SHORT COMMUNICATION

Fat reducing effects of *Nelumbo nucifera* leaf extract in overweight patients

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ABSTRACT

The leaf of *Nelumbo nucifera* (Family Nelumbonaceae) has been widely included in the diet of Chinese people from the time of the Min Dynasty. In this study, a randomized double-blind trial (n = 60) was performed to determine the effects of extract from sun dried *Nelumbo nucifera* leaves (NnEx), which included quercetin-3-glucuronide (Q3GA) as the main components, in overweight patients (24 kg/m\(^2\) < body mass index < 28 kg/m\(^2\)) during 12 weeks. For both men and women, compared with those in the non-intervention control groups, the whole body fat was significantly decreased after NnEx ingestion, and men also significantly reduced visceral fat and waist circumference after NnEx ingestion compared with those in the control group.

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1. Introduction

Obesity is the most common metabolic disturbance worldwide and predisposes toward the development of cardiometabolic disturbances, such as type 2 diabetes
DM2 (Spiegelman and Flier 2001), hypertension, and dyslipidemia (Fruhbeck et al. 2018). High hepatic glucose production, impaired insulin secretion, and insulin resistance are typically found in patients with obesity, and all are involved in the pathogenesis of metabolic syndrome (MS). In particular, the hypertrophy of adipocytes is linked to the development of insulin resistance and MS (Engin 2017, Frankenberg et al. 2017). In recent years, a marked increase in the risk of cardiovascular disease worldwide has been associated with an increasing prevalence of DM2 and obesity (Holscher et al. 2016). Arteriosclerotic calcification is greater in individuals with MS, DM2, and type 1 diabetes mellitus, and this impairs conduit vessel compliance and function, thereby increasing the risks of dementia, stroke, heart attack, limb ischemia, renal insufficiency, and amputation of a lower extremity (Stabley and Towler 2017).

*Nelumbo nucifera* (Family Nelumbonaceae) leaf was first described as a food material during the Tang Dynasty, and has been widely included in the diet of Chinese people from the time of the Min Dynasty. Dumotier (1829) separated the Family Nelumbonaceae from Family Nymphaceae. In the previous in vitro studies, *Nelumbo nucifera* leaf extract solution significantly decreased triglyceride accumulation during adipogenesis in preadipocytes (Siegener et al. 2010). Extract from *Nelumbo nucifera* Gaertn (Family Nymphaceae) has been shown to promote lipolysis in the white adipose tissue of mice through β-adrenergic receptor (Ohkoshi et al. 2007). Here, we describe the results of a randomized double-blind trial that was designed to determine the effects of long-term consumption of extract from *Nelumbo nucifera* leaves (NnEx) in overweight patients. The effects of the consumption of 1 or 2 g/day NnEx for 12 weeks on indices of adiposity and blood metabolic parameters were compared with those of no consumption of NnEx in diet.

## 2. Results and discussion

### 2.1. Beverage component analysis

NnEx was obtained and analyzed by the method described in supplementary material, and the phytochemical composition analysis indicated that NnEx contained mainly quercetin and its glucosides (Table S1). The experimental beverages were produced by the method described in supplementary material, and the results of the analysis of the experimental beverages, including the pH and flavor, are presented in Table S2.

### 2.2. Baseline characteristics of the study participants

Ninety-five Chinese participants (23 kg/m² < BMI < 30 kg/m²) were recruited referred to Asia overweight standard. When according to the Chinese overweight/obesity standard (Gao et al. 2016), it included 7 obese (BMI ≥ 28 kg/m²), 60 overweight (24 kg/m² < BMI < 28 kg/m²), and 28 normal weight people (BMI ≤ 24 kg/m²). All of the 95 volunteers were allocated to the three study groups: a control group (C group), a low intervention group intaking 1 g NnEx / day (L group), and a high intervention group intaking 2 g NnEx / day (H group). Seven participants in the C group, and 6 and 9 participants in the L and H group, respectively, withdrew because of discontinued intervention. Because several reports found a correlation between
diseases and overweight/obesity (Gao et al. 2016) (Fruhbeck et al. 2018), the experimental data of 60 overweight subjects (C group: 22; L group: 22; H group: 16) from those who completed the trial were used in this study to evaluate the effect of NnEx ingestion (Table S3). Obese people were excluded because of the low number of subjects. Normal weight people were also excluded (Figure S1). None of the participants showed adverse reactions to the experimental beverages containing NnEx during the 12-week study period, indicating that NnEx is safe to ingest for a long period of time.

2.3. Safety

Quercetin is one of the main components of NnEx, and many toxicity tests have been performed using this substance. Pamukcu et al. reported that it is an intestinal and bladder carcinogen when included at a 0.1% concentration in the diet of outbred rats for 58 weeks (Pamukcu et al. 1980). However, Hirono et al. assessed the results of the inclusion of 1% or 5% quercetin in the diet of ACI rats for 540 days, and found no carcinogenicity (Hirono et al. 1981), and no carcinogenicity or reproductive toxicity were shown by another study in which Fischer rats were fed a 0.2% quercetin diet (100 mg/kg/day) for 64 weeks (Stoewsand et al. 1984).

Examination of single oral administration of NnEx to rats (supplementary material) showed that the LD50 value was >6 g/kg in both male and female rats and that this dose does not cause severe toxicity. Additionally, repeated oral administration of NnEx at 4 g/kg for 13 weeks (supplementary material) did not cause any histopathological abnormalities indicating that this dose is safe for longer-term use in rats. The quercetin component of NnEx is <10%, therefore 1 g/kg/day of NnEx in the diet would contain a concentration of quercetin that is below the toxic level in rats. Furthermore, in the clinical study, the participants consumed 1 or 2 g NnEx daily, which should also be a safe level.

2.4. Reduction in whole-body fat, visceral fat and waist circumference for overweight men

For men in the L group, in which participants consumed 1 g/day NnEx, the body fat significantly reduced by 6.5% (-1.8% from 27.5%, p = 0.003) and by 9.5% (-2.6% from 27.5%, p = 0.019) after 6 and 12 weeks, respectively (Table S4). It is a larger decrease in body fat during 12 weeks intervention by 1 g/day NnEx even than during an ultradistance triathlon, in which reported a reduction of body fat by 7.7% (-1.1% from 14.2%) (Knechtle et al. 2008). The waist circumference significantly decreased 1.3 cm and 2.3 cm after 6 and 12 weeks, respectively, without change in plasma LDL-cho and HDL-cho (Li et al. 2017, Srivastava 2018). While in the H group, in which participants consumed 2 g/day NnEx, not only the body fat and waist circumference significantly decreased, but also the visceral fat significantly reduced by 6.5% (-0.9% from 13.8%, p = 0.037) and by 9.4% (-1.3% from 13.8%, p = 0.023) after 6 and 12 weeks, respectively, together with the BMI significantly decreased after 6 and 12 weeks (Table S4).
Visceral fat is an important part of whole-body fat and has been researched for its relationship with many metabolic diseases. Adipocyte size in visceral adipose tissue is significantly related to HOMA-IR in male obese human, while none markers in subcutaneous adipose tissue contributed to HOMA-IR (Verboven et al. 2018). Another report indicates the physiological characteristics, such as the size and number of adipocytes, lipolysis reactivity, lipid storage capacity and the production of inflammatory cytokines, B cell accumulation are related to the increased cardiovascular metabolic risk associated with visceral obesity (Tchernof and Despres 2013) and metabolic impairment in aging adipose tissue (Camell et al. 2019). Although some reports described a similar decrease in body weight and BMI by nature product intervention such as Moro (Citrus sinensis (L.) Osbeck) orange juice (Cardile et al. 2015), the effect of visceral fat or body fat reduction was little analyzed except in this study. Therefore, in clinical practice, in addition to BMI, waist circumference measurement is useful to identify and manage overweight patients with high risk of complications (Tchernof and Despres 2013). The body fat reduction effect of NnEx consumption in this study is thought because of its lipolysis function. While further research is needed to find out which specific indicators of visceral fat are affected by the ingestion of NnEx.

2.5. Reduction in whole-body fat for overweight women

For women both in the L and H groups, in which participants consumed 1 g/day or 2 g/day NnEx, the body fat significantly reduced by 10.5% (-3.7% from 35.2%, $p = 0.009$) and by 8.6% (-2.9% from 33.6%, $p = 0.027$) only after 12 weeks compared to control group, without significant change in visceral fat, waist circumference and hip circumference (Table S5).

The lack of a significant decrease of visceral fat in women may reflect the lower ration of visceral fat in women compared to men in this study (Table S4 S5). However, the body fat in women in this study was higher than men and had significant decreases after NnEx ingestion, but there was no change seen in waist and hip circumference in women. It has sex difference that the distribution of subcutaneous fat. Adult women have 48% less waist fat than men, and girls have considerably more peripheral fat than boys whether measured as extremity or hip fat from early childhood to young adulthood (Taylor et al. 2010). NnEx ingestion decreased the whole-body fat in women, as same as men for whom the significant effect was reflect in waist circumference and visceral fat, while for women the effect might be in other body parts.

3. Conclusions

NnEx has anti-obesity effects for both men and women. For men, NnEx significantly reduced body fat including visceral fat, together with reducing waist circumference. For women, NnEx significantly reduced body fat but except visceral fat and did not affect waist circumference.
Author contributions

ZSJ designed the study and helped prepare the manuscript. LY, XW and HY performed the clinical trial. HD and TK assisted with the data collection. XG assisted with the data analysis. EL performed the data analysis and wrote the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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