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The potency of Berastagi sweet orange (*Citrus sinensis*) peel extract for obesity treatment: An experimental study in male rats

WINDA RIZKI PEBRINA BATUBARA[1], TRI NUGRAHA SUSILAWATI[2,♥], DONO INDARTO[1,3,4]

[1]Clinical Nutrition Postgraduate Program, School of Graduates, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia
[2]Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia.
Tel./Fax.: +62-271-664178, ♥email: tri.susilawati@staff.uns.ac.id
[3]Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia
[4]Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia

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Abstract. *Batubara WRP, Susilawati TN, Indarto D. 2023. The potency of Berastagi sweet orange (*Citrus sinensis*) peel extract for obesity treatment: A study in the rats. Nusantara Bioscience 15: 251-257.* Obesity is an abnormal accumulation of body fat and is a trigger factor for various degenerative diseases. Obese continues to grow every year in the world. This study aimed to investigate the effects of Orange Peel Extract (OPE) on Body Weight (BW), obesity index (Lee Index), Body Fat Percentage (BFP) and visceral fat in obese-model rats. Thirty male Wistar rats were divided into 5 groups: controls (negative/NC given aquadest and positive/PC given orlistat 12.3 mg/kgBW/day) and interventions (OPE 1-3) given OPE 250, 500 and 750 mg/kgBW/day, respectively. All rats were fed a high-fat, high-fructose (HFHFr) diet for 28 days. Statistical analysis was performed using ANOVA and Friedman test with $p<0.05$. The average BW in all groups increased significantly after 14 and 28 days of the intervention ($p=0.005$). A significant decrease in the obesity index average was found in the OPE 2 and 3 groups ($p=0.028$ and $p=0.034$) compared to the NC group. The OPE 1-3 and PC group significantly decreased in average BFP compared with the NC group on the 28th day of intervention ($p<0.05$). In conclusion, OPE can reduce obesity induced by HFHFr. Future research could be directed to evaluate the OPE side effects for obesity treatment.

Keywords: Berastagi sweet orange, *Citrus sinensis* L., obesity, orange, orange peel extract

INTRODUCTION

Obesity is one of the epidemic diseases that has continued to increase over the years (Haththotuwa et al. 2020). It had been predicted that by 2022, 38% of the world's population aged >5 years will be obese with a Body Mass Index (BMI) ≥25 kg/m² (Lobstein et al. 2023). Obesity in men is characterized by body fat percentage (BFP) >25% and in women >35% (Chusyd et al. 2016). Obesity is a condition of abnormal body fat accumulation caused by unbalanced energy intake (Chusyd et al. 2016; Panuganti et al. 2023). In addition, several other factors cause obesity, including genetic factors, hormonal and psychological factors, gut microbiota, diseases and drugs, chemical exposure and the environment (Safaei et al. 2021). Not only changing body image (Godoy-Izquierdo et al. 2020; Gow et al. 2020), obesity is also the cause of several chronic diseases such as diabetes mellitus, dyslipidemia, cancer and heart disease (Ramón-Arbués et al. 2019; Friedenreich et al. 2021). Fat accumulation in the body can occur when caloric intake exceeds the amount of calories burned. Fat accumulation in subcutaneous adipose tissue, skeletal muscle, liver, and other metabolic organs can increase Body Weight (BW) (Frank et al. 2019). The accumulation of visceral fat can also reduce adiponectin levels resulting in reduced oxidation of triglycerides in adipose tissue and liver, called adiposopathy. This causes an increase in body weight beyond normal limits, which over time can become obesity (Fonseca et al. 2020).

Obesity treatment involves pharmacologic therapy, non-pharmacologic therapy, and bariatric surgery (Wolfe et al. 2016). Commonly used non-pharmacological treatments involve various dietary methods such as *kenyang* diets (Isyrofi et al. 2022), and low macronutrient diets, such as low fat or low carbohydrate diets combined with exercise. Non-pharmacological treatments are usually effective but often fail because patients do not follow the program consistently (Higuera-Hernández et al. 2018). Pharmacological therapy with drugs is also less effective because it usually requires higher costs and prolonged treatment (Kosmalski et al. 2023). At the same time, bariatric surgery can be performed if the BMI criteria is ≥40 kg/m2. However, the surgery costs a lot of money and poses side effects such as causing anaemia (Fink et al. 2022). Those are why other alternatives are needed to treat obesity, and hopefully, they will be more effective and efficient than other treatments. Therefore, the use of phytochemicals in natural materials should be considered.

Citrus fruits belonging to the Rutaceae family are among the most abundant fruits in the world (Zayed et al. 2021). Berastagi sweet orange (*Citrus sinensis* L.) is one of the oranges that are easy to find and spread in almost all regions in Indonesia. Previous researchers worldwide have found that citrus peels have a variety of substances (vitamins, minerals, and phytochemicals) that have good health benefits. Still Berastagi orange peel has not yet been investigated regarding its potential in reducing variable obesity. However, citrus processing produces a large amount of waste every year. It is known that orange peel is the main waste of citrus fruit, causing serious environmental pollution if not handled properly. Sweet orange peel waste is one of the natural materials that contain various nutrients that are not healthy. Nutrients found in sweet orange peel extract include vitamin C and magnesium (Batubara et al. 2023) and the phytochemical substances in flavonoid glycosides, phenolic compounds, pectin, and essential oils (Li et al. 2019; Huang et al. 2020), which are thought to have beneficial effects for the treatment of obesity. This study aimed to investigate the effects of sweet Orange Peel Extract (OPE) administration on BW, Lee Index, BFP and visceral fat in rats on a high-fat, high-fructose (HFHFr) diet.

MATERIALS AND METHODS

Extraction of sweet orange peel

Sweet orange peels were extracted from previous research (Batubara et al. 2023). The sweet orange used was originally from Berastagi, Sumatera, Indonesia and the fruits were obtained from a traditional market in Surakarta, Central Java, Indonesia. Berastagi sweet orange has the following specifications; round-shaped orange fruit with an average weight of 190-490 grams; the diameter of the fruit is 7.2-10.3 cm; the surface color is yellow, yellowish green and orange; the skin tends to be shiny; the surface texture is slightly smooth to rough; the skin is relatively thick 6.2-7.75 mm and more difficult to peel. Other characteristics include smooth skin surface, fragrant aroma, and sweet taste. The preparation of OPE using the maceration method carried out at the Phytochemistry Laboratory of Universitas Setia Budi had been performed previously (Depari et al. 2021) with minor modifications. Fresh sweet orange peels were cut and dried at room temperature for five days. About 1500 grams of sweet orange peel is crushed to produce about 1360 grams of simplicia. The simplicia was then dissolved in 96% ethanol (Shisam Mas Chemical Pharmacy) in a ratio of 1:10 for two days with occasional stirring. The ethanol as a solvent was referred to previous research, which stated that nutritional content from orange peel, such as vitamin C and magnesium dissolved in ethanol solvent (Uçak and Khalily 2022). The simplicia was then filtered using filter paper and put into a rotary evaporator (IKA RV 8 series with semi-automatic model and power intensity of 24 V made in USA) with a temperature of 70°C and a rotation of 70 rpm to evaporate the ethanol in the extract and then, put into oven blower (Binder WTB FD 56 made in Indonesia) to get a thick extract. The extract was weighed with an analytical balance, and the yield (%) was calculated. The OPE was stored in glass bottles and kept at 2-4°C before further analysis.

Animals and experimental design

The laboratory of Universitas Sebelas Maret, Central Java, Indonesia, provided laboratory experimental research using male albino rats. The samples in this study were 30 white male Wistar rats (*Rattus novergicus*) aged 4-5 weeks, weighed 100-200 g, in good health, with normal activity and behavior, and no anatomical abnormalities. The number of samples was calculated using the formula of Arifin and Zahiruddin (2017) n=DF/(k+1), with DF represents minimum (10) or maximum (20), k number of groups, and n is the number of samples in each group, then the maximum value plus 20% reserved. Based on the formula, the number of samples in this study was 6 rats/group. The selected male rats were randomly divided into 5 groups: Negative Control (NC), Positive Control (PC), and three intervention groups (OPE 1-3). Animal maintenance was carried out in the Technical Implementation Unit of The Integrated Laboratory of Universitas Sebelas Maret. All rats were housed in one room with a 12-hour light-dark cycle at a room temperature of 23±2°C and humidity of 50±10%. All rats were acclimatized by feeding standard comfeed BR-2 (PT. Japfa Comfeed Indonesia Tbk.) and drinking ad-libitum for seven days before treatment. The Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret, approved the research protocol with Ethical Clearance No. 89/UN27.06.11/KEP/EC/2023.

OPE intervention

Preparation of HFHFr feed referred to previous research (Sundari et al. 2022). HFHFr was prepared in the Technical Implementation Unit of The Integrated Laboratory of Universitas Sebelas Maret from a mixture of 32 grams BR-2 feed, 28 grams duck egg yolk, 40 grams beef tallow, 12 grams chicken liver, and 4 grams butter and it consisted of 54.64% fat and 10% fructose. All rats were fed HFHFr (10% body weight) for 28 days. After the rats were obese (Lee Index >300), each group underwent further experiment for 28 days; i.e.: OPE groups 1-3 were given OPE 250, 500 and 750 mg/kgBW/day, respectively; the PC group was given orlistat (Novell Pharmaceutical Laboratories) 12.3 mg/kgBW/day, and NC group was given aquadest. All test materials were diluted in 2 mL of aquadest and then given to the test animals orally using a blunt needle sonde every morning. The dose of OPE given refers to research conducted by Depari et al. (2021) with an effective dose of 1000 mg/kgBW in reducing sugar levels in obese rats accompanied by diabetes mellitus, besides that, the dose of orlistat is a standard dose from humans converted to rats (Ardiansyah et al. 2018).

Measurement of BW, Lee Index, BFP, and visceral fat

The BW and naso-anal length of rats were measured and recorded weekly. The measurements of BW and naso-anal length were taken using digital scales (Joil accuracy rate of 0.1 gr, PT Arta Joil Tappa, Indonesia) and tape measure (Onemed PT. Jayamas Medica Industri Tbk Indonesia). Lee Index and BFP measurements were obtained with the following formula (Kora et al. 2000; Lee et al. 2011):

Lee Index (Obesity index) =
{Body weight (g)1/3/Naso-anal length (cm)} × 102
TM index = {Body weight (g)1/3/Naso-anal length (cm)}
Body fat content = 0.581 × TM index-22.03

Visceral fat measurement was performed on the last day of the intervention. Rats were sacrificed, then dissected, and wet fat on the abdominal part of the rat was taken and weighed using digital scales (Joil accuracy rate of 0.1 gr, PT Arta Joil Tappa, Indonesia).

Statistical analysis

The data obtained were processed using SPSS (Statistical Program for Social Science) version 22 and presented as mean ± Standard Deviation (SD). Normal distributed data such as Mean BW and BFP between groups were analyzed using a one-way Analysis of Variance (ANOVA) and followed by a Least Significance Different (LSD) test. The duration of OPE intervention within groups was measured using Repeated Measure ANOVA followed by Tukey's Post Hoc test. For data that is not normally distributed, such as the mean Lee Index and Visceral Fat non-parametric test:, the Friedman test was used, followed by the Willcoxon and Mann Whitney test. A *p*-value of <0.05 means that there was a significant difference between groups.

RESULTS AND DISCUSSION

Effect of OPE administration on BW

There were significant (*p*=0.005) BW changes in all groups after 0, 14, and 28 days of HFHFr diet (Figure 1.A). Figure 1.B shows that the average BW of rats increased at the end of the intervention period, and only the OPE2 group decreased statistically significantly (*p*>0.05).

Effect of OPE administration on Lee Index

Figure 2.A showed that the administration of HFHFr diet significantly increased the Lee Index of rats, and at the end of modeling, the whole group was declared obese (Lee Index higher than 300 g/cm^3). The OPE administration significantly reduced the rats' Lee Index at the end of the intervention. A significant decrease in the mean Lee Index was found in the OPE2 and OPE3 groups (*p*=0.028 and p=0.034) compared to the NC group from day 0 to day 28 of the intervention (Figure 2.B).

Effect of OPE administration on BFP

Mean BFP in all groups changed significantly on 0, 14, and 28 days of intervention (p<0.05) (Figure 3.A). Figure 3.B shows that OPE administration significantly decreased the mean BFP in all intervention groups (OPE 1-3) and the PC group compared to the NC group on day 28 of the intervention (p<0.05). The mean ± SD BFP values in the intervention groups and PC group were as follows (3.32±1.69), (2.51±1.92), (2.33±1.54) and (3.26±1.4).

Effect of OPE administration on visceral fat mass

There was no significant difference (p>0.05) in the visceral fat mass of rats in the control and the intervention groups (Table 1). Although there was no statistically significant difference between the control and the intervention groups, the average visceral fat weight in the intervention group (OPE 1-3) was lower than that in the PC group.

Table 1. Mean differences in visceral fat mass of rats with obesity with or without OPE administration

Groups	Mean ± SD (g) After 28 days of the intervention	*P*
OPE1	1.54±0.49	0.756
OPE2	1.30±1.09	
OPE3	1.83±0.63	
PC	1.85±0.89	
NC	1.30±0.47	

Note: Group differences were tested using Kruskal-Wallis (*p*<0.05)

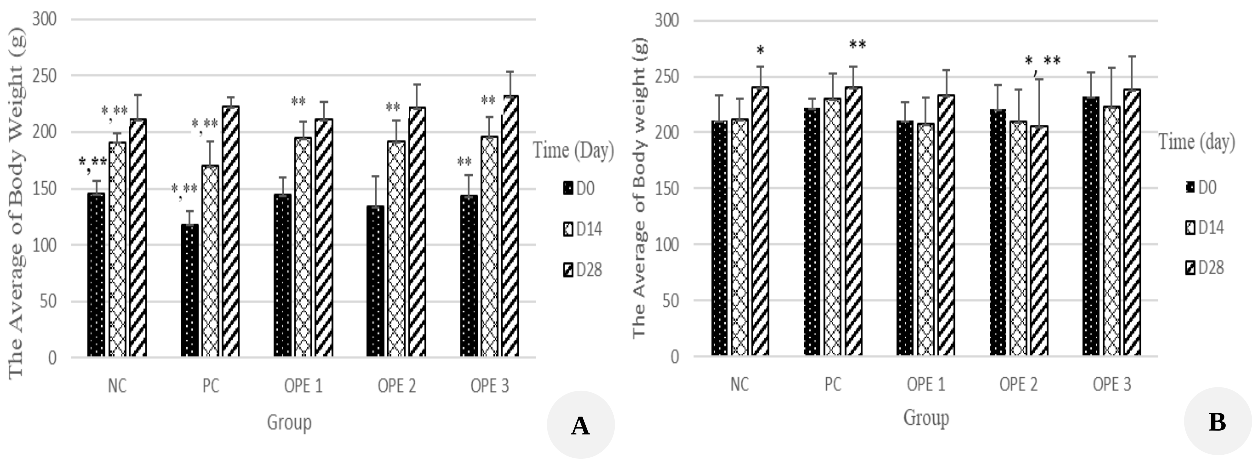

Figure 1. BW changes among all groups. A. Comparative effects (in each group) of HFHFr administration on body weight changes from days 0, 14 and 28 obesity induction. B. Comparative effect (in each groups) of OPE administration on BW from day 0, 14 to 28 of the intervention. *: significance of the change in average body weight compared to NC, **: significance of the change in average body weight compared to PC. (*p*<0.05)

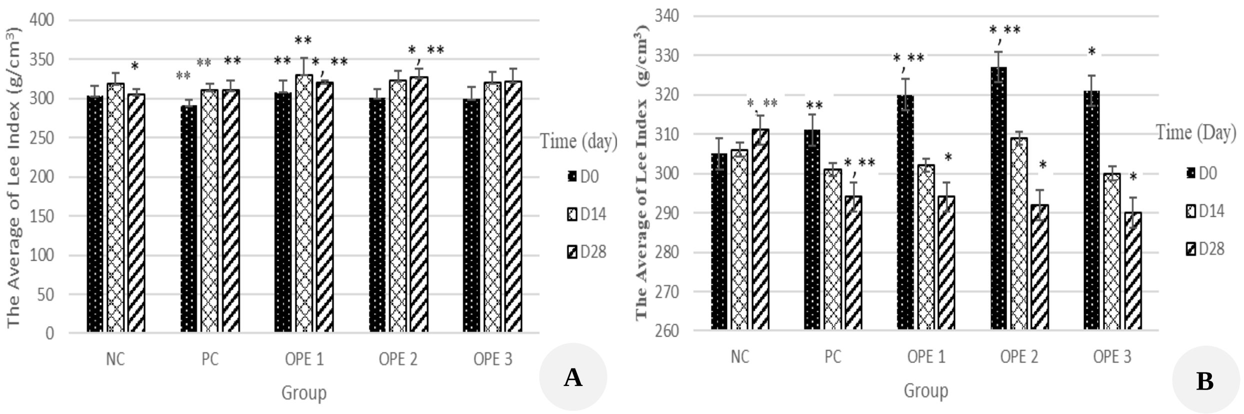

Figure 2. Lee Index changes among groups: A. Comparative effect of OPE administration among male rats with HFHFr on day 0, 14, and 28 obesity induction. B. Comparative effect of OPE administration within male rats' groups on Lee Index from day 0, 14 to 28 interventions. *: significance of the change in average body weight compared to NC, **: significance of the change in average body weight compared to PC. (p<0.05)

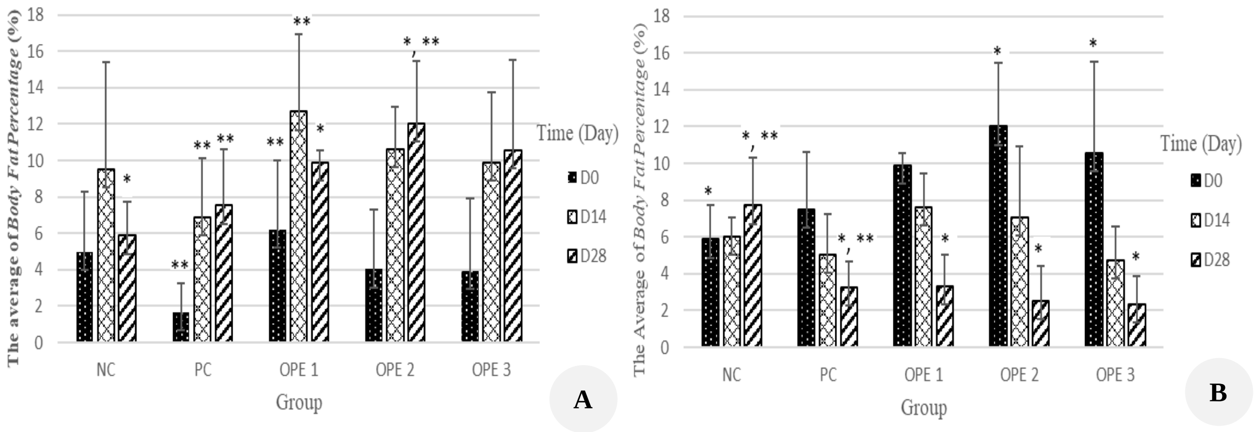

Figure 3. BFP changes among groups: A. Comparative effect of OPE administration among male rats with HFHFr on day 0, 14, and 28 obesity induction. B. Comparative effect of OPE administration within male rats' groups on BFP from day 0, 14 to 28 interventions. *: significance of the change in average body weight compared to NC, **: significance of the change in average body weight compared to PC. (p<0.05)

Discussion

Our study investigated the impact of OPE on BW, Lee Index, BFP, and visceral fat in obesity-induced male rats. Berastagi sweet orange is one of the Washington Navel Orange varieties grown in Berastagi Area, North Sumatra Province, Indonesia. Berastagi sweet orange is spread in almost all regions in Indonesia at a relatively affordable price. In general, only the pulp is consumed and the peel becomes waste. Preliminary research has been conducted to determine the nutritional content of OPE and it was found that OPE contains 1556.6 mg/100 g vitamin C and 2397.45 mg/kg magnesium which has beneficial effects in reducing obesity variables (Batubara et al. 2023).

Determining the Lee Index is a relatively easy and inexpensive way to determine obesity status by comparing BW with the naso-anal length of rats. Body mass index is influenced by various factors such as age, gender, and demographics (Purnell 2015). In this study, after being given OPE for 28 days, BW increased significantly. The Lee Index and BFP of rats decreased significantly, but there was no significant difference in visceral fat between groups. The average BW of all groups increased significantly on 14 and 28 days of the intervention, and OPE 2 was a significant difference between the NC and PC groups. This study aligns with research that found that OPE enriched with essential oils caused weight gain in test animals (Montalbano et al. 2019). Although all of OPE (172) did not cause weight loss, the weight gain in the intervention groups was lower than that in the NC group. Hesperidin is one of the phytochemical substances from the flavonoid glycoside group commonly found in citrus peels (da Silva et al. 2019). Hesperidin combined with hesperetin, has been proven to stimulate the release of cholecystokinin (CCK) in the intestinal endocrine cell STC-1, which is responsible for stimulating the digestion of fat and protein in the intestine. CCK is a peptide hormone that regulates appetite and satiety by signaling to the brain to reduce food intake. Therefore, stimulating CCK secretion by hesperidin and hesperetin can suppress appetite and reduce food intake, helping to control body weight and treat obesity (Kim et al. 2013; Gouveia et al. 2022). In the process of weight loss, there is a possibility that there will be an increase in body weight after a few days of treatment called the phenomenon of weight fluctuation and can occur for several reasons, including; the presence of fluid retention, hormonal changes, and the weight of undigested food and waste in the digestive system (Lean and Malkova 2016; Conti 2018; Livovsky et al. 2020). However, this weight fluctuation is normal and temporary, so a longer treatment time is needed to lose weight. This is in line with the previous research, which proved that administering Pacitan sweet orange peel extract at 500 mg/kg BW and 750 mg/kg BW for six weeks can significantly reduce BW (Samsudin et al. 2017). In addition, the increase in body weight in all groups was directly proportional to the increase in body length in mice, so the researchers concluded that the determination of obesity in this study could not only focus on BW variables but needed to be compared with body length (Lee Index).

In this study, we found that there was a decrease in Lee Index and BFP in the intervention group, which also align with previous studies showing that OPE was effective in reducing Lee Index and BFP, but at the same time, visceral fat in the intervention group was also significantly reduced (Gosslau et al. 2018; Huang et al. 2022; Lee 2023). The vitamins and minerals (vitamin C and magnesium) contents in OPE are thought to cause of the decrease in the Lee and BFP indices in this study. Ascorbic acid or vitamin C has a good effect on treating visceral obesity because it reduces BW, visceral adipose tissue and visceral adipocyte size (Liu et al. 2017) and with hesperidin, can activate peroxisome proliferator-activated receptor-α (PPARα), the target enzyme responsible for the β-oxidation of fatty acids in visceral adipose tissue and liver (Lee et al. 2019; Xiong et al. 2019). When activated, PPARα regulates the expression of genes involved in peroxisomal oxidation of α and β very long-chain fatty acids (VLCFA) and branched-chain fatty acids (Pettersen et al. 2019) as well as inhibits pyruvate oxidation by increasing pyruvate dehydrogenase kinase-4 (PDK4), increasing fatty acid and glycerol oxidation (Zhang et al. 2014) and may contribute to weight loss by increasing fatty acid oxidation and decreasing glucose oxidation. With an intracellular distribution of 99%, Mg is the fourth most abundant mineral in the human body (Asbaghi et al. 2021). Magnesium is important in various bodily functions, including energy metabolism, glucose utilization, protein synthesis, fatty acid synthesis and breakdown, muscle contraction, and overall cellular function (Fiorentini et al. 2021). Magnesium is also important in weight loss as it is involved in the production of ATP through oxidative phosphorylation in the mitochondria (Schwalfenberg and Genuis 2017). Studies have shown that adequate magnesium intake, a healthy diet, and intense exercise can help lose weight. Magnesium may also help reduce stress and improve sleep (Zhang et al. 2022), indirectly contributing to weight loss.

Visceral fat metabolizes faster than subcutaneous fat, which means it is harder to target with exercise alone or diet alone, so it can take a long time to reduce visceral fat if only relying on one treatment (Liu et al. 2017). This aligns with a previous study, which mentioned that it took at least 6 weeks to see a significant difference in visceral fat between the control and the treatment groups given fermented sweet orange peel (Liu et al. 2017). OPE can reduce obesity, but this study has several limitations that should be considered; the intervention period was only 4 weeks, which is relatively short compared to most intervention trials on obesity. The weight loss effect could be even greater if the OPE treatment is given over a longer observation period. In addition, there is a need to analyze the potential of other nutrients in OPE that may strengthen the effect of OPE in reducing obesity.

In conclusion, there's a discrepancy between the results of previous studies and this study regarding visceral fat. This is because visceral fat is the type of fat stored deep in the abdominal cavity, encasing organs including the liver and intestines and is considered dangerous as it is associated with a higher risk of type 2 diabetes, insulin resistance, heart disease and certain cancers (Covassin et al. 2018; Chait and den Hartigh 2020). This study reinforces the beneficial effects of OPE to ameliorate HFHFr diet-induced obesity. However, further studies are needed to identify other anti-obesity components and comprehensively understand the cellular and molecular mechanisms of OPE effects in obesity. This study demonstrates the potential of OPE to be developed into a functional food for managing HFHFr diet-induced obesity.

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