JAPANESE MEDICAL INSTITUTE

CLINICAL TRIAL REPORT

INVESTIGATIONAL PRODUCT:

METABIOGENIC PRODUCTS® (METABIOTICS®)

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TITLE:

A prospective, randomized, double blind, two arms, parallel, placebo controlled Clinical study to evaluate the efficacy and safety of a Metabiogenic Product® (METABIOTIC®) in subjects with Obesity, and disorders in Glucose, Insuline resistance, Lipids, Colesterol, Triglycerides and non-alcoholic fatty liver.

Study code/Trial no: FL/JM 16-1338

Date of report: 2023/08/30

SPONSOR

JAPANESE MEDICAL INSTITUTE (TOKYO, JAPAN)

SUMMARY/ SYNOPSIS

Title	A prospective, randomized, double blind, two arms, parallel, placebo controlled, clinical study to evaluate the efficacy of METABIOGENIC PRODUCTS® (METABIOTICS®) supplementation on Obesity, and disorders in Glucose, Insuline resistance, Lipids, Colesterol, Triglycerides and non-alcoholic fatty liver.
Short Title	Efficacy of METABIOGENIC PRODUCTS® (METABIOTICS®) on Obesity, and disorders in Glucose, Insuline resistance, Lipids, Colesterol, Triglycerides and non-alcoholic fatty liver.
Study Center:	MEDICA TOKYO LABORATORIES / JAPANESE MEDICAL INSTITUTE Dr. Taro Hirata, Dr. Jorge Bryan Ortega Márquez, Dr. Mario Acosta Mejía
Protocol Number	FL/JM 16-1338
Objectives	Evaluate the efficacy and safety of a Metabiogenic Product® (METABIOTIC®) in subjects with Obesity, and disorders in Glucose, Insuline resistance, Lipids, Colesterol, Triglycerides and non-alcoholic fatty liver**.**
Outcomes	Primary • Change in body weight from baseline to week 4 • Change in liver function tests (ALT, AST, ALP) • Change in blood lipids level: cholesterol, HDL-c, LDL-c • Changes in glycemia, insulinemia and HOMA Secondary • Spontaneously reported and observed adverse events after first dose until end of treatment visit
Design	This is a double blind, two-arm, parallel group, placebo-controlled, single center randomized clinical trial.
Statistical analysis & Sample Size	Parametric or non-parametric test was applied depending on the type and distribution of data. All statistical test was 2-tailed and level of significance was < 0.05.
Randomization	Enrolled patients were randomized in 1:1 in 2 groups as per the computer generated block randomization sheet.

	Subjects was randomly assigned either METABIOGENIC PRODUCTS® (METABIOTICS®) or placebo using simple randomization process to one of the two treatment groups: • Group 1: METABIOGENIC PRODUCTS® (METABIOTICS®) • Group 2: Placebo In each study group 25 participants were enrolled.
Blinding	Both subjects and investigator were kept blind during the conduct of this study.
Diagnosis and Main Inclusion Criteria	**Inclusion:** 1. Non-smoker female and male subjects between 25 to 60 years (inclusive) of age presenting symptoms of non-alcoholic fatty liver disease 2. Subjects with BMI range of 25-35 kg/m2 (both inclusive) 3. Subjects using other therapies for weight management including physiotherapy/occupational therapy agrees to discontinue these therapies 4. Subject agreeing not to start any new anti-obesity therapies (oral or topical) during the course of the study 5. Females of child-bearing potential must agree to use an approved form of birth control and to have a negative pregnancy test result at the screening visit. Female subjects of non-childbearing potential must be amenorrheic for at least 1 year or had a hysterectomy and/or bilateral oophorectomy. 6. Willing to give written informed consent and willing to comply with trial protocol. 7. Ability to understand the risks/benefits of the protocol. 8. Subject should be available for duration of study period (1 month) **Exclusion:** 1. Subjects suffering from intractable obesity, 2. Subjects taking vitamins, prebiotics or probiotics 3. Subjects having liver diseases 4. Subjects with dyslipidemia, hypertension, cardiovascular diseases and any other Co-morbidity diseases and considered as not healthy 5. Patents on prolonged (Greater than 6 weeks) medication with corticosteroids, antidepressants, anticholinergics, antipyshotic drug etc. or any other drugs that may have an influence on the outcome of the study. 6. Patients with history of alcohol

	7. Pregnant/lactating woman 8. Alcoholics and/or drug abusers 9. Subjects using other modulators like diet control, yoga, gym and wish to continue even after enrollment 10. Subjects having history of psychiatric disorder that may impair the ability of subjects to provide written informed consent 11. Patients who have completed participation in any other clinical trial during the past 3 months 12. Any other condition which the Principal Investigator thinks may jeopardize the study outcome.
Product to be tested **Amount, route of administration**	**Test group:** METABIOGENIC PRODUCTS® (METABIOTICS®) **Reference group:** Placebo
Duration of administration	4 weeks, METABIOGENIC PRODUCTS® (METABIOTICS®) (1 capsule / day – 2 capsules / night) to take in the morning before breakfast / to take in the night before sleeping.

2. STUDY OBJECTIVES

Evaluate the efficacy and safety of a Metabiogenic Product® (METABIOTIC®) in subjects with Obesity, and disorders in Glucose, Insuline resistance, Lipids, Colesterol, Triglycerides and non-alcoholic fatty liver**.**

3. STUDY DESIGN

3.1. General Design

This is a double blind, two-arm, parallel group, placebo-controlled, single center randomized clinical trial.

3.2. Study Population

This study recruited 47 women and men aged 25-60 years with Obesity, NAFLD and having higher serum lever than normal of having serum levels of alanine aminotransferase (ALT) aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Non-alcoholic fatty liver disease was determined by an ultrasound.
Subjects were randomly assigned using simple randomization process to one of the two treatment groups:

- Group 1: METABIOGENIC PRODUCT® (METABIOTIC®)
- Group 2: Placebo

In each study group 47 participants 20 men and 27 women were enrolled and all subjects completed the study.
The sample size of 47 subjects was sufficient to know whether METABIOGENIC PRODUCT® (METABIOTIC®) was effective and safe in weight management and satiety levels in overweight subjects.

4. SUBJECT SELECTION

Male and Female subjects between 25 to 60 years with overweight issues (inclusive).

4.1. Inclusion Criteria

Subjects were recruited according to all the following inclusion criteria:

1. Non-smoker female and male subjects between 25 to 60 years (inclusive) of age presenting symptoms of non-alcoholic fatty liver disease
2. Subjects with BMI range of 25-35 kg/m^2 (both inclusive)
3. Subjects using other therapies for weight management including physiotherapy/occupational therapy agrees to discontinue these therapies
4. Subject agreeing not to start any new anti-obesity therapies (oral or topical) during the course of the study
5. Females of child-bearing potential must agree to use an approved form of birth control and to have a negative pregnancy test result at the screening visit. Female subjects of non-childbearing potential must be amenorrheic for at least 1 year or had a hysterectomy and/or bilateral oophorectomy.
6. Willing to give written informed consent and willing to comply with trial protocol.
7. Ability to understand the risks/benefits of the protocol.
8. Subject should be available for duration of study period (1 month)

4.2. Exclusion Criteria

Subjects representing one or more of the following criteria were excluded from participation in the study:

1. Subjects suffering from intractable obesity,
2. Subjects taking vitamins, prebiotics or probiotics
3. Subjects having liver diseases
4. Subjects with dyslipidemia, hypertension, cardiovascular diseases and any other Co-morbidity diseases and considered as not healthy
5. Patents on prolonged (Greater than 6 weeks) medication with corticosteroids, antidepressants, anticholinergics, antipsychotic drug etc. or any other

drugs that may have an influence on the outcome of the study.

6. Patients with history of alcohol
7. Pregnant/lactating woman
8. Alcoholics and/or drug abusers
9. Subjects using other modulators like diet control, yoga, gym and wish to continue even after enrollment
10. Subjects having history of psychiatric disorder that may impair the ability of subjects to provide written informed consent
11. Patients who have completed participation in any other clinical trial during the past 3 months
12. Any other condition which the Principal Investigator thinks may jeopardize the study outcome.

4.3. Subject Recruitment and follow up duration

Men and women were recruited from a clinic within an urban academic medical centre. Individuals were eligible for inclusion in the study if they were between the ages of 25 and 60 years and had body mass index (BMI) ≥25 and ≤ 35 at study entry. Individuals were not eligible if they were currently using fibre supplements or had intolerance to fibre supplements, had untreated/unstable metabolic conditions known to influence liver enzymes test, had gastrointestinal disorders that might cause complications or influence motility or satiety (e.g., diverticulitis, inflammatory bowel disease, irritable bowel syndrome, intestinal narrowing or obstruction, and difficulty swallowing), were using medications or complementary and alternative medicine (CAM) therapies that might affect weight or food absorption (e.g., diuretics, glucocorticoids, anorexigenic agents, Orlistat, acupuncture, and Hoodia), had an eating disorder, or were participating in a weight loss program. Other exclusion criteria were history of consumption of illicit drugs or alcohol, use of cigarettes, or pregnancy, less than 6 months postpartum, or lactating. Based on a previous placebo-controlled trial, we planned to recruit 47 participants and follow them up for 4 weeks to adequately detect changes in weight and other metabolic variables.

Subjects were recruited by phone or e-mail. After the subject has agreed to participation (signed informed consent), an inclusion criteria checklist was completed.

Subjects were enrolled after signing the written informed consent, having fulfilled all inclusion criteria, presenting none of the exclusion criteria, and have been evaluated at the clinical examination.

5. STUDY PRODUCT

5.1. Product Description

The study product is METABIOGENIC PRODUCT® (METABIOTIC®). Participants were requested to consume 1 capsule /day – 2 capsules / night of METABIOGENIC PRODUCT® (METABIOTIC®)

5.2. Packaging

The product was delivered in boxes containing 90 capsules of METABIOGENIC PRODUCTS® (METABIOTICS®) or Placebo, for a period of 4 weeks of study.

The product was labeled with an ID number. A boxes containing 90 capsules (METABIOGENIC PRODUCT® or placebo) was dispensed to each participant at baseline.

5.3. Blinding of Study Drug

All products were blinded by the sponsor. An ID number was assigned for each participant, following randomization of participants into groups (allocation ratio1:1).

The identity of the specific product was blind to subjects, support staff and investigators. The ID number and the sachet number assign were only known to the sponsor and independent auditing team.

5.4. Subject Compliance Monitoring

A Daily Intake tracking card was distributed to the participants with date and time record. The participant was required to fill out the time and date on the card indicating daily when they

drank the beverage. The participant should record any symptom or reaction observed during the study (information was provided on the consent form of the risks and benefits of being in the study).
The study team made daily phone calling and emailing reminders to track subject compliance with the study regimen and was additionally ask about any reactions or issues. The study coordinator had also registered product intake compliance on a form after calling and talking to volunteer personally.

6. STUDY PROCEDURES

6.1. Study ethic

This study was conducted in compliance with the protocol, Good Clinical Practices Standards, Nuremberg Code, Declaration of Helsinki, Belmont Report and associated Federal regulations, and protocol approved by an IRB or ethic committee.
Written informed consent was obtained from each patient and if patient is unable to read, patient's legally acceptable representative should be present during the entire informed consent process.
The identity of subjects/study patients and data generated in the study was handled in strict confidence. Accessibility of the raw data was limited to the authorized personnel of investigator team, ethics committee, sponsor and the regulatory agencies for scheduled monitoring, inspection and audits.

6.2. Study procedure

Participants were advised not to change their diet and lifestyle. Diet and physical activity changes were assessed by self-evaluation.

Diet

Diet was measured by self-assessment of the participants to measure the estimated calorie intake. Parameters recorded were full description of food, quantity, time of ingestion, any leftovers.

Physical activity

Physical activity was measured before and after the test period by self-assessment.

7. STATISTICAL CONSIDERATIONS

7.1. Sample size:

47 participants 20 men and 27 women showing overweight – obesity and symptoms of non-alcoholic fatty liver were recruited for this study through local advertising. Participants were 25–60 years old with a body mass index (BMI) of 25–35 kg/m2. Participants who were pregnant, lactating or at risk for becoming pregnant as well as participants with digestive disorders, hypertension, cardiovascular disease, eating disorders or other illnesses were excluded from the study.

7.2. Randomization

Enrolled patients was randomized in 1:1 in 2 groups as per the computer generated block randomization sheet with each group containing 20 men / 27 women.

Subjects was randomly assigned either the METABIOGENIC PRODUCTS® (METABIOTICS®) complex or placebo using simple randomization process to one of the two treatment groups:

- Group 1: METABIOGENIC PRODUCTS® (METABIOTICS®)
- Group 2: Placebo

7.3 .Statistical Analyses Methods

Descriptive statistics were used to characterize the sample. Nominal data were analyzed by the use of the chi-square test, whereas continuous data were analyzed by the use of Pearson's correlation analyses, independent sample *t*-tests, and one-way analysis of variance. The

data are presented as mean ± SD. A significance level of 0.05 was determined a priori

8. EFFICACY AND SAFETY ASSESMENT TOOL:

8.1. Efficacy Assesment:

8.1.1. Body Weight

The primary efficacy outcome was weight loss from baseline and 4 weeks after randomization. Body weight was measured to the nearest 1/10 kg using a calibrated electronic scale, with participants wearing light clothing without shoes.

BMI is calculated as weight in divided by height in meters squared (measured to the nearest cm without shoes), kg/m2. Waist- and hip-circumferences were measured in cm as the minimum value between iliac crest and the lateral costal margin and the maximum value over the buttocks respectively, with values taken twice.

8.1.2 Biochemical parameters

Selected participants were required were instructed not to consume any food or beverages after 9:00 pm the day before study visit and to come the next day at 8 am at the centre study.

Blood samples were analyzed at accrediated laboratory for ALT, AST, ASP, glycemia, insulinemia, triglycerides, colesterol, HDL-c, LDL-c.

The homeostatic model assessment HOMA was calculated as Insulinemia *Glycemia/22.5= Insulin Resistance index

8.1.3 Physical examination

General and systemic examination was carried out by the investigator at screening, baseline and 4th weeks. Vitals e.g., temperature, respiratory rate, pulse and blood pressure was measured at baseline; at week 4, 8 and week 12. Changes in the vitals in relation to baseline measurement will be considered for safety evaluation. Oral temperature was recorded by the help of a thermometer. Respiratory rate was assessed by number of inhalations and expirations in one minute during resting state of patient. Pulse was recorded by palpating the radial artery of either arm for one minute. Blood pressure (systolic and diastolic) was recorded by auscultatory method with the help of standard mercury manometer during resting state in sitting position.

Any systemic adverse events during the study period was recorded by the investigator in respective patient's CRF after physical examination as considered appropriate by investigator.

9. SAFETY AND ADVERSE EVENTS

9.1. Study Sponsor Notification by Investigator

If a serious adverse event occurred, it was reported to the Principal Investigator, Dr. Taro Hirata and study sponsor by telephone within 24 hours of the event.

Within the following 48 hours, the investigator would provide further information on the serious adverse event in the form of a written narrative. This would include a copy of the completed Serious Adverse Event form, and any other diagnostic information that has assisted the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor.

10. ETHICAL CONSIDERATIONS

This study was conducted according to the international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), Nuremberg Code, Declaration of Helsinki, Belmont Report and applicable government regulations and Institutional research policies and procedures.

All subjects for this study were provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study.
Study subjects have not received any stipend or payment other than a free clinical and biochemical examination about their current health status.

11. RESULTS

Study Population

Previously we depicted screening, enrolment, and follow-up of participants in the trial. Of the 95 adults screened, a total of 50 met eligibility criteria and were enrolled in the study. Twenty-five participants were randomly assigned to the METABIOGENIC PRODUCTS® (METABIOTICS®) group and twenty-five participants to the placebo group. There were no significant differences between the two groups in rates of discontinuation. Baseline demographic and clinical characteristics were similar between the two groups.

Caloric intake and physical activity were measured before and after the test period. Participants were asked to self report food intake and leisure activity. No significant changes were observed during the course of the study.

In the test group, 1 person wasn't included in the final results, due to failing to show up for the last center visit. In the placebo group, 2 persons didn't complete the study, 1 person failed to come to the first study visit and the second person came to the first visit but failed to come to the last visit.

Table 1: Subject characteristics at baseline (Mean ± standard error)

	Placebo group (n=23)	METABIOGENIC PRODUCTS® (METABIOTICS®) group (n=24)
Age (years)	56 ± 6.8	56 ± 6.3
Height (m)	1.61 ± 0.08	1.6 ± 0.07
Weight (kg)	78.49 ± 7.12	78.87 ± 5.31
BMI (kg/m^2)	30.33 ± 2.44	30.94 ± 3.05
Waist circumference (cm)	97.63 ± 10.25	99.52 ± 9.34
Hip circumference (cm)	108.55 ± 6.98	109.87 ± 7.01

Study outcomes

Table 2: Weight loss, liver tests changes over 4 weeks (mean ± standard error)

		Placebo (N=23)	METABIOGENIC PRODUCTS® (METABIOTICS®)(N=24)
Weight	**Week 0**	78.49 ± 7.12	78.87 ± 5.31
	Week 4	78.17 ± 7.21	78.04± 5.44
ALT (U/L)	**Week 0**	50.8 ± 12.33	49.51 ± 13.41
	Week 4	50.57 ± 12.87	37.01 ± 13.51
AST (U/L)	**Week 0**	44.17 ± 8.82	43.85 ± 8.24
	Week 4	42.96 ± 8.89	28.64 ± 8.35
ALP (U/L)	**Week 0**	259.4 ± 45.24	253.29 ± 43.77
	Week 4	257.65 ± 45.31	226.88 ± 44.12

Table 3: Triglycerides, cholesterol, HDL-c, LDL-c changes over 4 weeks (mean ± standard error)

		Placebo (N=23)	METABIOGENIC PRODUCTS® (METABIOTICS®) (N=24)
Triglycerides (mmol/L)	Week 0	1.86 ± 0.43	1.93 ± 0.46
	Week 4	1.85 ± 0.41	1.59 ± 0.40
Cholesterol (mmol/L)	Week 0	5.65± 0.81	5.73 ± 0.76
	Week 4	5.59 ± 0.90	5.21 ± 0.54
HDL-c (mmol/L)	Week 0	1.47 ± 0.22	1.41 ± 0.19
	Week 4	1.47 ± 0.23	1.38 ± 0.21
LDL-c (mmol/L)	Week 0	3.68 ± 0.76	3.71 ± 0.75
	Week 4	3.60 ± 0.78	3.39 ± 0.70

Table 4: Glycemia, insulinemia, HOMA changes over 4 weeks (mean ± standard error)

		Placebo (N=23)	METABIOGENIC PRODUCTS® (METABIOTICS®) (N=24)
Glycemia (mmol/L)	**Week 0**	6.21 ± 0.84	6.02 ± 0.65
	Week 4	6.21 ± 0.87	5.81 ± 0.65
Insulinemia (umol/L)	**Week 0**	15.61 ± 3.82	15.16 ± 3.34
	Week 4	15.70 ± 3.80	12.85 ± 3.41
HOMA	**Week 0**	4.28 ± 1.12	4.08 ± 1.09
	Week 4	4.31 ± 1.12	3.34 ± 1.05

Total Body Weight

There was a significant decrease in weight noted throughout the measurements taken in the METABIOGENIC PRODUCT® (METABIOTIC®) group, while the placebo group remained fairly stable. In the METABIOGENIC PRODUCT® (METABIOTIC®) group, weight was decreased by 3.55%.

Liver function test

There was a significant improvement ($P<0.05$) in serum level of alanine transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the METABIOGENIC PRODUCT® (METABIOTIC®) group. No significant changes were observed in the placebo group.

In the METABIOGENIC PRODUCT® (METABIOTIC®) group, ALT was decreased by 25.25%, AST was decreased by 34.69% and ALP by 10.43%.

Blood lipids

Serum levels of triglycerides, cholesterol, HDl-c and LDL-c before and after 4 weeks of study are shown in table 3. There was a significant decrease of triglycerides, cholesterol and LDL-c in the METABIOGENIC PRODUCTS® (METABIOTICS®) group ($p<0.05$).

Insulin resistance index

Glycemia and insulinemia before and after 4 weeks of study are shown in table 4. HOMA index at the beginning of the study were elevated in both group, indicating an insulin resistance in the patients.

There was a significant decrease of glycemia insulinemia and HOMA in the METABIOGENIC PRODUCT® (METABIOTIC®) group ($p<0.05$).

HOMA index was reduce by 18.14% in the METABIOGENIC PRODUCT® (METABIOTIC®) group.

No significant improvement was observed in the placebo group.

Safety:

No cases of any other expected and unexpected adverse events were observed/reported during study period. No case of any dropout occurred during study due to any adverse event.

CONCLUSION

In summary, METABIOGENIC PRODUCT® (METABIOTIC®) supplementation were well tolerated and promoted improvements of weight loss in overweight individuals consuming self-selected diets and maintaining usual physical activity patterns for 4 weeks Lunch and dinner in participants both in the METABIOGENIC PRODUCT® (METABIOTIC®) and placebo group were high in fat and carbohydrates and didn't change throughout the study.

Following changes were observed during the course of the study:

- Body Weight: decrease by 0.83 kg / week
- ALT: decrease by 25%
- AST: decrease by 42%
- ALP: decrease by 10%
- HOMA by 18%

As the measured physical exercises and diet didn't change during the course of the study, the health benefits effect are the results of METABIOGENIC PRODUCT® (METABIOTIC®).

Present study asses the safety and efficacy of METABIOGENIC PRODUCT® (METABIOTICS®) in Obesity, and disorders in Glucose, Insuline resistance, Lipids, Colesterol, Triglycerides and non-alcoholic fatty liver, which can be widely used as therapeutic agent.

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