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# Beneficial Effect of Food Substitute Containing L-Arginine, ω-3 Poly Unsaturated Fatty Acid, and Ribonucleic Acid in Preventing or Improving Metabolic Syndrome: A Study in 15 Overweight Patients and a Study of Fatty Acid Metabolism in Animals

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***Summary*** **This study was conducted to investigate whether or not a food substitute (Dr. BAANs®) containing three bioactive components L-arginine, ω-3 polyunsaturated fatty acid, and ribonucleic acid, supplied orally to 15 overweight patients, may have efficacy to prevent or improve the metabolic syndrome of these patients. To provide supporting data for this clinical study, the *in vivo* fatty acid metabolism of obese mice was analyzed using $^{125}$I labeled 15-(p-iodophenyl)-9-methylpentadecanoic acid (9MPA) in the tissues' lipid pool. After 3 months of intervention, the results showed that there were improvements observed in liver functions, lipid profiles and metabolic syndrome marker. Significant differences were also found in the values of blood pressure, body weight, percentage of body fat, and body mass index. In the animal study, the tissue uptake of $^{125}$I-9MPA at 10 min after injection was higher in obese mice than in the control mice and the treatment with Dr. BAANs® in obese mice decreased the uptake significantly. The final product metabolite of p-iodophenylacetic acid in obese mice was increased significantly by the treatment. In conclusion, this food substitute may have a beneficial effect for the prevention or improvement of metabolic syndrome.**

***Key Words***: **L-arginine, ω-3 poly unsaturated fatty acid, ribonucleic acid, metabolic syndrome, fatty acid metabolism**

## Introduction

Metabolic syndrome is a cluster of interrelated risk factors (visceral obesity, hyperlipidemia, hyperglycemia, and hypertension) that increase susceptibility to cardiovascular disease and type-2 diabetes. The underlying causes of this syndrome are obesity, physical inactivity, and genetic factors [*1*].

There is general agreement that lifestyle changes primarily focused on weight reduction are the first line approach in patients with metabolic syndrome. Among

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conventional preventive methods, a low calorie diet (1500 kcal) and exercise have been practiced as therapy and preventive methods against metabolic syndrome as typified by obesity. On the other hand, a low-calorie diet without exercise will cause a decrease in basal metabolism, which can cause a lack of nutritional substances and minerals necessary for the body; therefore, an excessively strict diet without medical supervision is extremely dangerous [*2*, *3*].

At the 40th meeting of the Japan Society of Adult Diseases in 2006, Koide *et al.* [*4*] addressed a preventive method against lifestyle diseases in a new concept; Bio Activating Advanced Nutrients (BAANs) theory. The theory of this method is not like a conventional diet therapy. It is designed to stimulate protein synthesis by energy generation in the process of expediting the burn-off of fatty acids as derivatives of body fat break-down, which promotes the intra-vital metabolic cycle as a result. The principal ingredients are three bioactive components: L-arginine, which is present at high levels in meat, ω-3 poly unsaturated fatty acid, which is a main component of fish oil, and ribonucleic acid (RNA), which is found at high numbers in beer yeast.

Recently, the radio labeled fatty acid tracer 15-(p-iodophenyl)-9-methylpentadecanoic acid (9MPA) was developed for metabolic analysis. This tracer is converted to an intermediate metabolite, 3-methylnonanoic acid (3MNA), after 3 cycles of β-oxidation. 3MNA continuously undergoes α and β-oxidation to yield the metabolite p-iodophenylacetic acid (PIPA). Therefore, 9MPA is well suited to study fatty acid uptake and oxidation *in vivo* [*5*, *6*]. In this study for assessing fatty acid metabolism in obese, leptin deficient ob/ob mice were used, which is an animal model for obesity, insulin resistance, diabetes, and non-alcoholic steatohepatitis [*7*]. In ob/ob mice, the absence of the leptin signaling in the hypothalamus causes obesity due to increases in food intake and decreases in energy expenditure.

The aims of the present study were to evaluate whether or not the food substitute (Dr. BAANs®) administered orally in overweight patients may have efficacy to prevent or improve metabolic syndrome after 3 months of treatment, and also to evaluate *in vivo* fatty acid metabolism in obese mice, and obese mice treated by this food substitute.

## Materials and Methods

*Clinical study*

*Subjects.* Fifteen adult patients of both genders participated in the experiment. All of the patients met the following inclusion criteria: age 20–60 years old, obesity with body mass index (BMI) ≥25 kg/m$^2$. The patients met with one or more of the following criteria: hypertension, hyperglycemia, hyperlipidemia, or abnormal liver markers. The exclusion criteria were a history of thyroid disease, current pregnancy, an unstable medical condition, or the current use of medications known to affect lipid metabolism, blood pressure, blood glucose, or any medication known to affect weight or appetite. The present study was carried out in accordance with the Declaration of Helsinki and with the prior approval by the ethical committee of the Bio Research Center of Niigata City, Japan. The protocol and aim of the study were fully explained to the participants, who gave their written consent.

*Food substitute.* The food substitute (Dr. BAANs®) used in this study was donated by BBK Bio Corp in Japan. The validated composition of each sachet was: 1000 mg L-arginine, 150 mg ω-3-poly unsaturated fatty acid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the ratio of 2:1, 280 mg of all kinds of RNA from beer yeast, 420 mg triglyceride, 12.0 g lactoprotein, and 12.0 g carbohydrates. One sachet was solveled into 250 ml drinking water and contained 170 kcal.

*Study design.* An evaluation period of 3 months was determined in order to observe changes in body weight or body fat percentage, blood pressure level, blood glucose level, lipid levels, and hepatic function before and after the substitution of food with Dr. BAANs®. During the study all participants received counseling for their intake every day as follows: for breakfast they drank 1 sachet of Dr. BAANs® (containing 170 Kcal/250 ml). The participants were asked not to modify their dietary habits for lunch and dinner or their lifestyle during the whole experimental period. At the beginning of the study and every month until one month after withdrawal of the food substitute, blood samples were taken in the morning, after a 12 h fast and 15 min of rest, using blood collection tubes in order to observe the initial and final levels of glucose, triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and glycosylated hemoglobin (HbA1c). For adiponectin and plasminogen activator inhibitor-1 (PAI-1) only the pretreatment levels, the levels after 3 months of treatment, and the levels 1 month after withdrawal of the food substitute were measured. Liver function was also checked by measuring the aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltransferase (γ-GTP) levels at the baseline, and every month until one month after withdrawal of the food substitute.

The prevalence of hyperlipidemia, hypertension, hyperglycemia, and abnormal levels of liver function were registered before and after the experimental period. Hypercholesterolemia was defined as having TC levels more than 220 mg/dl, hypo-high density lipoproteinemia was defined as having HDL-C values lower than 40 mg/dl. Hypertriglyceremia was defined as having TG values higher than 150 mg/dl; Hyper-low density lipoproteinemia was defined as having LDL-C values higher than 140 mg/dl. All these

parameters were defined according to the guidelines of the Japan Society of Internal Medicine [*8*]. Hypertension was defined as having values of systolic blood pressure (SBP) more than 140 mmHg or diastolic blood pressure (DBP) more than 90 mmHg according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) criteria [*9*].

Obesity was defined as BMI values more than 25 kg/m$^2$; abnormal values for the percentage of body fat was defined as more than 25%; and abnormal waist circumference was defined as values more than 85–90 cm according to new criteria for obesity in Japan from the Japan Society for the Study of Obesity [*10*]. Abnormal values of AST, ALT, and γ-GTP were defined as values more than 40 IU, 35 IU, and 50 IU, respectively. Hyperglycemia was defined as values of blood glucose more than 110 mg/dl. An HbA1c abnormal value was defined as value more than 5.8%.

*Anthropometric measurements.* Waist circumference was measured midway between the lower rib margin and the iliac crest in standing position after normal expiration. Height was measured using a rigid stadiometer. Weight of the subjects (in light clothing) was measured on a calibrated balance scale. Percentage of body fat was measured using a body fat analyzer. BMI was calculated as weight (kg) divided by height (m) squared (kg/m$^2$). Blood pressure was measured using a sphygmomanometer by an ausculatory method.

*Laboratory analysis.* Blood, for plasma analyses, was drawn into sterile glass tubes with 100 units of freeze-dried sodium heparin and centrifuged at 4,000 g for 5 min at 4°C. TC, TG, LDL-C, and HDL-C concentrations were determined using standard enzymatic procedures; AST, ALT, γ-GTP, and glucose levels were measured with enzymatic kits using spectrophotometric methods. Adiponectin was measured by ELISA and PAI-1 was measured by an enzymatic method.

*Safety assessments.* All adverse events were collected from participants every month by medical examinations.

*Animal study*

*Materials.* $^{125}$I-Labeled 9MPA (7.4 GBq/mg), $^{125}$I-labeled 3MNA (7.4 GBq/mg), and $^{125}$I-labeled PIPA (7.4 GBq/mg) were donated by Daichii Radioisotope Laboratories Ltd. (Tokyo, Japan).

*Animals.* Obese 8-week-old B6V-Lep ob/ob mice ($n$ = 6), weighing about 48 grams, and 8-week-old C57 Bl/6 mice ($n$ = 3), weighing about 25 grams, were purchased from Charles River Laboratories, Kanagawa, Japan. Three of the B6V-Lep ob/ob mice were treated with Dr. BAANs®. The C57 Bl/6 mice served as a control group. The animals were housed three per cage and allowed free access to food and water ad libitum. Mice were housed in a temperature-controlled room under a 12-h light dark (7 a.m. to 7 p.m.) cycle. For the treatment of obese mice, 1 ml / 25 g BW Dr. BAANs® was given daily for 1 week. The doses of the present study were selected on the basis of preliminary studies.

*$^{125}$I Radioactivity in the tissues lipid pool.* $^{125}$I-9MPA (741 KBq (20 μCi) was injected intravenously into the above 3 groups of mice (control, obese, and obese treated with Dr. BAANs®). At 10 min after injection, the mice were sacrificed. Lipid extraction from tissues (myocardium, skeletal muscle, visceral fat and subcutaneous fat) was performed according to a modified version of the method developed by Folch *et al.* [*11*]. Briefly, tissue specimens were homogenized and extracted twice with chloroform/methanol (2:1, v/v). The resulting organic, aqueous, and solid phases were separated. Radioactivity distribution in the organic phase was assayed by thin layer chromatography (TLC) on a reverse phase plate (C18 Silicagel Spotfil), together with $^{125}$I-labeled standard fatty acids (9MPA, 3MNA, and PIPA).

*Statistical analysis*

Data are expressed as mean ± SD. Group comparisons were made by ANOVA, followed by Tukey's multiple comparison test to identify differences among various groups. $p$<0.05 was considered statistically significant.

## Results

*Clinical study*

All of the 15 subjects (100%) completed the study. Another evaluation that was taken from the history of each subject showed that nothing changed in their dietary habits and amount of food they ate. The baseline characteristic of the participants and changes in parameters after treatment are summarized in Table 1. After 3-months intervention body weight, BMI (Fig. 1a), and percentage of body fat were reduced significantly.

In accordance with JNC 7, high blood pressure prevalence was assessed in total samples before and after treatment with Dr. BAANs® (Table 2). The results show that the initial hypertension prevalence was diminished after intervention in both SBP and DBP values and the reduction of blood pressure in systolic and diastolic were 7.2 ± 13.3 and 5.0 ± 10.6 mmHg, respectively. In Fig. 1b we showed the reduction of blood pressure in systolic for each subject.

The prevalence on the abnormal values of liver function (AST, ALT, and γGTP), were diminished after intervention (Table 2). The most important changes were observed in ALT, the initial prevalence of abnormal values was 47%; and after intervention it decreased to 27%. No subject in this study changed from normal value to abnormal value after intervention.

Table 1. Baseline characteristics of patients and changes in parameters after treatment

| Variable | Mean ± SD | | |
|---|---|---|---|
| | Before | After | Change |
| | $n = 15$ | $n = 15$ | |
| Age (years) | 42.4 ± 9.3 | 42.4 ± 9.3 | |
| Height (m) | 169.6 ± 9.4 | 169.6 ± 9.4 | |
| Weight (kg) | 77.7 ± 12.6 | 76.5 ± 12.1* | −1.2 ± 1.8 |
| Waist circumference (cm) | 92.2 ± 7.3 | 90.8 ± 6.4 | −1.4 ± 3.9 |
| BMI (kg/m$^2$) | 26.9 ± 2.4 | 26.5 ± 2.5* | −0.4 ± 0.6 |
| Body fat (%) | 29.6 ± 4.3 | 26.4 ± 4.6* | −3.2 ± 1.7 |
| SBP (mmHg) | 126.8 ± 19.7 | 119.6 ± 13.9* | −7.2 ± 13.3 |
| DBP (mmHg) | 82.1 ± 15.4 | 77.1 ± 15.1 | −5.0 ± 10.6 |
| AST (IU) | 26.8 ± 11.3 | 23.5 ± 7.2 | −3.3 ± 6.7 |
| ALT (IU) | 35.7 ± 22.0 | 26.9 ± 12.9* | −8.8 ± 12.2 |
| γ GTP (IU) | 71.9 ± 55.2 | 53.3 ± 37.0* | −18.6 ± 22.6 |
| TC (mg/dl) | 228.6 ± 44.7 | 218.2 ± 35.5 | −10.4 ± 21.7 |
| LDL-C (mg/dl) | 131.1 ± 41.4 | 123.7 ± 31.9* | −7.4 ± 18.1 |
| HDL-C (mg/dl) | 59.9 ± 17.8 | 59.1 ± 18.1 | −0.8 ± 6.3 |
| TG (mg/dl) | 164.2 ± 122.3 | 129.8 ± 78.8* | −34.4 ± 67.0 |
| Blood glucose (mg/dl) | 96.9 ± 18.1 | 96.7 ± 21.2 | −0.2 ± 10.0 |
| HbA1c (mg/dl) | 5.1 ± 0.5 | 5.0 ± 0.4 | −0.1 ± 0.2 |
| Adiponectin (μg/ml) | 3.8 ± 4.2 | 4.3 ± 4.5* | 0.58 ± 0.64 |
| PAI-1 (ng/ml) | 59.3 ± 37.1 | 44.0 ± 25.2* | −14.3 ± 23.4 |

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; AST = aspartate amino transferase; ALT = alanine transaminase; γ-GTP = gamma glutamyl transpeptidase; TC = total cholesterol; LDL-C = low density lipoprotein-cholesterol; HDL-C = high density lipoprotein-cholesterol; TG = triglyceride; HbA1c = hemoglobin A1c; PAI-1 = plasminogen activator inhibitor-1. *$p<0.05$ vs before treatment

Table 2. Prevalence of abnormal values before and after treatment

| Variable | Before (%) $n = 15$ | | After (%) $n = 15$ | |
|---|---|---|---|---|
| | Normal | Abnormal | Normal | Abnormal |
| SBP | 10 (67) | 5 (33) | 12 (80) | 3 (20) |
| DBP | 10 (67) | 5 (33) | 11 (73) | 4 (27) |
| AST | 14 (93) | 1 (7) | 15 (100) | 0 (0) |
| ALT | 8 (53) | 7 (47) | 11 (73) | 4 (27) |
| γ GTP | 8 (53) | 7 (47) | 9 (60) | 6 (40) |
| TC | 6 (40) | 9 (60) | 9 (60) | 6 (40) |
| LDL-C | 9 (60) | 6 (40) | 11 (73) | 4 (27) |
| HDL-C | 2 (13) | 13 (87) | 2 (13) | 2 (13) |
| TG | 9 (60) | 8 (40) | 11 (73) | 4 (27) |
| Blood glucose | 13 (87) | 2 (13) | 14 (93) | 1 (7) |
| HbA1C | 14 (93) | 1 (7) | 14 (93) | 1 (7) |

SBP = systolic blood pressure; DBP = diastolic blood pressure; AST = aspartate amino transferase; ALT = alanine transaminase; γ-GTP = gamma glutamyl transpeptidase; TC = total cholesterol; LDL-C = low density lipoprotein-cholesterol; HDL-C = high density lipoprotein-cholesterol; TG = triglyceride; HbA1c = hemoglobin A1c.

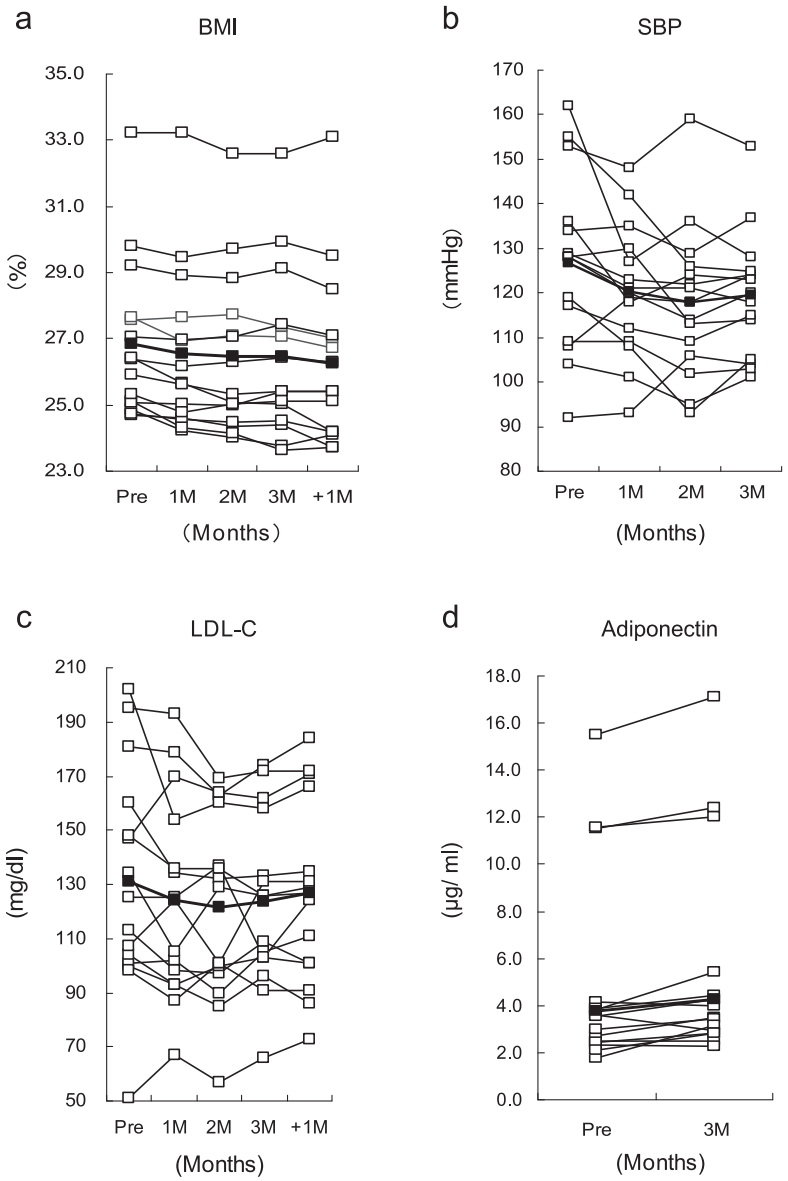


Fig. 1. Body mass index (BMI) (a), systolic blood pressure (SBP) (b), low density lipoprotein-cholesterol (LDL-C) (c) and adiponectin (d) values in each subject from baseline until the end of intervention. Open square is value of each patient; close square is average in each value.

On the other hand, when the effects of Dr. BAANs® on hyperlipidemia prevalence were assessed, the initial abnormal levels of TC, LDL-C, and TG were diminished after treatment (Table 2). At the end of the treatment period there were significant differences in LDL-C and TG levels (Table 1 and Fig. 1c). While for blood glucose and HbA1c values, no significant changes were observed throughout the experimental period (Table 1). The metabolic syndrome marker adiponectin (Fig. 1d) was increased significantly ($3.8 \pm 4.2$ μg/ml vs $4.3 \pm 4.5$ μg/ml, $p<0.05$) while PAI-1 was decreased significantly ($59.3 \pm 37.1$ ng/ml vs $44.0 \pm 25.2$ ng/ml, $p<0.05$).

In the overall safety assessment, no adverse events (including nausea, vomiting, diarrhea, weakness, fatigue, syncope) were noted in any of the subjects.

*Fatty acid metabolism in animals*

The uptake of $^{125}$I-labeled compound (%) into the tissues is summarized in Table 3. The myocardial uptake at 10 min after injection of $^{125}$I-9MPA was higher in obese mice than in control mice. Treatment with Dr. BAANs® in obese mice decreased the uptake significantly (Table 3). These results were the same as found in other tissues (skeletal muscle, and visceral and subcutaneous fat). The level of final product of 9-MPA metabolite, PIPA in those tissues were markedly reduced in obese mice but recovered with treatment (Fig. 2

Table 3. Distribution of $^{125}I$ radioactivity in tissues following 10 min intravenous injection of $^{125}I$-labeled 9MPA

| Tissue | 9MPA % | 3MNA % | PIPA % | 3MNA + PIPA/9MNA % |
|---|---|---|---|---|
| Myocardium | | | | |
| Control mice ($n$ = 3) | 3.6 ± 1.2 | 17.9 ± 9.3 | 11.8 ± 1.2 | 8.3 ± 0.3 |
| ob/ob mice ($n$ = 3) | 19.9 ± 0.3* | 9.0 ± 0.7 | 1.3 ± 0.4* | 0.5 ± 0.1* |
| ob/ob mice + Dr BAANs ($n$ = 3) | 9.9 ± 2.5*# | 6.6 ± 0.5 | 9.5 ± 3.2# | 1.7 ± 0.6*# |
| Skeletal muscle | | | | |
| Control mice ($n$ = 3) | 1.9 ± 0.4 | 4.2 ± 1.5 | 24.8 ± 3.0 | 16 ± 5.1 |
| ob/ob mice ($n$ = 3) | 15.2 ± 2.6* | 12.2 ± 1.3* | 3.7 ± 0.3* | 1.1 ± 0.2* |
| ob/ob mice + Dr. BAANs ($n$ = 3) | 12.2 ± 3.8* | 10.2 ± 2.4* | 24.8 ± 9.0# | 3.2 ± 1.9* |
| Visceral fat | | | | |
| Control mice ($n$ = 3) | 8.7 ± 0.4 | 5.9 ± 0.3 | 31.1 ± 7.1 | 4.2 ± 0.9 |
| ob/ob mice ($n$ = 3) | 10 ± 0.2* | 4.1 ± 0.1* | 1.6 ± 0.8* | 0.5 ± 0.1* |
| ob/ob mice + Dr. BAANs ($n$ = 3) | 5.0 ± 0.1*# | 3.4 ± 0.7* | 5.6 ± 0.8*# | 1.7 ± 0.1* |
| Subcutaneous fat | | | | |
| Control mice ($n$ = 3) | 1.8 ± 1.1 | 2.5 ± 1.8 | 4.1 ± 0.7 | 4.8 ± 3.1 |
| ob/ob mice ($n$ = 3) | 8.7 ± 0.4* | 4.0 ± 0.1 | 2.3 ± 0.2 | 0.7 ± 0.01 |
| ob/ob mice + Dr. BAANs ($n$ = 3) | 6.1 ± 0.3*# | 3.4 ± 0.5 | 7.8 ± 1.0*# | 1.8 ± 0.3 |

Results are the means ± SD. *$p$<0.05 vs control; #$p$<0.05 vs ob/ob mice (without treatment)

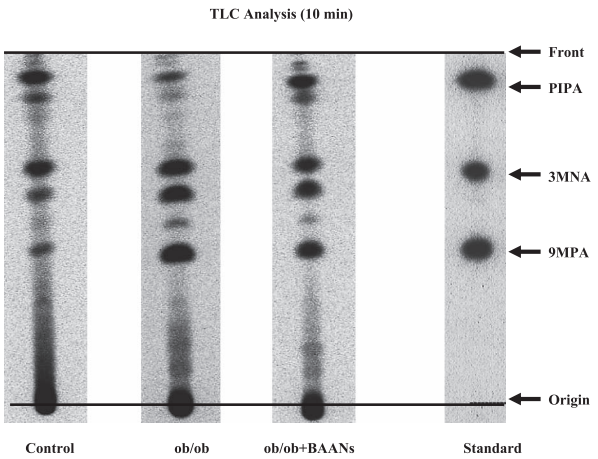


Fig. 2. Representative examples of TLC analysis of 9MPA in control, ob/ob, and ob/ob mice treated with Dr. BAANs® at 10 min after $^{125}I$-9MPA injection. Summary of radioactivity from intermediate metabolites above 9MPA to PIPA were defined as metabolites processed by β-oxidation.

and 3). The ratio of 9MPA metabolites (3MNA + PIPA) to 9MPA non metabolites in myocardial tissue in obese mice was significantly increased by treatment (Table 3). These results indicate the recovery of the slower conversion of metabolites following treatment.

## Discussion

The aims of the present study were to evaluate whether or not the food substitute (Dr. BAANs®) administered orally in overweight patients may have efficacy to prevent or improve metabolic syndrome after 3 months of treatment. After 3 months, comparing the initial and final data showed that the parameters of metabolic syndrome criteria were improved, while in the animal study the final product of 9-MPA metabolite PIPA in obese mice was increased significantly by treatment, indicating the recovery of the slower conversion metabolites in obese mice.

This food substitute contains three bioactive components: L-arginine, ω-3 polyunsaturated fatty acid, and RNA. Previous studies in animals and humans have demonstrated the efficacy of dietary arginine supplementation, the physiological precursor of nitric oxide (NO), in reducing the mass in obese patients [*12*, *13*]. Numerous studies have demonstrated the beneficial effect of acute and chronic L-arginine supplementation on endothelium derived nitric oxide production and endothelial function. L-arginine has also been shown to reduce systemic blood pressure in some forms of experiments involving hypertension [*14*]. As a precursor for the syntheses of NO, L-arginine plays pivotal role in nutrition and metabolism. Arginine is classified as an essential amino acid for young mammals, and conditionally essential for adults, particularly at times of trauma and disease. Some evidence also showed that enteral or parenteral administration of arginine reverses endothelial dysfunction associated with major cardiovascular risk factors (hypercholesterolemia, smoking, hypertension, diabetes, obesity/insulin resistance, and aging), and ameliorates many common cardiovascular disorders (coronary and

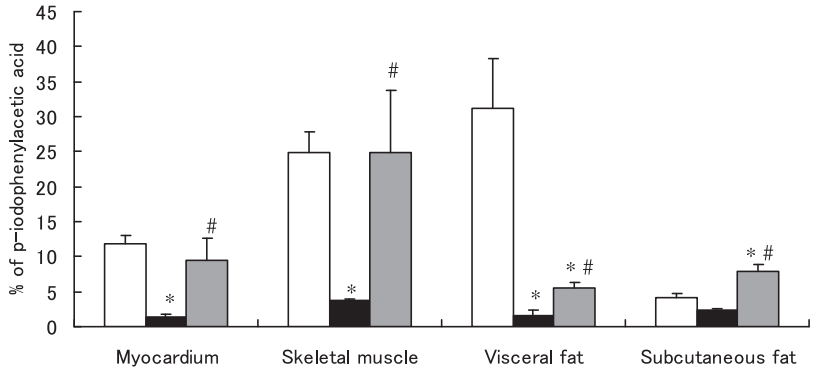


Fig. 3. The final product of 9-MPA metabolite PIPA in each tissue. White bar control mice, Black bar ob/ob mice, gray scale bar ob/ob mice treated with Dr. BAANs®, * $p<0.05$ vs control; # $p<0.05$ vs ob/ob mice.

peripheral arterial disease, ischemia/reperfusion injury, and heart failure) [*15*].

Another active component of this food supplement is ω-3 polyunsaturated fatty acid. Some epidemiological, observational, and randomized controlled clinical studies have reported the beneficial effects of ω-3 polyunsaturated fatty acid on cardiovascular disease [*16*]. It is known that metabolic syndrome gives a 2 to 3 fold increased risk for coronary heart disease, a similar risk for future ischemic stroke, and a much greater risk for future diabetes [*17*]. The potential mechanism by which ω-3 fatty acid may reduce the risk of cardiovascular disease involves reducing the susceptibility of the heart to ventricular arrhythmia, and promoting antithrombosis, nitric oxide-induced endothelial relaxation and hypotriglyceridemic. ω-3 polyunsaturated fatty acid also retard growth of atherosclerotic plague, and may also have a small, dose-dependent hypotensive effect [*18*, *19*].

The third active component of Dr. BAANs® is RNA. There are many food supplements on the market that contain this component. RNA is synthesized endogenously and has important effects on the growth and development of cells that have a rapid turn over, such as those in the immune system and the gastrointestinal tract. In healthy people dietary nucleotides are probably not essential, and in fact most will be metabolized and rapidly excreted from the system. However, under certain circumstances (e.g. in the sub-well, diseased, or under conditions of stress or poor diet) dietary nucleotides may be semi-essential for optimizing the function of the gastrointestinal and immune systems. The dietary sources of nucleotides are nucleoproteins and nucleic acid, and these are present at varying levels in food (but not in fruit or vegetables). Rapidly dividing tissues require a constant supply of nucleotides in order to manufacture essential nucleic acids. Exogenous supplies of nucleotides may optimize tissue function particularly during recovery from mucosal injuries when the endogenous supply may limit the synthesis of nucleic acids [*20*, *21*].

In the previous study, Koide *et al.* [*4*] reported that repetitive dosing of Dr. BAANs® in twenty test subjects (metabolic syndrome patients or candidates) for one months decreased body weight by 3.8 kg and reduced body fat by 2.4%. In the present study the same results were observed that after 3-months intervention, body weight, BMI, and percentage of body fat were reduced significantly. The possible explanation for this result is that the components of this food substitute may stimulate protein synthesis by energy generated in the process of expediting the burn-off of fatty acids, which results in the improvement of the intravital metabolic cycle and protein synthesis.

In this study, comparing the initial and final data and the prevalence of abnormal values before and after treatment, improvements were observed in blood pressure (SBP: 7.2 ± 13.3 mmHg, DBP: 5.0 ± 10.6 mmHg decrease), lipid profiles, and all items of hepatic functions (Tables 1 and 2). As a result, metabolic syndrome in the all subjects was observed to either improve significantly or tended to improve. These results suggested that this food substitute had a beneficial effect on blood pressure, hepatic function, and lipid profiles.

The value of adiponectin in the blood, a marker of metabolic syndrome, was significantly increased (0.58 ± 0.64 μg/ml) and the PAI-1 value was decreased significantly (14.3 ± 23.4 μg/ml). From these results, it was anticipated that adiponectin expression, which is a beneficial adipocytokine, was increased in fat cells. This substance has been reported to have anti-inflammatory and anti-atherogenic properties [*22*]. Obese patients generally have low levels of adiponectin and hence may be deprived of its protective effects against metabolic syndrome [*23*, *24*]. While the levels of PAI-1, which is a non-beneficial adipocytokine, were decreased. Circulating PAI-1 increases in obese subjects with metabolic syndrome, as well as in patients with type II diabetes [*25*]. The levels of PAI-1 were higher in relation to metabolic syndrome. Interventional studies reported that if insulin resistance is improved, plasma PAI-1

levels decrease. Treatment with insulin-sensitizing drugs like metformin or thioglitazone decrease plasma PAI-1 levels in subjects with type II diabetes and to some extent in normal obese subjects [*26*, *27*].

In support of the present clinical study, evaluation of the fatty acid metabolism *in vivo* in obese mice was also performed and compared with obese mice treated by the food substitute Dr. BAANs® by analysis of the distribution of an $^{125}$I-labeled metabolite in the lipid pools of various tissues. In myocardium, skeletal muscle, visceral and subcutaneous fat, the final product of 9-MPA metabolite PIPA in obese mice was significantly increased by the dietary intervention, and it was indicated that this dietary supplement enhanced fatty acid β-oxidation in these tissues. The slower conversion in the obese mice was compatible with the very poor myocardial β-oxidation activity of palmitic acid. In this animal study, we fed the food substitute to the mice for only one week because we realize that body weight may be reduced after long dietary intervention in obese animals and thus results will not be reliable.

Finally, the safety of this dietary supplement in this human study was demonstrated by the absence of the elevation of AST levels throughout the evaluation period and also lack of reports of adverse effects in the population studied.

In conclusion, the present results demonstrate that this food substitute, which contains L-arginine, ω-3 polyunsaturated fatty acids, and RNA, may have a beneficial effect on the prevention or improvement of metabolic syndrome. These results suggest that this food substitute could be used as a supplement for overweight patients to prevent or improve metabolic syndrome; however, these findings deserve more research in view that the present study was performed in a small number of subjects and the short duration of the study.

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