or 0.05 by Wilcoxon’s signed rank test or paired t-test: * $p < .05$, *** $p < .001$

3.5. Serum Plasminogen Activator Inhibitor-1, TNF- α , and IL-6 in the Control, Intervention/Non-MS, and Intervention/MS Groups

Table 2 shows the serum plasminogen activator inhibitor-1, TNF- α , and IL-6 in the control, intervention/non-MS, and intervention/MS groups, respectively. Serum TNF- α had significantly decreased after intervention compared with the baseline in the intervention/non-MS and intervention/MS groups, respectively ($p < 0.001$, $p < 0.01$). Serum IL-6 had significantly decreased after intervention compared with the baseline in the intervention/non-MS and intervention/MS groups ($p < 0.01$)

4. Discussion

We conducted a non-randomized controlled trial by providing a Japanese-style healthy lunch to middle-aged men at a workplace cafeteria. The aim of this study was to determine the effect of Japanese-style healthy lunch on metabolic syndrome outcomes (blood pressure, serum TNF-alpha, IL-6, serum lipids); with an overall goal of enabling the prevention and improvement of MS by healthy lunch intake; this should help to reduce MS medical expenses. The finding, following the 12-week healthy lunch intervention, showed that it contributed to the prevention/improvement of MS. Furthermore, it contributed to the normalization of the “bad” adipokines (TNF- α and IL-6) which is unique to the MS.

MS has been shown to be closely related to dietary habits [17, 18]. Previous studies have reported that MS is inversely correlated with potassium, vitamin D, carotene, and insoluble dietary fiber [19, 20, 21, 22]. These nutrients are mainly contained in vegetables. The healthy lunch taken in this study satisfied two thirds of the amount of Japanese recommended daily vegetables. However, the menu was not a single dish, but a Japanese type of menu for the day, consisting of staple food (Japanese: Shushoku), side dishes (Japanese: Fukusai), main dishes (Japanese: Shusai), and soup stock. It is thought that the

factor contributing to the prevention/improvement of MS in this study was the increase in vegetable intake and the balanced diet. Bahari et al. reported an association between the intake of vegetables, fruits, fish, small fish, natto, and fried tofu; and reduction in the prevalence of MS, in a Japanese food group, in a cohort study [13]. Conversely, Akter et al. reported that Western style breakfast patterns may offer some protection against Japanese MS [23]. Moreover, Arisawa et al. reported that high fat/Western dietary pattern may be positively associated with insulin resistance in the Japanese population [24]. As findings of these reports showed, dietary factors have significant impact on MS; consequently, it can be inferred that the contents of diet are important.

In our study, especially among participants with MS, abdominal circumference and systolic blood pressure decreased (Figure 2). In blood markers, improvement effect on lipid metabolism was more evident than the effect on glycometabolism (Figure 4). Serum glucose concentration was not significantly reduced. The reason for this is unknown; however, that the intervention period of healthy lunch was as short as 3 months, and that interindividual variability of serum glucose was large, in the intervention group, might explain this. However, the serum TNF- α and IL-6, in the intervention group decreased significantly after the intervention (Table 2). Chronic inflammation involving the whole body has attracted attention as a basic pathology of MS; and it has been clarified that adipose tissue, in obesity, is also involved in the development of inflammatory changes [25, 26]. TNF- α interferes with the action of insulin. Increased visceral fat increases the secretion of glucose and brings about insulin resistance; which is one of the factors that causes or exacerbates diabetes [27, 28]. IL-6 and TNF- α have been found to be higher in participants with MS and those with insulin resistance. These factors are both known to promote lipolysis and the secretion of FFAs, which contribute to the increase in hepatic glucose output and insulin resistance as well as impaired adipocyte differentiation, and promote inflammation [28]. In our study, it is inferred that the intake of

healthy lunch contributed at least, to the normalization of cytokines, because, these inflammatory markers declined after the intervention. Calorie restriction is known to accompany a reduction in inflammatory markers, including IL-6 [29, 30, 31]. In a short-term study of obese diabetic men by Khoo et al., 8 weeks of calorie-restricted low-fat, high-protein, and reduced carbohydrate diet led to a significant decrease in plasma IL-6 levels [32]. Ghalandari et al. reported that in comparison with simple advice to modify carbohydrate intake, a calorie-restricted and moderate carbohydrate diet supplemented with psyllium has better effects on plasma insulin and pro-inflammatory cytokines in patients with type 2 diabetes [33]. In this study, energy intake after intervention did not decrease significantly. Moreover, the amount of carbohydrates in the diet was not particularly restricted. In this study, except for the intake of the healthy lunch, participants could freely partake in other diet. We consider that the normalization of the blood pressure and serum cytokines was relevant; considering that this was just one meal. In another similar study of ours, we reported that those who had more healthy lunches had decreased serum lipids and blood pressure [34]. However, the analysis in this study, which compared between MS/non-MS, revealed that TNF- α and IL-6, which are also specific markers for MS, were reduced.

Holdstock et al. reported that the rate of BMI decrease showed a significant positive correlation with the increase rate of blood ghrelin, adiponectin, and showed a significant negative correlation with blood insulin and leptin concentrations [35]. In this study, BMI did not decrease significantly after intervention in the intervention group. We think that it is necessary to re-evaluate this causal relation. However, there is no other report stating that ghrelin concentration rises in response to a healthy diet in Japanese men. In the future, we need to clarify the relationship of intervention of long-term healthy lunch with BMI and ghrelin and leptin concentrations.

The findings of this research showed that the intake of healthy lunch at the cafeteria, by workers, contributed to the prevention/improvement of MS; suggesting that this might be a useful remedy that may not increase the burden on the participants. Thus, it is thought that this research might also lead to the opportunity for participants to practice with the healthy menu at home. We therefore consider the findings to be valuable in promoting improvement of diet in the food environment at the workplace.

However, in this study, the limitation is the small number of participants. In future, we plan to increase the number of people and also consider behavioral changes for the targeted participants after the intervention. Furthermore, this research did not verify the effect of specific nutrients. Further studies are therefore needed to examine the effect of specific nutrient on MS.

5. Conclusions

We conducted a non-randomized controlled trial by providing a Japanese-style healthy lunch to middle-aged men

at a workplace cafeteria. We obtained an effective outcome by demonstrating that the ongoing intake of a Japanese-style healthy lunch decreased blood pressure, serum TNF- α , IL-6, and serum lipids. In addition, dietary fiber increased in the intervention group. The result of this research showed that the intake of healthy lunch at the cafeteria used by workers contributed to the prevention/improvement of MS; suggesting that this might be a useful remedy that may not increase the burden on the participants.

Abbreviations

AC: Abdominal circumference; Alb: Albumin; BFP: Body fat percentage; CRP: C-reactive protein; DBP: Diastolic blood pressure; EDTA: Ethylenediaminetetraacetate; ELISA: Enzyme-linked immunosorbent assay; FFA: Free fatty acid; Glu: Glucose; HbA1C: Hemoglobin A1C; HDL-Chol: High-density lipoprotein; LDL-Chol: Low-density lipoprotein; MS: Metabolic syndrome; SBP: Systolic blood pressure; T-Chol: Total cholesterol; TG: Triacylglycerol; TP: Total protein; UA: Uric acid.

Ethics Approval and Consent to Participate

This study was approved by University of Shizuoka (No. 20-5). All selected participants provided written informed consent for their participation.

Consent for Publication

Not applicable

Availability of Data and Materials

Data are available from the corresponding author on reasonable request.

Funding

This study was supported by University of Shizuoka.

Acknowledgments

We are grateful to the city hall workers for participation in the study.

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Competing Interests

The authors of the manuscript declare no conflicts of

interest.

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