



## Research article

## The effect of red ginseng and ginseng leaves on the substance and energy metabolism in hypothyroidism rats

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## ABSTRACT

**Background:** Recent studies have revealed that the properties Traditional Chinese Medicine is mostly associated with are substance and energy metabolism. Our study aimed to compare the effect of red ginseng (RG) (warm property) and ginseng leaves (GL; cold property) on the substance and energy metabolism of rats with hypothyroidism.

**Materials and methods:** Rats were administered propylthiouracil intraperitoneally for 20 d to cause hypothyroidism. The reference group was orally administered *Aconiti Lateralis Radix Praeparata* [FZ (Fuji in Chinese)], while both the RG and GL groups were orally administered crude drugs. The rectal, tail, toe, and axilla temperature of the rats were assayed every 3 d. Oxygen consumption, carbon dioxide production, heat production, and energy expenditure were measured via TSE phenoMaster/LabMaster animal monitoring system. Adenosine monophosphate-activated protein kinase, Na<sup>+</sup>-K<sup>+</sup>-ATPase, fumarase, pyruvic acid and cyclic adenosine monophosphate/cyclic guanosine monophosphate were determined.

**Results:** The lower levels of triiodothyronine, tetraiodothyronine, and thyrotropin-releasing hormone and the higher level of thyroid stimulating hormone revealed the successful establishment of a hypothyroidism model. Oxygen consumption, carbon dioxide production, heat production, and energy expenditure in the FZ and RG groups were obviously increased. The activity of Na<sup>+</sup>-K<sup>+</sup>-ATPase and fumarase in the FZ and RG groups was significantly increased. The cyclic adenosine monophosphate/cyclic guanosine monophosphate level in the FZ and RG groups was increased, while the GL group showed the opposite.

**Conclusion:** Our research provides a new way to explore the efficiency of Chinese medicine on the basis of the relationship between drug property and effects on substance and energy metabolism.

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## 1. Introduction

The theory of Traditional Chinese Medicine (TCM) mainly includes four properties, five flavors, and channel tropism, etc. The four properties are referred to as cold, cool, hot, and warm. A recent study revealed that the flavor of the drug was related to specific efficacy, while the property of drug was mainly concerned with the body's energy and substance metabolism by different pathways [1]. Generally, drugs with hot or warm properties are used for the treatment of cold disease, and the drugs with cold or cool properties are used for the opposite.

Ginseng, the dried root and rhizome of *Panax ginseng* Meyer, is held in high esteem in Asian countries as a herbal medicine used to treat various diseases such as atherosclerosis, hypertension, and cancer [2,3]. Red ginseng (RG) is produced by steaming the raw fresh ginseng. During the steaming process, its warm property is significantly reinforced. But the dry leaves of *P. ginseng* Meyer exhibit a totally different property to RG, e.g., RG attributes to the warm property and ginseng leaves (GL) attribute to the cold. In addition, RG and GL contain similar ginsenosides, but their proportions of the main individual ginsenosides are different: ginsenoside-Re is higher in GL and ginsenoside -Rg1, and -Rb1 are higher in the ginseng root. From the perspective of meridian

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tropisms, RG is mostly used for the spleen, lung, and heart, while GL is used for the lung and stomach. *Aconiti Lateralis Radix Praeparata* [FZ (FuZi in Chinese)] is the root of *Aconitum carmichaelii* Debx, and is generally used as a hot property drug.

Thyroid hormones are essential for normal mammalian development and are well known to play fundamental roles in the cardiovascular, nervous, immune, and reproductive systems [4]. Thyroid hormone is a set of amino acids containing iodine, including triiodothyronine (T3) and tetraiodothyronine (T4), which play an important role in promoting tissue metabolism and physical growth, besides improving nerve excitability [5–8]. Hypothyroidism is one of the most common endocrine diseases, and its main clinical feature is a low basal metabolic rate caused by the insufficient synthesis, secretion, or biological effect of thyroid hormones [9]. A decrease of T3, T4, or thyrotropin-releasing hormone (TRH), accompanied by an increase in thyroid stimulating hormone (TSH) are considered as the assessment criteria of hypothyroidism patients who manifest clinical symptoms of low basal metabolic rate, such as fatigue, sluggishness, chills, and hypothermia [10]. Hypothyroidism belongs to the category of “consumptive disease” [11], and patients show diminishing energy metabolism. Clinically, FZ is utilized extensively in prescriptions to cure hypothyroidism [12,13]. In addition, the prescriptions which contain RG are also used in the treatment of hypothyroidism, such as *Gan-caorenshen* decoction and *Buzhong yiqi* decoction [14,15]. In TCM, hypothyroidism is mostly regarded as a cold disease and drugs with warm or hot properties are used for its treatment.

To date there are no reports concerning the effects of RG and GL on hypothyroidism. Previously, the effects of RG and GL on substance and energy metabolism in healthy animals have been studied [16]. In the present work, rats with hypothyroidism induced by propylthiouracil (PTU) were used to evaluate the effect of RG (with warm property) and GL (with cold property) on hypothyroidism based on basal metabolic rate, endocrinium, substance metabolism, and energy metabolism.

## 2. Materials

### 2.1. Chemicals and reagents

A UV–2100 spectrophotometer was purchased from Unico Co. (Shanghai, China). Imagequant was purchased from Tanon Technology Co. (Shanghai, China). Normal saline was obtained from Kelun Pharmaceutical Ltd. (Heilongjiang, China). PTU [Batch Number (No.): BCM4329V] was obtained from Sigma-Aldrich (St. Louis, MO, USA). A Microplate Reader was purchased from Kate Biological Medical Electronics Technology Co. Ltd. (Shenzhen, China). A T3 assay kit (Batch No. 20150901, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), T4 assay kit (Batch No. 20150903, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), TSH assay kit (Batch No. 20150829, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), TRH assay kit (Batch No. 20150829, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), TSH assay kit (Batch No. 20150905, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), cyclic adenosine monophosphate (cAMP) assay kit (Batch No. 20150903, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), cyclic guanosine monophosphate (cGMP) assay kit (Batch No. 20150905, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), adenosine monophosphate-activated protein kinase (AMPK) assay kit (Batch No. 20150828, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), fumarase (FUM) assay kit (Batch No. 20150903, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), Na<sup>+</sup>-K<sup>+</sup>-ATPase assay kit (Batch No. 20150903, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), protein quantitative assay kit

(Batch No. 20150829, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China). BCA Protein Assay Kit (Kangwei Shi Ji Biotechnology Institute, Beijing, China), tissue protein extraction kit (Kangwei Shi Ji Biotechnology Institute, Beijing, China), Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis Gel kit (Kangwei Shi Ji Biotechnology Institute, Beijing, China), AMPK antibody was purchased from Abcam (Cambridge, UK), phosphor-activated protein kinase lactate (pAMPK) antibody was purchased from Cell Signaling Technology (Shanghai, China), and an ECL kit (Thermo Scientific, St. Illinois, Rockford, USA).

### 2.2. Animals

Rats for experiment were purchased from the laboratory animal center of Changsheng Bio-Technique Co. Ltd. (Benxi, Liaoning, China), qualified no. SCXK 2010-0001. Animals were kept in an air-conditioned room (22°C; relative humidity, 55%) and fed *ad libitum* with standard feed and water in the course of the study. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study protocol was approved by the ethics regulations of Liaoning University of Traditional Chinese Medicine, China (131/2010).

### 2.3. Plant materials

RG (Batch no. 20150727) was purchased from Dalian Drug store, China, September, 2015. GL (Batch no. 20151012) were obtained from Kuan Dian, Liaoning province of China. FZ (Batch no. 151003) was purchased from Dalian Drug store, China, September, 2015. All the samples were identified by Professor Bing Wang, College of Pharmacy, Liaoning University of Traditional Chinese Medicine as the dried roots and leaves of *P. ginseng* Meyer and the roots of *A. carmichaelii* Debx. All plant substances have been deposited at the College of Pharmacy, Liaoning University of Traditional Chinese Medicine, China.

## 3. Methods

### 3.1. Sample preparation

A 300 g sample of *A. carmichaelii* was immersed in a 10-fold volume of distilled water for 1 h and boiled for 1 h. After being filtered, an eight-fold volume of distilled water was added and boiled again for 1 h. The filtrate was combined and concentrated to a final volume of 1.38 g/mL crude drug. RG and GL were dried at 50°C, pulverized to pass through a 150 mesh sieve and then the powder was dissolved in distilled water to meet the final concentrations of 0.83 g/mL crude drug of RG and GL. The samples were kept at 4°C before usage.

The doses of FZ, RG, and GL were calculated through equivalent dose ratio of human to rat according to body surface area. The 1-d doses of RG and GL for humans are 9 g according to the 2015 edition of Chinese Pharmacopoeia [17]. Therefore, the equivalent single doses of RG and GL for rats were calculated as 0.83 g/kg/d, while a double dose for rats was 1.66 g/kg/d. The 1-d dose of FZ for humans is 15 g according to the 2015 edition of Chinese Pharmacopoeia [17], while the equivalent five-fold dose for rats was calculated as 6.9 g/kg/d. To choose the appropriate dosage, two doses of RG and GL were tested in the preliminary experiment (Figs. S1–S4) and 1.66 g/kg of RG and GL were selected for the following experiments.

### 3.2. Animals and drug administration

Fifty healthy male specific pathogen free grade Sprague Dawley rats, weighing 180–220 g, were housed for 5 d in an animal room prior to the experiments. Rats were randomly divided into five groups ( $n = 10$  per group): the FZ group, the RG group, the GL group, the blank group, and the control group. The control group, FZ, RG, and GL groups were injected intraperitoneally with PTU (10 mg/kg/d), while the blank group was injected with distilled water of the same volume. After 4 h the FZ group was intragastrically (i.g.) administered with 6.9 g/kg crude drug for 20 d, which was five times the human equivalent dose according to the 2015 Chinese Pharmacopoeia [17]. The RG group and GL group were administered i.g. with 1.66 g/kg of their suspension for 20 d, which was twice the human equivalent dose according to those recorded in 2015 Chinese Pharmacopoeia. The doses of FZ, RG, and GL were calculated through equivalent dose ratio of human to rat according to body surface area [18]. The blank group and control group were administered i.g. the equivalent volume of distilled water for 20 d.

### 3.3. Determination of rat rectal temperature, tail temperature, toe temperature, and axilla temperature

Rectal temperature was measured with electronic rectal thermometer at 4:00 PM on the 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup>, 13<sup>th</sup>, 16<sup>th</sup>, and 19<sup>th</sup> d. Simultaneously, tail temperature, toe temperature, and axilla temperature were measured with an infrared thermometer.

### 3.4. Blood and tissue sampling

At 1 h after the last drug administration, the rat was intraperitoneally injected chloral hydrate (0.3 mL/100 g body weight). After anesthesia the abdominal cavity was exposed, then blood was collected from the abdominal aorta and cryogenically centrifuged at 2,500 rpm/min for 15 min to separate the plasma from the blood cells. The dissection of the liver was performed immediately after the blood was collected. About 0.1 g of the liver was homogenized with nine times the volume of saline and then centrifuged at a speed of 3,500 rpm/min for 10 min. All the dissected parts of the liver were taken from the same spot of the liver as much as possible and frozen at  $-80^{\circ}\text{C}$  before usage.

### 3.5. Determination of oxygen consumption, carbon dioxide production, heat production, respiratory exchange rate, and energy expenditure

Determination of oxygen consumption ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), heat production (H), respiratory exchange rate (RER), and energy expenditure (EE) were performed at 8:00 AM of the 19<sup>th</sup> d of drug administration using TSE phenoMaster/LabMaster (TSE Systems, Thuringia, Germany) at the Small Animal Phenotyping Core Facility (CMU, University of Geneva, Geneva, Switzerland), under a 12 h light–dark cycle. EE was calculated by using 1-min samples for quantification of  $\text{VO}_2$  and  $\text{VCO}_2$ . The formula for the calculation of EE was  $\text{EE} = (3.815 + 1.232 \times \text{VCO}_2/\text{VO}_2) \times \text{VO}_2 \times 0.001$ . The rats in different groups were individually placed in the respiratory chambers and recorded every 30 min for 24 h.  $\text{VO}_2$ ,  $\text{VCO}_2$ , and EE was calculated according to the manufacturer's guidelines. The RER was estimated by calculating the ratio of  $\text{VCO}_2:\text{VO}_2$ . Values were adjusted by body weight to the power of 0.75 ( $\text{kg}^{-0.75}$ ) where mentioned. The room temperature was controlled at  $22.0 \pm 1.0^{\circ}\text{C}$  in an isolated environment. The sampling flow rate was continuously controlled at 0.25 L/mL, with flow speed set at 1.0 L/min. This was continuously detected for 24 h to measure the  $\text{VCO}_2$ ,  $\text{VO}_2$ , H, RER, and EE of each group [19,20].

### 3.6. Determination of T3, T4, TRH, TSH, cyclic adenosine monophosphate/cyclic guanosine monophosphate, pyruvic acid in plasma and AMPK, $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ , FUM in liver

All the indexes were measured strictly according to their measurement kits.

### 3.7. Western blot analysis

The total proteins were extracted from the liver tissue using a protein extraction kit. The protein content was detected by the bicinchoninic acid method using a protein assay kit. Equal protein amounts were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were then incubated with the primary antibodies at  $4^{\circ}\text{C}$  overnight with gentle shaking, followed by incubation with the peroxidase-labeled secondary antibody for 1 h at  $37^{\circ}\text{C}$ . The bands were detected with an enhanced chemiluminescence kit and visualized with imagequant.

### 3.8. Statistical analysis

Measurement data were expressed as mean  $\pm$  standard deviation, and were analyzed by analysis of variance with the least significant difference test by using SPSS version 20.0 (Chicago, IL, USA). Values of  $p < 0.05$  were considered statistically significant.

## 4. Results

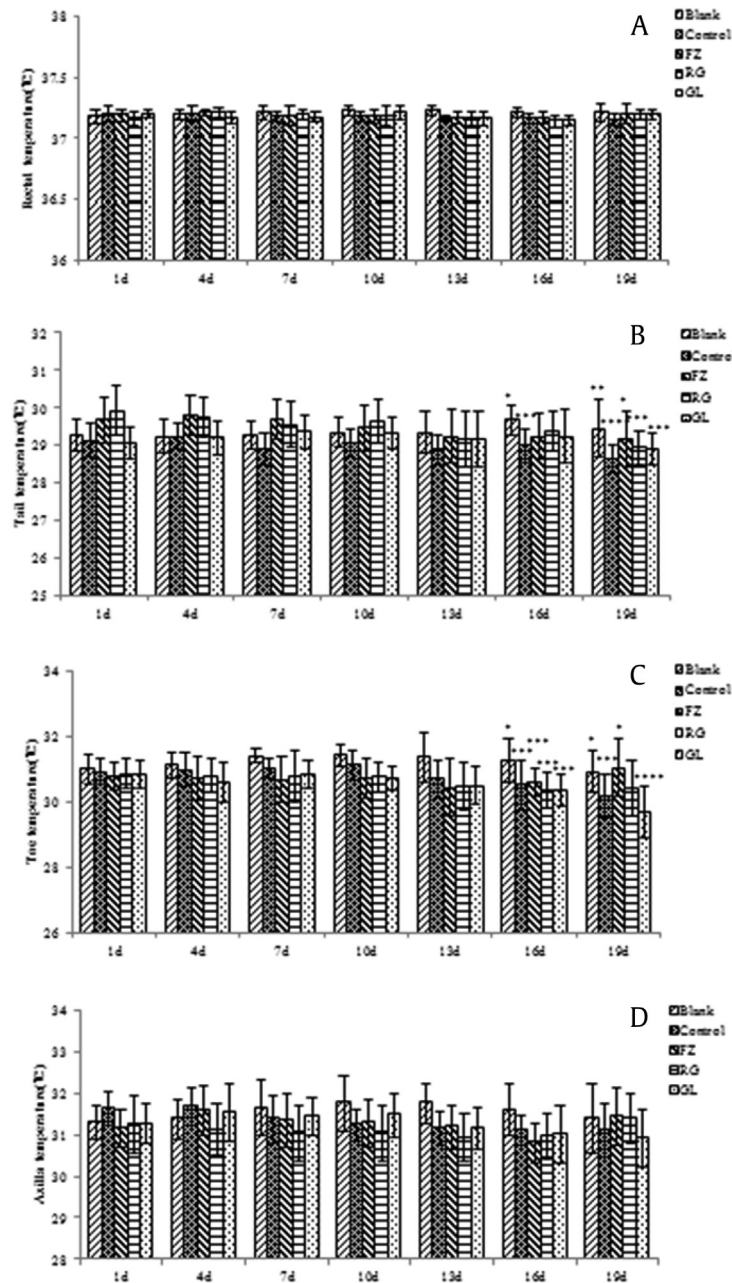
### 4.1. The variation of rectal temperature and surface temperature

Studies have demonstrated that hypothyroidism patients suffer from cold extremities. The variation of rat body temperature was determined by four aspects: rectal temperature, tail temperature, toe temperature, and axilla temperature. Results indicated that the rat rectal and axilla temperature in all five groups showed no statistical difference within 19 d of drug administration.

The tail temperature of the control group showed an obvious decrease on the 16<sup>th</sup> d of drug administration ( $p < 0.05$ ) compared with the blank group. On the 19<sup>th</sup> d of drug administration, the tail temperature of control group significantly decreased as compared with blank group ( $p < 0.01$ ), while the FZ group was markedly increased as compared with the control group ( $p < 0.05$ ). The toe temperature of the rats in the control group obviously decreased on the 16<sup>th</sup> d of drug administration as compared with the control group. On the 19<sup>th</sup> d of drug administration, the toe temperature of FZ group was significantly higher than the control group ( $p < 0.05$ ), while the GL group showed a decreasing tendency (Figs. 1A–1D).

### 4.2. Influence on basal metabolic rate ( $\text{VO}_2$ , $\text{VCO}_2$ , H, RER, EE) of rats

To elucidate the metabolic changes in rats, the phenoMaster/LabMaster (TSE Systems) was used and the results are shown in Figs. 2A–2E. The data disclosed that  $\text{VO}_2$ ,  $\text{VCO}_2$ , H, and EE significantly decreased in the control group as compared to the blank rats on the 19<sup>th</sup> day of drug administration ( $p < 0.01$ ) during the daytime. In addition, the  $\text{VO}_2$ ,  $\text{VCO}_2$ , H, and EE were significantly increased in the FZ and RG groups as compared with the control group ( $p < 0.01$ ) during the daytime. However,  $\text{VO}_2$ ,  $\text{VCO}_2$ , H, and EE in the blank group did not show any significance as compared to the control group during the night time, but  $\text{VO}_2$ ,  $\text{VCO}_2$ , H, and EE in the FZ group significantly increased as compared with the control group (Figs. 3A–3E). RER of all five groups showed no significant difference during the 24 h.



**Fig. 1.** Effects of the Aconiti Lateralis Radix Praeparata, red ginseng, and ginseng leaves after administrating to rats. (A) Effect on rectal temperature. (B) Effect on tail temperature. (C) Effect on toe temperature. (D) Effect on axilla temperature. Cumulative values are reported as mean $\pm$ S.E. for 10 rats in each group. \*  $p < 0.05$  compared to control group. \*\*  $p < 0.05$  and \*\*\*  $p < 0.01$  compared to blank group, using analysis of variance with the least significant difference analysis. FZ, Aconiti Lateralis Radix Praeparata; GL, ginseng leaves; RG, red ginseng.

#### 4.3. T3, T4, TSH, and TRH assays in plasma

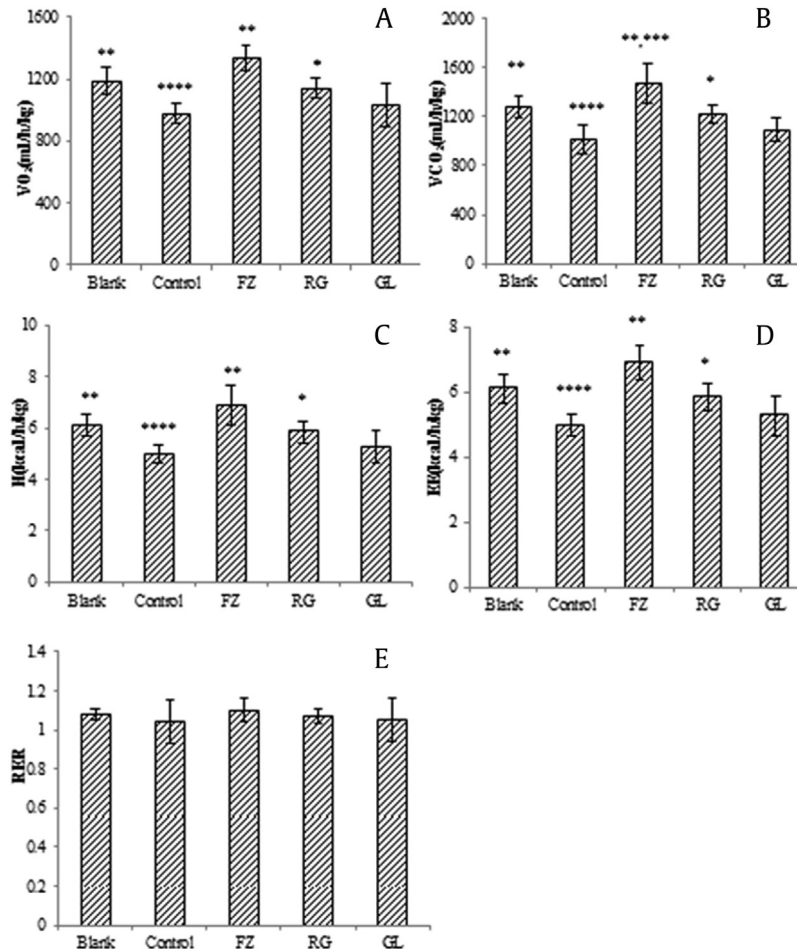
The decrease of T3, T4, and TRH and the increase of TSH are considered as important indicators to clinically evaluate hypothyroidism patients. Our results showed that the levels of T3, T4, and TRH in the control group were completely decreased, while the level of TSH in the control group was increased as compared to the blank group ( $p < 0.01$ ), which showed that the hypothyroidism model was established (Figs. 4A–4D). The levels of T3 in the FZ and RG groups were increased compared with the control group, but the GL group showed no statistical differences. In addition, the level of T4 in the FZ, RG, and GL groups were statistically increased compared to the control group. The content of TSH in the FZ group

was statistically decreased compared with the control group ( $p < 0.05$ ), but the RG and GL groups showed no significant difference compared with the control group (Fig. 4C). The content of TRH in the FZ, RG, and GL groups was increased compared with the control group [ $p < 0.05$  (Fig. 4D)].

#### 4.4. Hepatic AMPK level

To explore the influence of RG and GL on the energy metabolism, the hepatic AMPK content was measured by the enzyme-linked immunosorbent assay method. As shown in Fig. 5A, a distinct difference between the blank group and control group was observed ( $p < 0.01$ ). In addition, the FZ, RG, and GL groups can increase the





**Fig. 2.** Effects of blank, control, Aconiti Lateralis Radix Praeparata, red ginseng, and ginseng leaves during the daytime after administrating for 19 d. (A) Effect on oxygen consumption. (B) Effect on carbon dioxide production. (C) Effect on heat production. (D) Effect on energy expenditure. (E) Effect on respiratory exchange rate. Cumulative values are reported as mean  $\pm$  standard error for 10 rats in each group. \*  $p < 0.05$  and \*\*  $p < 0.01$  compared to controls. \*\*\*  $p < 0.05$  and \*\*\*\*  $p < 0.01$  compared to blank group, using analysis of variance with the least significant difference analysis. EE, energy expenditure; FZ, Aconiti Lateralis Radix Praeparata; GL, ginseng leaves; H, heat production; RER, respiratory exchange rate; RG, red ginseng; VCO<sub>2</sub>, carbon dioxide production; VO<sub>2</sub>, oxygen consumption.

content of AMPK significantly as compared with control group ( $p < 0.01$ ); however, the effect of GL group was weaker than the FZ and RG groups.

The Western blot results showed that the AMPK level of the control group was significantly reduced compared with the blank group. The AMPK and pAMPK levels of the FZ, RG, and GL groups were increased compared with the control group ( $p < 0.01$ ), but the AMPK and pAMPK levels of GL group were lower than the FZ and RG groups (Figs. 5B–5D).

#### 4.5. Hepatic Na<sup>+</sup>-K<sup>+</sup>-ATPase activity assay

Na<sup>+</sup>-K<sup>+</sup>-ATPase was closely related to the decomposition of ATP. Fig. 6 demonstrated that the Na<sup>+</sup>-K<sup>+</sup>-ATPase activity in liver showed a significant difference between the blank group and the control group, with a weaker Na<sup>+</sup>-K<sup>+</sup>-ATPase activity observed in the control group ( $p < 0.01$ ). The Na<sup>+</sup>-K<sup>+</sup>-ATPase activity of the FZ and RG groups was obviously increased as compared with the control group ( $p < 0.01$ ), while there was no difference between the GL and control groups.

#### 4.6. Hepatic FUM level

To determine whether aerobic oxidation of glucose could be influenced, the critical enzyme FUM in rat liver was measured. The

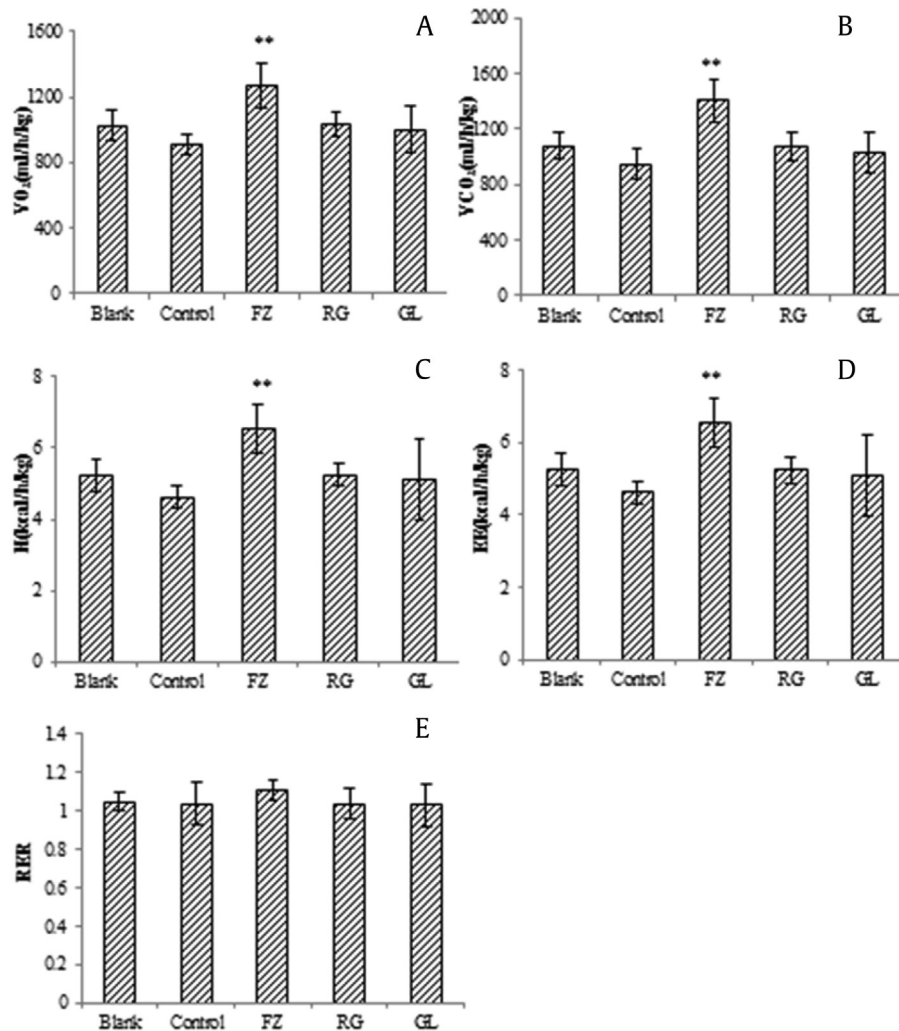
results showed that the FUM level in the control group was obviously decreased as compared with the blank group ( $p < 0.05$ ), indicating that the effect of aerobic oxidation of rats induced by PTU was weaker than the normal rats. The hepatic FUM of the FZ and RG groups was significantly increased as compared with the control group ( $p < 0.01$ ), while there was no difference between the GL and control groups (Fig. 7).

#### 4.7. The pyruvic acid in plasma

Pyruvic acid is the final product of glycolysis, which is utilized for further ATP production. Our results showed that there was statistical differences between the blank and control groups ( $p < 0.01$ ) and the blank group showed a higher level of pyruvic acid (Fig. 8). In addition, the FZ, RG, and GL groups could increase the content of pyruvic acid significantly as compared with the control group ( $p < 0.05$ ).

#### 4.8. The level of cAMP/cGMP in plasma

cAMP and cGMP are second messengers, which are involved in various cellular functions. As showed in Fig. 9A, the level of cAMP tended to decrease in the control group as compared with the blank group ( $p < 0.01$ ), while the FZ, RG, and GL groups were increased significantly as compared with the control group. The results of



**Fig. 3.** Effects of blank, control, Aconiti Lateralis Radix Praeparata, red ginseng, and ginseng leaves during the night after administrating for 19 d. (A) Effect on oxygen consumption. (B) Effect on carbon dioxide production. (C) Effect on heat production. (D) Effect on energy expenditure. (E) Effect on respiratory exchange rate. Cumulative values are reported as mean  $\pm$  standard error for 10 rats in each group. \*  $p < 0.05$  and \*\*  $p < 0.01$  compared to controls, using analysis of variance with the least significant difference analysis. EE, energy expenditure; FZ, Aconiti Lateralis Radix Praeparata; GL, ginseng leaves; H, heat production; RER, respiratory exchange rate; RG, red ginseng;  $VCO_2$ , carbon dioxide production;  $VO_2$ , oxygen consumption.

cGMP showed no statistical difference between the blank and control groups, while the FZ, RG, and GL groups were increased significantly as compared with the control group (Fig. 9B). The cAMP/cGMP of the control group had a tendency to diminish, however it showed no statistical significance. The cAMP/cGMP of the FZ and RG groups had a tendency to increase as compared with the control group, while the GL group showed the contrary (Fig. 9C).

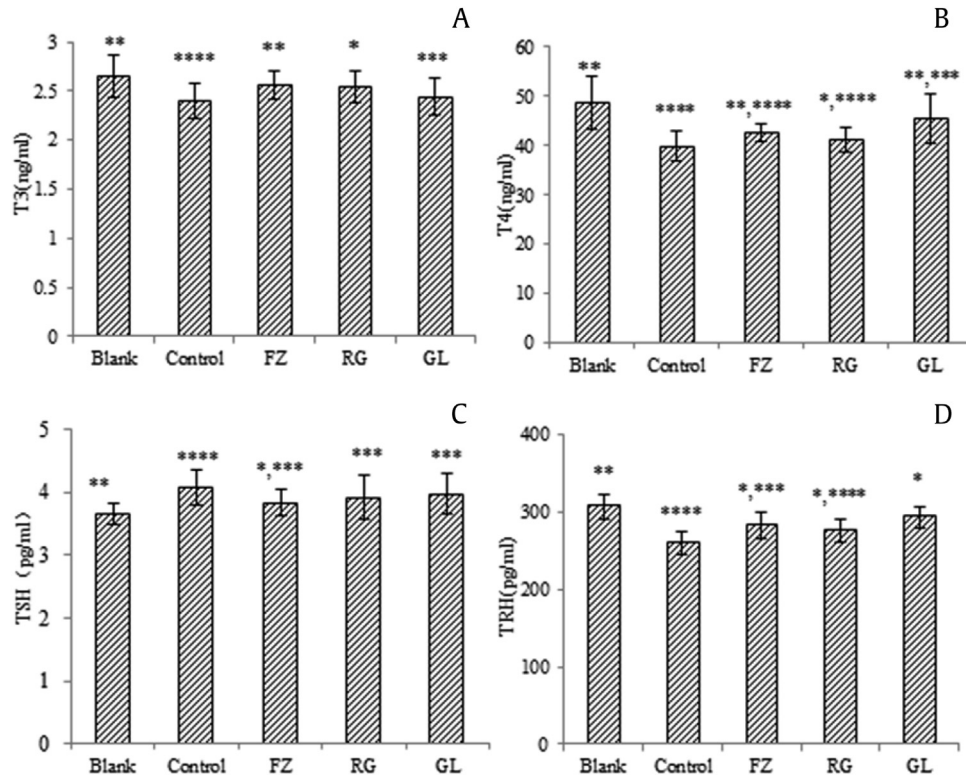
## 5. Discussion

Our present study investigated for the first time the effects of RG and GL on the substance and energy metabolism of hypothyroidism rats based on the difference in the drug properties. Thyroid hormones are involved in the regulation of tissue metabolism, and any alteration of thyroid hormones is suspected to cause metabolic disorders [20]. Numerous researches have demonstrated that hypothyroidism patients were liable to display symptoms of low basal metabolism coupled with a decrease in T3, T4, and TRH, and an increase in TSH in plasma [4,21]. Therefore, the successful establishment of a hypothyroidism model in this study was verified by

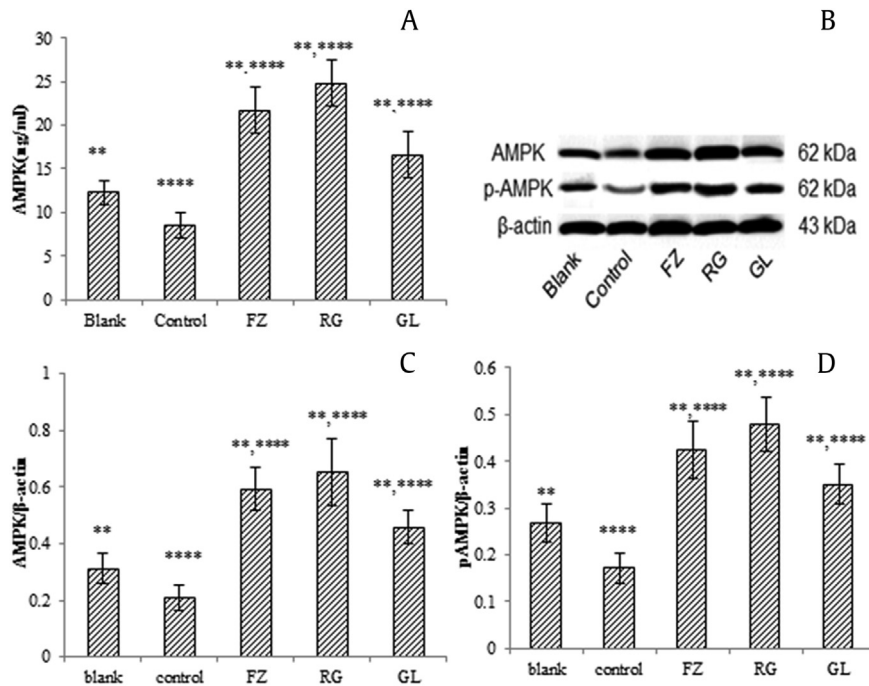
evaluating the content of T3, T4, TSH, and TRH; and PTU was used as it is commonly used to establish a hypothyroidism model. The levels of T3, T4, and TRH were significantly increased after administration of FZ (hot property) and RG (warm property) for 20 d, but decreased after administration of GL (cold property), which demonstrated that the different properties of RG and GL had different effects on hypothyroidism rats. The level of TSH decreased after administration of FZ (hot property) for 20 d, which also showed that TCM with hot property had a positive therapeutic effect on hypothyroidism rats.

To further understand the mechanism of RG and GL on the EE, the  $VO_2$ ,  $VCO_2$ , H, and EE were measured using the phenoMaster/LabMaster (TSE Systems) for 12 h in the daytime and 12 h at night. The results indicated that the  $VO_2$ ,  $VCO_2$ , H, and EE of hypothyroidism rats were decreased as compared with the blank group during the 12 h daytime, while rats in the FZ and RG groups could increase their basal metabolism. However, GL with cold property showed no effect.

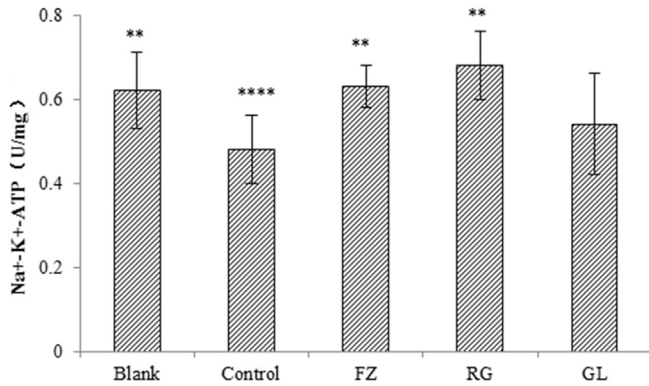
The major pathways of glycometabolism are glycolysis and oxidation, which is mostly associated with the tricarboxylic acid (TCA) cycle. Glycolysis is an effective way to replenish energy in



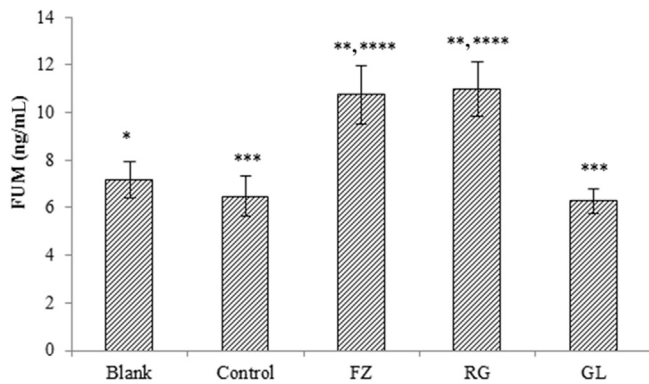
**Fig. 4.** Levels after administrating for 20 d. (A) Triiodothyronine. (B) Tetraiodothyronine. (C) Thyroid stimulating hormone. (D) Thyrotropin-releasing hormone content in plasma. Cumulative values are reported as mean  $\pm$  standard error for 10 rats in each group. \*  $p < 0.05$  and \*\*  $p < 0.01$  compared to controls. \*\*\*  $p < 0.05$  and \*\*\*\*  $p < 0.01$  compared to blank group, using analysis of variance with the least significant difference analysis. T3, triiodothyronine; T4, tetraiodothyronine; TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone.



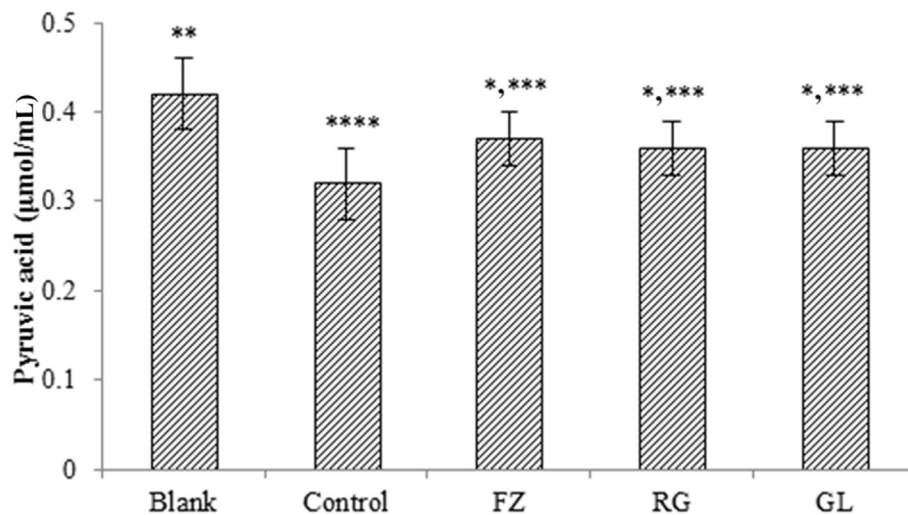
**Fig. 5.** Liver analyses after 20 d. (A) Adenosine monophosphate-activated protein kinase (AMPK) content. (B) The level of AMPK and phosphor-activated protein kinase lactate. (C) AMPK content. (D) pAMPK content. Cumulative values are reported as mean  $\pm$  standard error for 10 rats in (A) and three rats in each group (B, C, D). \*  $p < 0.01$  compared to controls. \*\*  $p < 0.01$  compared to blank group, using analysis of variance with the least significant difference analysis. AMPK, adenosine monophosphate-activated protein kinase; FZ, Aconiti Lateralis Radix Praeparata; GL, ginseng leaves; pAMPK, phosphor-activated protein kinase lactate; RG, red ginseng.



**Fig. 6.** The hepatic Na<sup>+</sup>-K<sup>+</sup>-ATPase activity after administrating for 20 d. Cumulative values are reported as mean ± standard error for 10 rats in each group. \*  $p < 0.05$  and \*\*  $p < 0.01$  compared to controls. \*\*\*  $p < 0.05$  and \*\*\*\*  $p < 0.01$  compared to blank group, using analysis of variance with the least significant difference analysis. FZ, Aconiti Lateralis Radix Praeparata; GL, ginseng leaves; RG, red ginseng.



**Fig. 7.** The hepatic fumarase content after administrating for 20 d. Cumulative values are reported as mean ± standard error for 10 rats in each group. \*  $p < 0.05$  and \*\*  $p < 0.01$  compared to controls. \*\*\*  $p < 0.05$  and \*\*\*\*  $p < 0.01$  compared to blank group, using analysis of variance with the least significant difference analysis. FUM, fumarase; FZ, Aconiti Lateralis Radix Praeparata; GL, ginseng leaves; RG, red ginseng.

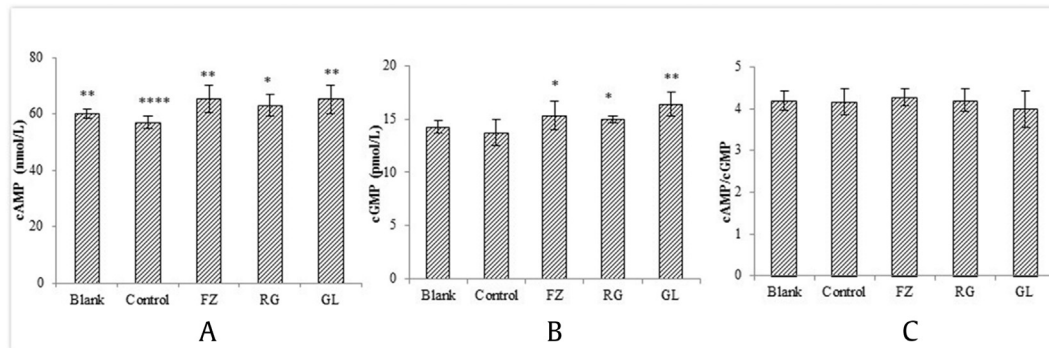


**Fig. 8.** The content of pyruvic acid in plasma after administrating for 20 d. Cumulative values are reported as mean ± standard error for 10 rats in each group. \*  $p < 0.05$  and \*\*  $p < 0.01$  compared to controls. \*\*\*  $p < 0.05$  and \*\*\*\*  $p < 0.01$  compared to blank group, using analysis of variance with the least significant difference analysis. FZ, Aconiti Lateralis Radix Praeparata; GL, ginseng leaves; RG, red ginseng.

anaerobic conditions. ATP is synthesized by oxidative phosphorylation of glucose in mitochondria, which is effective for the metabolic process and produces more ATP molecules from a given amount of glucose when compared to glycolysis. The transformation of sugars, fats, and amino acids can be achieved with pyruvic acid through acetyl-CoA and TCA. Therefore, pyruvic acid plays an important role for the metabolism of the three major nutrients. The increase of pyruvic acid indicated the acceleration of glucose metabolism and the enhancement of energy metabolism of the body. Our study showed that the pyruvic acid content of the control group was significantly decreased as compared with the blank group on the administration for 20 d, indicating that the glycolysis of hypothyroidism rats was weaker than the normal rats. However, the pyruvic acid content of the FZ, RG, and GL groups was obviously increased compared with the control group, demonstrating that FZ, RG, and GL could elevate the effect of glycolysis. FUM is the Krebs cycle enzyme, which mediates the reversible conversion of fumarate to malate [22]. Loss of FUM activity results in accumulation of intracellular fumarate, which, in turn, affects multiple signaling pathways [23]. The high content of FUM could accelerate the TCA cycle to generate more energy. In the work presented here, the FUM content in the control group was obviously decreased compared with the blank group, suggesting that the TCA cycle of the hypothyroidism rats induced by PTU was weaker than the normal rats. In addition, the hepatic FUM of the FZ and RG groups was significantly increased compared with the control group, while there was no difference between the GL and control groups. Thus, the hot property FZ and warm property RG could enhance the glycometabolism of hypothyroidism rats. On the contrary, the GL with cold property was of no avail to hypothyroidism rats.

Energy supply with demand is essential for the survival and function of organisms. AMPK regulates the cellular energy balance in response to energy demand and supply. From the biochemical point of view, AMPK is an enzyme that regulates adenine nucleotide metabolism and homeostasis in a wide range of organisms, by catalyzing the interconversion reaction:  $ATP + AMP \rightleftharpoons 2ADP$  [24,25]. AMPK is activated by any stress stimulation which could cause the decreasing level of ATP in cells [26]. Once activated, AMPK phosphorylates and regulates key enzymes in all branches of





**Fig. 9.** Levels in plasma after administrating for 20 d. (A) cAMP. (B) cGMP. (C) cAMP/cGMP. Cumulative values are reported as mean  $\pm$  standard error for 10 rats in each group. \*  $p < 0.05$  and \*\*  $p < 0.01$  compared to controls. \*\*\*  $p < 0.05$  and \*\*\*\*  $p < 0.01$  compared to blank group, using analysis of variance with the least significant difference analysis. cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; FZ, Aconiti Lateralis Radix Praeparata; GL, ginseng leaves; RG, red ginseng.

metabolism, as well as transcription factors that regulate gene expression, to redirect cellular metabolism away from anabolic, ATP-consuming pathways to energy-generating catabolic pathways. In addition, the activation of the AMPK pathway could regulate fat metabolism and increase glucose uptake [27]. Researches have shown that the activation of AMPK played important roles in glycometabolism and adipose metabolism *in vivo* [28]. The influence on the hepatic AMPK content and pAMPK level were determined in our study, showing a distinct difference between the blank and control groups, with a higher level observed in the blank group. This indicated that the ability of hypothyroidism rats to breakdown ATP was weaker than that of the normal rats. In particular, the level of AMPK and pAMPK in the FZ, RG, and GL groups was significantly higher than the control group, revealing that FZ, RG, and GL could activate the AMPK of the hypothyroidism rats, which was their function. Nevertheless, the effect of GL was weaker than FZ and RG, which may be related to the cold property of GL.

$\text{Na}^+\text{-K}^+\text{-ATPase}$  is a plasma membrane-associated ion pump protein that actively extrudes three  $\text{Na}^+$  ions from the cell while importing two  $\text{K}^+$  ions for the hydrolysis of every ATP molecule [29]. ATP synthase is a central bioenergetic engine for all organisms and represents the smallest molecular motor, which was optimized in the course of evolution.  $\text{Na}^+\text{-K}^+\text{-ATPase}$  not only maintains the different gradient concentrations of ions, but also adjusts the transportation of amino acids and glucose [29]. Many researches have shown that as the  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity is increased, the energy consumption and H could be regulated upwards. Our results indicated that the control group was significantly decreased as compared with the blank group after drug administration for 20 d, showing that the energy consumption and the H process of the hypothyroidism rats was weaker in accordance with the pathogeny of hypothyroidism patients. Scholars have proposed a hypothesis that the hot property of TCM could accelerate energy consumption and H. Further, the research showed that  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity was significantly increased when FZ with hot property and RG with warm property were administrated for 20 d, while GL showed no effect on its activity.

cAMP and cGMP are the intracellular second messengers, which play a broad and critical role on cellular metabolism and physiological effect. Under normal circumstances the metabolism of cAMP and cGMP is presented in a dynamic equilibrium and, although they are interactive, have opposite transmission effects. Goldberg proposed that their interactive effect could be generalized as *Yin* and *Yang* in TCM. Another study explicitly put forward the theory that cAMP belongs to *Yang* and cGMP belongs to *Yin* [30,31]. The theory of TCM considers that the properties of hot and warm belong to Yang, while the properties of cold and cool belong to Yin.

As a result, cAMP, cGMP, and cAMP/cGMP are considered as the major indexes for evaluating the property of TCM. To accurately elucidate the effects of the accumulation of cAMP/cGMP, our study showed that the level of cAMP was decreased in the control group, while significantly increased after the administration of FZ, RG, and GL for 20 d. The results are consistent with the inference that cAMP belongs to *Yang*. The level of cGMP in the control group had no statistical difference with the blank group, but increased significantly after administration of FZ, RG, and GL for 20 d. The cAMP/cGMP levels in the control group had a tendency to decrease, however, showed no statistical significance. The cAMP/cGMP had tendency to increase compared with the control group after administration of FZ and RG for 20 d, while the GL group exhibited oppositely. Therefore, it could be inferred that the TCM with hot properties could increase the cAMP/cGMP levels of hypothyroidism rats through higher-regulation of levels of cAMP and its hot property belongs to *Yang*. However, TCM with cold properties could reduce the cAMP/cGMP of hypothyroidism rats through higher-regulation of the level of cGMP, suggesting that the cold property belongs to *Yin*.

## 6. Conclusion

In TCM, drug property is a unique standard to indicate a drug's efficacy. Recent research indicated that the drug properties were primarily associated with the metabolism of substances and the energy of organism [1], which provides a new way to explore the efficacy of Chinese drugs. As we know, hypothyroidism is a kind of metabolic disease. However, ginseng was rarely used on the effects of hypothyroidism. Although the active constituents of both RG and GL are ginsenosides, their types and proportions are different. According to the principle of TCM, the properties of RG and GL are different, and hypothyroidism is regarded as a disease with cold property.

Generally, the drugs with hot or warm properties are used for the treatment of diseases with cold property. However, its action mechanism is unclear. It is for the first time that our results revealed that drugs with hot or warm properties, such as FZ and RG, could ameliorate diseases with cold properties by way of regulation of the metabolism of substances and the energy of the organism, which further confirmed that RG and GL have different pharmacological actions.

## Conflicts of interest

None of the authors declare any conflicts of interests in this study.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jgr.2016.11.005>.

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