EXPERIMENTAL STUDY

Effect of Linguizhugan decoction on hyperlipidemia rats with intermittent fasting

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Abstract

OBJECTIVE: To explore the effect of Linguizhugan decoction on hyperlipidemia rats with caloric restriction.

METHODS: The hyperlipidemia model of rat was induced by high fat diet for 8 weeks. After the model was established, 26 rats were randomly divided into 4 groups: the control group (n=6), the model group (n=6), the intermittent fasting (IF) group (n=8), and the IF and herbal medicine (IFH) group (n=6). IF group was applied intermittent fasting every other day. The IFH group was given Linguizhugan decoction every day and intermittent fasting every other day. Blood samples were taken at the end of 16 weeks, and serum ghrelin and lipid was tested.

RESULTS: Serum ghrelin in the IF group significantly increased (P<0.01). Serum ghrelin in IFH group was lower than the IF group (P<0.05), but higher than the model group (P<0.01).

CONCLUSION: Linguizhugan decoction may play a part in regulation of energy and appetite in hyperlipidemia rats with IF.

INTRODUCTION

Lingguizhugan decoction is a classical formula from Jin Gui Yao Lue. It is widely used to treat phlegm and fluid syndrome. In the previous studies, we have used this decoction combined with fasting therapy to treat obese, hyperlipidemia, insulin resistance, and 2 type diabetes, and found that patients were less hunger than those only with fasting therapy, and more likely to complete fasting therapy. Therefore, we predicted that Lingguizhugan decoction might have some effect on the regulation of energy and appetite during fasting therapy. Ghrelin is regarded as an index of food intake and energy homeostasis. In this research, serum ghrelin was investigated in the hyperlipidemia rats with intermittent fasting (IF) and Chinese herbal medicine.

METHODS

Animal and diet

A total of 26 Wistar rats [Number of animal license SOXK (Guangdong) 2008-0002], weighing (200±10) g obtained from Experimental animal centre of SUN YAT-SEN University were fed in a specific pathogen free cage in the Experimental Animal Center of North Campus of Sun Yat-sen University. After receiving adaptive feeding for 7 days, rats were divided into two groups randomly: 6 rats (control group) fed with normal diet and 20 with high fat diet (HFD) (basic diet with 30% of calories as fat) for 8 weeks.
78.3%, lard 10%, egg yolk power 10%, cholesterol 1%, bile salts 0.5%, propylthiouracil 0.2%, while calorie was 17.4 kcal/g, provided by Experimental Animal Center of Guangdong Province).

After HFD for 8 weeks, blood lipid was tested. Blood lipid of HFD rats was significantly higher than that of control group, suggesting that the model was regarded to be successful. Then, hyperlipidemia rats were divided into three groups: 1) model group: maintained on HFD. 2) IF group: fasting every other day. On fasting day, rats were fed no food but water from 8 am to 8 am the next day. On non-fasting day, rats were fed HFD and water freely. 3) IF with herbal medicine group: given Lingguizhugan decoction everyday by gavage, and intermittent fasting the same as IF group. After 8 weeks, rats were sacrificed under anesthesia, and fasting plasma were collected. All animal studies were approved by the Medical Ethics Committee of the first affiliated hospital of Sun Yat-sun University.

### Drugs and reagent
Lingguizhugan decoction, made from granule, was consisted of Fuling (Poria), Guizhi (Ramulus Cinnamomi), Baizhu (Rhizoma Atractylodis Macrocephalae), Zhigancao (Radix Glycyrrhizae, honey-fried) at the ratio of 12∶9∶6∶6. The powder was diluted by normal saline to make a new concentration of 0.344 g/mL. The dosage of administration via stomach was 3.44 g/kg·d⁻¹.

Ghrelin reagent was purchased from Uscn Life Science, Inc. (Wuhan, China). Serum lipid was tested by the laboratory department of the first affiliated hospital of Sun Yat-sun university.

### Statistical analysis
Statistical analyses were performed using SPSS 17.0 (SPSS Inc, Chicago, IL, USA). All the normal variables are expressed as mean±standard deviation (mean±SD). The comparison of two groups was analyzed by independent samples t test. *P*<0.05 was regarded as statistically significant.

### RESULTS

#### Comparison of serum lipid

As showed in the Table 1, levels of total cholesterol (TC), total glycerin (TG), low-density lipoprotein cholesterol (LDL-C) in the model group were all higher than the control group (all *P*<0.01). In the IF group, TC level was lower (*P*<0.01) and high-density lipoprotein cholesterol (HDL-C) was higher (*P*<0.01) as compared with the model group. Similarly, TC level in the IFH group was lower (*P*<0.01) and HDL-C was higher (*P*<0.01) than the model group. However, levels of TC, TG, HDL-C, LDL-C showed no statistical difference between the IF group and the IFH group (*P*>0.05).

**Comparison of serum ghrelin**
The ghrelin level in the model group [(314 ± 95) pg/mL] was lower than the control group [(503±129) pg/mL] (*P*=0.016). Compared with the model group, it was higher in the IF group [(683 ± 123) pg/mL] (*P*<0.0001). Compared with the IF group, ghrelin level in the IFH group [(490 ± 118) pg/mL] was lower (*P*=0.011), but it was still higher than the model group (*P*=0.011).

### DISCUSSION

Metabolic syndrome includes hyperinsulinemia, insulin resistance, visceral obesity, and dyslipidemia. Caloric restriction (CR) is important in the guideline of metabolic syndrome, obesity and type 2 diabetes. CR by 30% over a period of 3 months could improve insulin clearance, and play an important role in the normalization of insulin and lipid metabolism in liver.

Fasting as one type of CR has been applied in treating metabolic syndrome, obesity and type-2 diabetes in the past three years by us. During 7 days fasting, patients ate no food but water and juice and stopped taking any hypoglycemic drugs. Some patients might quit the fasting therapy because of great hunger, dizziness and fatigue. When we added Lingguizhugan decoction into fasting therapy, patients’ suffering lightened, and felt easier to complete the treatment. Therefore, we guess Lingguizhugan may play a role in regulation of appetite and energy metabolism.

From Table 1, it is showed that serum lipid in the IF and IFH group had no statistical difference. Compared with the model group, TC levels in two groups were
lower than the model group, while the HDL-C levels were all higher than the model group, indicating that IF could reduce TC and increase HDL-C. Here the herbal medicine showed no effect on the serum lipid. However, the serum ghrelin level was lower in the IFH group than the IF group.

Ghrelin was discovered in 1999 by Kojima and colleagues from extracts of rat gastric tissue.\(^4\) Stomach is a major source of circulating ghrelin (65%-90%),\(^5\) and the second is small intestine. Brain, pituitary, pancreas, kidney, lung, placenta, testis, and ovary also express detectable amounts of ghrelin.\(^6\) Ghrelin is the first and only gut peptide proven to have orexigenic properties. Endogenous ghrelin levels are closely related to meal patterns, rising before meals and falling within 1 h after meal.\(^7\) Both central and peripheral administration of ghrelin results in increased food intake.\(^8\) Ghrelin increases in the plasma during diet-induced weight loss in obese human.\(^9\) Suggesting that ghrelin may contribute to the sustained appetite associated with CR. In our study, we applied IF to rats, ghrelin was significantly higher than model group. When we add Lingguizhugan to IF rats, serum ghrelin level dropped compared to IF groups (\(P<0.01\)), suggesting that the decoction regulates appetite of IF rats, and may played a role in appetite regulation of patients so that they felt less hunger and able to complete the treatment easily. Lingguizhugan decoction is from \textit{Jin Gui Yao Lue}.\(^1\) It has the effect of warming Yang for resolving fluid retention and invigorating the spleen for eliminating dampness. In long-term CR, body gets energy mainly from fat, muscle and gluconeogenesis. Due to the decomposition of fat and muscle by fasting, patients felt great dizziness, tired and susceptible to cold, and this symptom is corresponding to Yang-deficiency syndrome. With the use of decoction, energy homeostasis maybe improved in hyperlipidemia patients with fasting, suggesting that Lingguizhugan decoction isn’t placebo but plays a part in fasting therapy. However, the mechanism of Lingguizhugan decoction on appetite and energy homeostasis is still unknown in the fasting patients. We propose that it may lie in the regulation of feeding center during fasting, so further research on this field will be needed in the future.

REFERENCES