VISCERAL FAT-REDUCING EFFECT OF SUPPLEMENT CONTAINING OLIVE LEAF EXTRACT (OLEAVITA) IN HEALTHY JAPANESE
— A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY —

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Abstract

Objectives: The objective of this research was to investigate the effectiveness of daily ingestion of a ME tablet, which contains olive leaf extract on BMI reduction and waist circumference reduction.
Methods: In this randomized, placebo-controlled, double-blind trial, 28 subjects were randomized. To evaluate this objective, visceral fat area and subcutaneous fat area were measured as the primary outcome. Weight, BMI, waist circumference and subjective reporting was also observed as the primary outcome.
Results: There was a significant difference in visceral fat area and BMI, and an improvement tendency in subcutaneous fat area and waist circumference compared to Placebo. In addition, sleeping quality showed significant difference with results from a questionnaire, in the intergroup comparison of 12-weeks of ingestion.
Conclusion: The present results suggest that daily ingestion of ME tablets containing olive leaf extract can reduce abdominal fat level, BMI and waist circumference.

Key Words: Olive leaf extract, obesity, BMI, abdominal fat

1. INTRODUCTION

Currently in Japan, the number of people who are considered as obese is rapidly rising. One of the main factors that trigger this problem is the changes in the social environment affecting eating habits such as the westernization of dietary habits and/or the inadequate exercise. Obesity is not simply defined as a state of excessive weight: it is also defined as a state of excessive body fat accumulation than a prescribed level. "Visceral fat obesity", a type of obesity that accumulates excessive fat to the abdominal cavity (inside of muscles), is thought to increase a risk of diabetes, high-blood pressure or abnormal lipid metabolism[1]. Metabolic syndrome is a state that people suffer from at least two diseases out of three (i.e. diabetes, high-blood pressure or abnormal lipid metabolism) concurrently[2].

Judging from the above, a prevention of obesity plays an important role in getting fit. For the prevention and treatment of obesity, it is necessary to improve the balance between energy intake (diet) and energy consumption (exercise); in other words, while reducing the energy intake from foods, we should boost the energy consumption. The simplest way for consuming energy should be doing exercise, but it is also practical to focus on the non-exercise activity thermogenesis (NEAT)[3] or increase the level of the resting metabolic rate (RTR). In the activity cycle of the intravital energy metabolism, sugar, fat and protein function as an ingredient for producing ATP (adenosine triphosphate) which works as a power source of humans. Therefore, an acceleration of the above cycle can contribute to the increase of the consumption level of energy[4].

On the other hand, as the contemporary society in Japan has chronic problem of health trouble such as obesity, many kinds of supplement focusing on improving our health condition are being sold. Recently, the olive leaf, which has been utilized as main food ingredient by Mediterranean area since ancient times, is drawing people's attention as functional food ingredients. It is considered that this attention is attributable to the report that the olive leaf extract contains functionalities such as hypoglycemic activity[5], vasodilator effect[6] and antioxidant activity[7]. In addition, the olive leaf extract is thought to have a close relationship with the adjustment function of energy metabolism[8]. Therefore it is expected to contribute to human's method of energy consumption, and eventually achieve some functionality for solving the obesity problem among modern people.

However, there are only a few reports that scrutinize the functionalities of olive leaf for humans, from a standpoint of prevention or improvement of obesity.

In this study, we elucidated the BMI reduction and waist circumference reduction effect of ingesting food

Table 1 Schedule for the study

Item \ Term	Screening	Pre Trial Test	Test period		
			4 w	8 w	12 w
Informed consent	●				
Selection and/or allocation	●				
Abdominal fat level	●	●			●
Anthropometric measurements	●	●			●
Subjective reporting		●	●	●	●
Ingestion of test foods			↔	↔	↔
Log			↔	↔	↔

● : Implementation
↔ : Daily practice during the test period

containing olive leaf extract (OreaVita), by adopting a randomized, placebo-controlled, double-blind study.

2. METHODS

2.1. Trial design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from twenty-four seven Inc. (Tokyo) at two centers (KUROSU HOSPITAL, Tokyo and JACTA, Tokyo). The study period was 12 weeks, from November 24th, 2015 to February 18th, 2016. This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all Subjects.

The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subject

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of CROee Inc. (Tokyo), recruited from October to November, 2015.

2.2.1. Inclusion criteria

(1) Healthy females aged between 35 and 59 years.

2.2.2. Exclusion criteria

(1) Individuals with a BMI $\geqq 30.0$ or < 22.0
(2) Individuals undergoing treatment of weight reduction;
(3) Individuals taking medication, including herbal medicines;
(4) Individuals judged to be unsuitable to participate in the trial by the doctor conducting the present study.

2.3. Randomization

Recruited subjects were 110 persons. Subjects who fulfilled eligibility criteria were 34 persons. The inclusion criteria were judged by the principle investigator.

All subjects were sequentially assigned based on a random number table to one of the masked products and randomized to group T (Test sample: 15) and group P (Placebo: 13). In the process of subject assignment, background factors such as age and BMI were taken into consideration to avoide biased distribution.

2.4. Description of test foods and blinding

The test food, "Megasurari ("ME")" and placebo were prepared by twenty-four seven Inc. The amount of daily intake was 2 tablets per day (1 tablet weighs 385 mg, therefore 2 tablets weigh 770 mg). The compositions of ME were olive oil, olive leaf extract (OreaVita), Fucoxanthin containing seaweed extract, etc., while Placebo was mainly consisted of Medium-chain triglyceride. Both tablets were indistinguishable in shape, color, odor or taste, and were managed by an identification symbol. All involved were blinded.

2.5. Experimental procedures

2.5.1. Experimental protocol

Subjects consumed 2 tablets of the supplement with hot or cold water every day for 12 weeks. Subjects were instructed as follows: to take the assigned foods as indicated; to maintain their usual lifestyles and habits; to avoid excessive amounts of food, drink, or alcohol; to maintain a daily record of lifestyle factors such as all the food items or number of steps they took for the day during the test period.

2.5.2. Outcome

The objective of this study is to elucidate the BMI reduction and waist circumference reduction effect of ingesting food containing olive leaf extract (OreaVita). To evaluate this objective, visceral fat area and subcutaneous fat area, and questionnaire were tested as the primary outcome.

The abdominal fat level was measured by means of CT images provided by Bright Speed (GE Healthcare). Several CT images around the umbilicus were obtained and single scan images at the precise point of the umbilicus were used for analysis. The visceral fat area and subcutaneous fat area was calculated from CT images using Power View Fat software (Vigoment Software Corp., Tokyo).

Height, body weight and BMI were scaled by AD-6228AP (A&D Company, Limited, Tokyo). Waist circumference, at the umbilical level, was measured

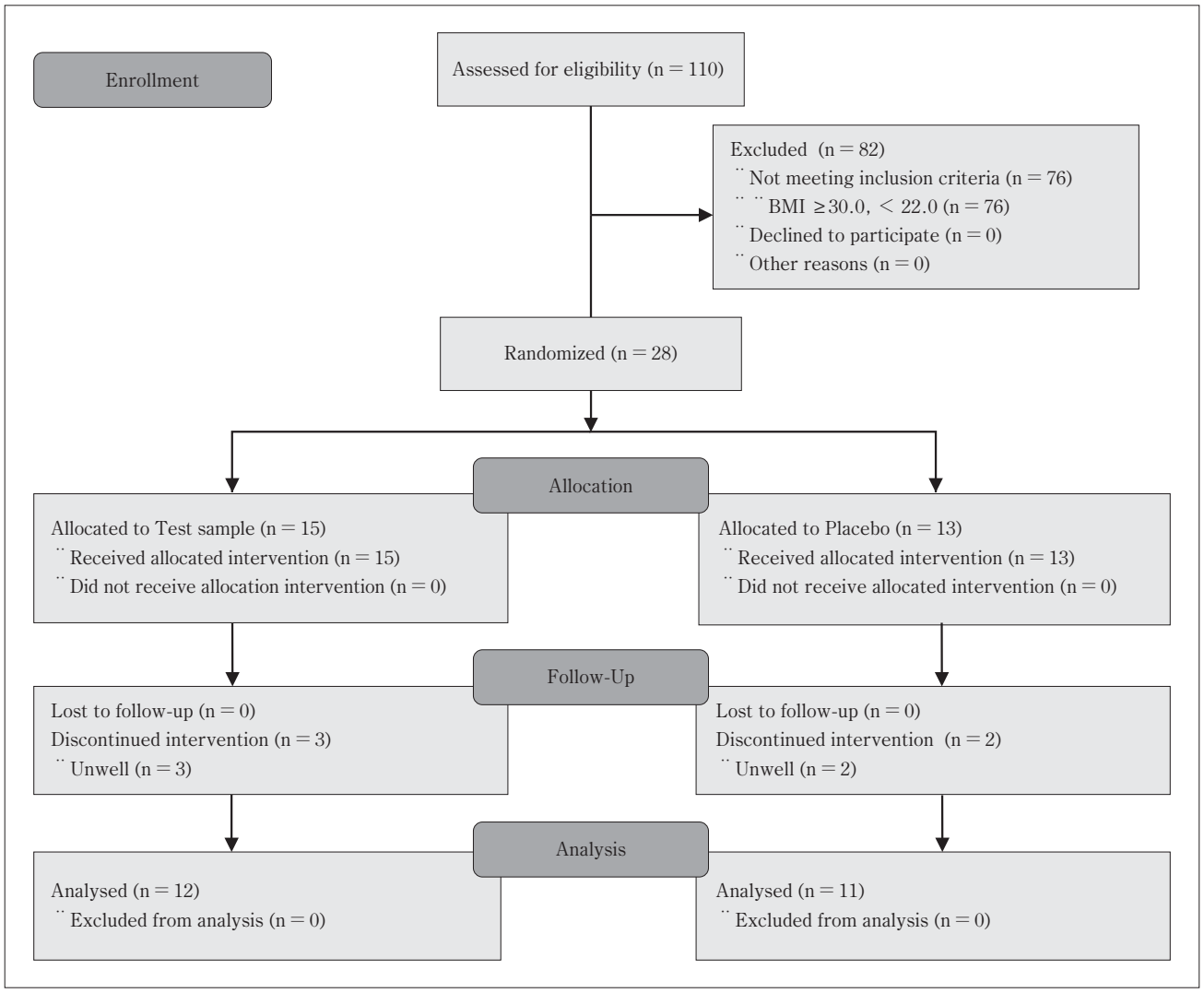

Fig. 1　Flow diagram of subject disposition

using a non-elastic anthropometric tape measure.

For subjective reporting, a questionnaire covered: Bowel movement, Sensitivity to cold, Clothing size variation, Dryness of skin, Fatigue, Mood, Frequency of activity, Sleeping quality, and Feeling lighter. Responses to each question were rated on an ordinal scale of 1 to 9, with higher scores indicating a better result.

To evaluate the safety of the test foods, adverse events were collected by means of a written questionnaire during the study.

According to the schedule shown **Table 1**, parameters on efficacy and safety were measured.

2.6. Data analysis

A full analysis set was adopted in the study and no sample size design was used. All statistics were expressed as mean ± standard deviation (SD).

Regarding the abdominal fat level, anthropometric measurements, and subjective reporting, changes from the baseline in the same group were assessed using the paired t-test. Student t-test was used for intergroup comparisons of changes from the baseline. Student t-test was used to compare subject backgrounds between groups. Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis.

Statistical analyses were performed using Statcel 3 (Yanai, 2011). The results were considered significant at the < 5% level in the two-sided test.

Table 2　Subject demographics

Item	Unit	ME	Placebo
Subjects	numbers	12	11
Age *	years	46.0 ± 7.0	46.5 ± 6.9
BMI *	kg/m^2	24.8 ± 1.7	25.3 ± 1.9

mean ± SD

* No significant differences

Table 3 Changes in abdominal fat areas, BMI, weight, and waist circumference

Item	Unit	Time points	Values		P-value [2]
			ME (n = 12) [1]	Placebo (n = 11) [1]	
Visceral fat area	cm^2	Baseline	84.2 ± 42.7	78.5 ± 34.6	
		12 - week	72.5 ± 43.1 **	76.7 ± 35.1	
		⊿ 0 - 12 w	− 11.7 ± 8.3	− 1.8 ± 13.9	0.049 #
Subcutaneous fat area	cm^2	Baseline	228.7 ± 48.2	211.3 ± 80.1	
		12 - week	217.7 ± 53.6 *	215.0 ± 79.0	
		⊿ 0 - 12 w	− 10.9 ± 15.6	3.7 ± 18.8	0.054 ‡
BMI	kg/m^2	Baseline	24.8 ± 1.7	25.3 ± 1.9	
		12 - week	24.6 ± 1.5	25.6 ± 2.0	
		⊿ 0 - 12 w	− 0.2 ± 0.5	0.3 ± 0.6	0.030 #
Weight	kg	Baseline	60.1 ± 5.5	64.0 ± 6.3	
		12 - week	59.5 ± 4.9	64.5 ± 6.6	
		⊿ 0 - 12 w	− 0.6 ± 1.4	0.5 ± 1.5	0.073 ‡
Waist circumference	cm	Baseline	89.3 ± 7.0	87.4 ± 7.1	
		12 - week	87.2 ± 6.0 **	87.7 ± 6.3	
		⊿ 0 - 12 w	− 2.1 ± 3.1	0.2 ± 2.9	0.078 ‡

Values are expressed as the mean ± SD.

1) * $p < 0.05$, ** $p < 0.01$ against baseline.

2) ‡ $p < 0.1$, # $p < 0.05$ between-group difference in change from baseline.

3. RESULTS

3.1. Participant demographics

The 28 subjects were randomly assigned to an intervention group and made a start with ingestion. 5 were withdrawn due to being unwell and the remaining 23 subjects completed the study. Thus, data obtained with 23 subjects (ME; 12, Placebo; 11) was used for the analysis of efficacy (**Fig. 1**). There were no significant differences in the age or BMI between groups (**Table 2**).

3.2 Abdominal fat level

Table 3 shows changes in abdominal fat areas. For the change, visceral fat area of the ME decreased significantly compared with the Placebo after 12 weeks of ingestion.

3.3. BMI, weight, and waist circumference

Table 3 shows the results of test analyses. After 12 weeks of ingestion, ME showed a significant difference in visceral fat area, subcutaneous fat area, and waist circumference, whereas Placebo showed no significant difference. Furthermore, significant difference was observed between the two groups of the change in visceral fat areas, and BMI after 12 weeks of ingestion. Subcutaneous fat area, weight, and waist circumference tended to decrease in the between-group comparison.

3.4. Questionnaire analyses

The results of questionnaire analyses are shown in **Table 4**. After 12 weeks of ingestion, ME showed a significant difference in "Bowel movement", "Dryness of skin", "Mood", "Frequency of activity", "Sleeping quality" and "Feeling lighter", whereas Placebo showed in "Sensitivity of cold", "Clothing size variation" and "Frequency of activity". In addition, in the between-group comparison there was a significant difference in "Sleeping quality" after 12-week of ingestion.

3.5. Safety

No adverse effects associated with the test product were observed in the course of the reporting.

4. DISCUSSION

We conducted a randomized, placebo-controlled, double-blind study for examining the efficacy of a supplement containing olive leaf extract (OreaVita). The objective of this study is to verify the weight reduction and waist circumference reduction effect of the supplement. As the primary outcome, the study showed the significant differences in visceral fat area and BMI compared to Placebo. It also showed an improvement tendency in subcutaneous fat area and waist circumference. In addition, several categories on the questionnaire conducted to search the subjective evaluation among ME, showed the improvement tendency, whereas the category "Sleeping quality" showed the significant differences in the between-group comparison. This result indicates the improvement of QOL. Furthermore, no adverse effects associated with ME were observed in the course of the reporting.

Table 4　Results of questionnaire analyses

Item	Time points	Scores		P-value [2]
		ME (n = 12) [1]	Placebo (n = 11) [1]	
Bowel movement	Baseline	5.2 ± 1.2	5.1 ± 1.4	
	4 − week	5.5 ± 1.2	4.5 ± 1.5	
	⊿ 0 − 4 w	0.3 ± 1.2	− 0.5 ± 1.4	0.110
	8 − week	5.8 ± 1.3	5.4 ± 1.4	
	⊿ 0 − 8 w	0.7 ± 1.6	0.3 ± 1.2	0.516
	12 − week	6.3 ± 0.8 *	5.6 ± 1.6	
	⊿ 0 − 12 w	1.2 ± 1.6	0.5 ± 1.6	0.357
Sensitivity to cold	Baseline	4.3 ± 1.6	4.8 ± 1.3	
	4 − week	4.9 ± 1.5 *	4.7 ± 1.3	
	⊿ 0 − 4 w	0.7 ± 0.9	− 0.1 ± 0.5	0.023 #
	8 − week	4.8 ± 1.9 †	5.3 ± 1.3	
	⊿ 0 − 8 w	0.5 ± 0.9	0.5 ± 1.2	0.919
	12 − week	5.3 ± 1.9 †	5.7 ± 1.6 *	
	⊿ 0 − 12 w	1.0 ± 1.6	0.9 ± 1.3	0.883
Clothing size variation	Baseline	4.4 ± 1.0	4.7 ± 0.5	
	4 − week	4.8 ± 0.9 †	4.9 ± 0.3	
	⊿ 0 − 4 w	0.4 ± 0.7	0.2 ± 0.4	0.325
	8 − week	5.3 ± 0.5 †	5.4 ± 0.5 **	
	⊿ 0 − 8 w	0.8 ± 1.3	0.7 ± 0.5	0.806
	12 − week	5.4 ± 0.8 †	5.3 ± 0.6 *	
	⊿ 0 − 12 w	1.0 ± 1.6	0.5 ± 0.7	0.393
Dryness of skin	Baseline	4.2 ± 0.9	5.2 ± 1.3	
	4 − week	4.5 ± 1.1	5.4 ± 1.1	
	⊿ 0 − 4 w	0.3 ± 0.7	0.2 ± 1.4	0.739
	8 − week	5.0 ± 0.9 *	5.4 ± 1.1	
	⊿ 0 − 8 w	0.8 ± 0.9	0.2 ± 1.3	0.186
	12 − week	5.2 ± 0.9 *	5.6 ± 1.3	
	⊿ 0 − 12 w	1.0 ± 1.5	0.5 ± 1.4	0.391
Fatigue	Baseline	4.1 ± 1.0	4.9 ± 1.8	
	4 − week	4.5 ± 0.7 †	5.2 ± 1.0	
	⊿ 0 − 4 w	0.4 ± 0.8	0.3 ± 1.5	0.772
	8 − week	5.3 ± 1.0 *	5.8 ± 0.8	
	⊿ 0 − 8 w	1.2 ± 1.3	0.9 ± 1.8	0.695
	12 − week	5.2 ± 1.3 †	5.5 ± 1.2	
	⊿ 0 − 12 w	1.1 ± 1.8	0.5 ± 1.6	0.453
Mood	Baseline	4.5 ± 1.0	5.2 ± 1.5	
	4 − week	4.8 ± 0.4	5.5 ± 1.6	
	⊿ 0 − 4 w	0.3 ± 0.8	0.3 ± 0.6	0.842
	8 − week	5.6 ± 0.5 *	6.1 ± 0.9 *	
	⊿ 0 − 8 w	1.1 ± 1.2	0.9 ± 1.0	0.721
	12 − week	5.7 ± 1.1 *	5.5 ± 1.1	
	⊿ 0 − 12 w	1.2 ± 1.8	0.4 ± 1.4	0.253
Frequency of activity	Baseline	4.5 ± 0.8	5.0 ± 1.2	
	4 − week	4.8 ± 0.8	5.2 ± 1.1	
	⊿ 0 − 4 w	0.3 ± 0.7	0.2 ± 0.8	0.610
	8 − week	5.4 ± 0.7 **	6.0 ± 1.0 *	
	⊿ 0 − 8 w	0.9 ± 0.9	1.0 ± 1.3	0.856
	12 − week	5.7 ± 1.1 *	6.1 ± 1.1 *	
	⊿ 0 − 12 w	1.2 ± 1.5	1.1 ± 1.5	0.904
Sleeping quality	Baseline	4.6 ± 1.1	6.1 ± 1.6	
	4 − week	5.2 ± 0.8 *	5.7 ± 1.7	
	⊿ 0 − 4 w	0.6 ± 0.7	− 0.4 ± 0.9	0.0099 ##
	8 − week	5.6 ± 0.7 **	6.4 ± 1.3	
	⊿ 0 − 8 w	1.0 ± 1.0	0.3 ± 0.9	0.090 ‡
	12 − week	5.9 ± 1.0 *	6.2 ± 1.6	
	⊿ 0 − 12 w	1.3 ± 1.6	0.1 ± 1.2	0.047 #
Feeling lighter	Baseline	3.8 ± 1.1	4.7 ± 0.8	
	4 − week	4.3 ± 1.0 **	4.5 ± 1.1	
	⊿ 0 − 4 w	0.6 ± 0.5	− 0.2 ± 0.8	0.009 ##
	8 − week	5.2 ± 0.7 **	5.5 ± 1.3 *	
	⊿ 0 − 8 w	1.4 ± 1.2	0.8 ± 0.9	0.199
	12 − week	5.3 ± 1.1 *	5.3 ± 1.6	
	⊿ 0 − 12 w	1.6 ± 1.8	0.5 ± 1.2	0.128

Scores are expressed as the mean ± SD.
1) † $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
2) # $p < 0.05$, ## $p < 0.01$ between-group difference in change from baseline.

Main Findings

In this study we examined the changes of visceral and subcutaneous fat area, BMI, weight and waist circumference of ME group after 12-week of ingestion of ME, compared to Placebo group. After 12 weeks, ME showed significant differences in the visceral fat area, subcutaneous fat area and waist circumference. On the other hand, the comparison between ME and Placebo showed significant differences in the visceral fat area and BMI, and showed significant improvement in the subcutaneous fat area, weight and BMI. In addition, as for the result of the questionnaire conducted to search the subjective evaluation, ME showed the significant improvement in six positive categories other than categories of "Sensitivity to cold", "Clothing size variation" and "Fatigue", and showed the between-group significant difference in "Sleeping quality". On the other hand, Placebo showed the significant differences in "Sensitivity to cold", "Clothing size variation" and "Frequency of activity" after 12-week of ingestion. Overall, it was indicated that the ingestion of ME contributed to the improvement of QOL.

The test product (ME) contains the olive leaf extract. It is believed that olive is the oldest herb historically: it is an oleaceous evergreen tree which has been cultivated since around B.C.3000 to B.C.2000. The main part used has been the fruit of olive, and its oil, which contains a lot of monounsaturated fatty acid known as oleic acid, has been widely used since ancient times.

The leaves, on the other hand, have been practically utilized as the ingredient of tea, its frequency of usage was lower than the fruits though. However, the function of the leaves started to be recognized after oleuropein, a type of polyphenol, was discovered from the olive leaf[9]. It is reported that oleuropein has a function of inhibiting plasma triacylglycerol elevation[10] and the reabsorption of bile acid in the intestine[11]. In addition, it is known that the bile acid induces expression of uncoupling protein 1 (UCP-1) existing in the inner mitochondrial membrane, through the intermediary of G-protein-coupled receptor (TGR 5) which functions as a receptor of the bile acid in the brown adipocyte, and eventually enhances the metabolic heat production[12][13]. There is also a report that emphasizes oleanolic acid, the triterpene contained in the olive leaf, is an agonist of TGR5 and therefore contributes to the energy metabolism[14]. In addition, the other study finding illustrates that oleuropein induces expression of UCP-1 in the brown adipocyte and promotes the secretion of noradrenaline and adrenaline[15]. Furthermore, there is a report that oleanolic acid significantly downregulates ACC, the key gene for fat synthesis, which could explain the inhibitory effect of oleanolic acid on fat production[16].

All of the above explanation about the functional mechanism support that the olive leaf extract is equipped with a wide variety of functions from conditioning of energy metabolism to metabolic heat production, and therefore it activates lipid metabolism. There are many in-vivo study reports that have resulted in similar findings[17][18].

Therefore, it is considered that in this study, the olive leaf extract contained in ME contributed to the activation of metabolism and improved the conditions of BMI and abdominal fat of the test subject. Although the subcutaneous fat level did not improved significantly compared to the visceral fat, this result could be explained by the fact that visceral fat is burnt prior to subcutaneous fat in lipid metabolism, and therefore the 12-week test period was too short for the consumption of subcutaneous fat. In addition, the positive results about QOL such as "Sleeping quality" may be yielded by the function of ME to activate the energy metabolism.

Secondary Findings

In this study, adverse events were collected by means of a written questionnaire during the study, and no abnormal change caused by ME was observed during the ingesting period. During the test period five test subjects discontinued the test. The reasons of discontinuance were personal ones such as illness (catching a cold), and it has nothing to do with the ingestion of ME.

These results indicated the safety of the ingestion of ME for the 12-week test period.

General Information

It is obvious that the cause of obesity is the lack of exercise and/or the decrease of energy consumption as well as overeating. As people get older and the skeletal muscle mass decreases, the level of energy metabolism will decline. As a result, if we eat as much as our youthful days, the surplus fat accumulates in our body and leads to the middle-aged obesity.

It is generally said that the basal metabolic expenditure consists of about 60% of total energy consumption of human body[19]. This fact means that if we increase the level of the basal metabolic expenditure, we can solve the problem of obesity without excess caloric restriction. Obesity not only leads to the diseases such as "lifestyle diseases", but also causes deterioration of the external appearance and eventually it can trigger serious degradation of QOL. If it is possible to improve the level of the energy metabolism by ingesting supplements as a mean for preventing or solving the obesity problem, it may contribute to the improvement of our health condition and QOL.

Limitations

For this study we used the food (supplement) containing the olive leaf extract which has recently started to draw attention of researchers. In the extract there are several functional ingredients found such as oleuropein or oleanolic acid, but these ingredients are under investigation and there are still many uncertainties as to their functional mechanism inside living organisms. Therefore, the mutual influences between ME and other food products is a matter of speculation.

In addition, the number of the test subjects was 23 (ME: 12, Placebo: 11) and it is rather small. Since there is a report that the functionality of the olive leaf extract tends to vary depending upon its blending methods or the sex of subject [20], it is desirable to further scrutinize its functions adopting a wider variety of test subjects and test methods.

5. CONCLUSION

In conclusion, we found out that the ingestion of the supplement (ME) containing the olive leaf extract for 12 weeks contributed to the decrease in abdominal fat level and BMI level, and the waist circumference reduction. In addition, no safety-related matter occurred during the 12-week test period.

CONFLICT OF INTEREST

All parts of this study were funded by twenty-four seven Inc. Hiroo Kojima is the principal. All authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

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