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Short communications

Effects of resveratrol on lipid metabolism in muscle and adipose tissues: A reevaluation in a pig model

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ABSTRACT

The body fat-lowering effect and mechanisms of action of resveratrol (0, 300, 600 mg resveratrol/kg diet) was evaluated in a pig model. Resveratrol improved serum lipid profiles, and decreased visceral adipose tissue weight and serum leptin level. In adipose tissue, peroxisome proliferator activated receptor γ (PPARγ) and fatty acid synthase (FAS) mRNA levels, and FAS and lipoprotein lipase (LPL) activities were decreased by resveratrol, while hormone sensitive lipase (HSL), adipose triglyceride lipase (ATGL) and carnitine palmitoyl transferase-1(CPT-1) mRNA levels, and HSL and CPT-1 activities were increased. In muscle tissue, PPARγ, FAS, acetyl-CoA carboxylase (ACC) and LPL mRNA levels and FAS and ACC activities were decreased by resveratrol, while HSL mRNA levels and CPT-1 activity were increased. Moreover, compared with the control group, LPL activity was decreased, and CPT-1 mRNA levels and HSL activity were increased in the 600 mg resveratrol/kg diet group. This study provides the very first evidence in a pig model that resveratrol could improve serum lipid profiles and decrease body fat deposition, which might be mediated by a reduction in fatty acid uptake and *de novo* lipogenesis, as well as an increase in fat mobilization and fatty acid oxidation in muscle and adipose tissues.

1. Introduction

Obesity, which is associated with several public health concerns, is dramatically increasing and gradually reaching epidemic levels, afflicting not only adults but also many adolescents and children (Alberdi et al., 2011). Although considerable efforts have been made to fight obesity, primary preventive measures and therapeutic options often fail (Poulsen et al., 2013). Therefore, novel treatment modalities still need to be investigated.

Resveratrol, a polyphenolic compound, is found in various plant species, including grapes, pistachios and berries (Changa, Leeb, & Sheua, 2012). The beneficial biological functions of

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Abbreviations: TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; GOT, aspartate aminotransferase; GPT, alanine aminotransferase; PPARγ, peroxisome proliferator activated receptor γ; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; LPL, lipoprotein lipase; HSL, hormone sensitive lipase; ATGL, adipose triglyceride lipase; CPT-1, carnitine palmitoyl transferase-1

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resveratrol are wide-ranging, including antioxidative (Cheng, Cheng, & Chang, 2013; Zhang et al., 2015), anti-inflammatory (Chiou et al., 2014; Cullberg, Foldager, Lind, Richelsen, & Pedersen, 2014), and skin (Lephart, Sommerfeldt, & Andrus, 2014) and bone (Poulsen et al., 2014) protection effects. Therefore, resveratrol seems to serve as an effective dietary supplement for regulating many aspects of body metabolism. Actually, resveratrol is now available on the market as a functional food.

Recently, many studies have shown that resveratrol has a body fat-lowering effect in rodents fed a high-calorie diet (Alberdi et al., 2011; Macarulla et al., 2009; Qiao et al., 2014). However, the efficacy remains controversial since some studies reported that resveratrol has no body fat-lowering effect in non-obese women (Yoshino et al., 2012), healthy obese men (Poulsen et al., 2013) and mice fed a high-fat diet (Price et al., 2012). In addition, the influence of resveratrol on ectopic fat deposition in muscle tissue is rarely reported. Therefore, the body fat-lowering effect of resveratrol still needs to be further investigated.

In addition to serving as an important livestock production animal, the pig is a better animal model than the rodent for human nutrition and metabolism investigation because of the similarity of anatomy and physiology between humans and pigs (Houpt, Houpt, & Pond, 1979). Although research on pigs requires large and rather expensive facilities, its use is a valuable tool when investigating the metabolism of phenolic compounds, such as anthocyanins (Wu, Pittman, & Prior, 2006) and phenolic acids (Wu et al., 2009).

Remarkably, our previous study using a pig model firstly found that intramuscular fat content and backfat depth were decreased by resveratrol (Zhang et al., 2015), indicating a decreased body fat mass. However, the underlying mechanisms responsible for the body fat-lowering effect of resveratrol in a pig model have not been investigated. Taking that into consideration, we hypothesized that resveratrol may change the lipid metabolism-related genes expression patterns and/or enzymes activities in muscle and adipose tissues, thus reducing body fat mass. The present study was conducted to test this hypothesis.

2. Materials and methods

2.1. Animals, diets and samples collection

All experiment procedures were approved by the Animal Care Advisory Committee of Sichuan Agricultural University. Twenty four barrows with average body weight of 78.06 kg were randomly divided into three dietary treatment groups: standard diet group (control group, n = 8) and standard diet supplemented with 300 or 600 mg resveratrol/kg diet groups (n = 8). The standard diet was formulated based on the NRC (1998) recommendation for the nutrient requirements of 75–100 kg pigs. Compositions and calculated nutrient contents of the standard diet are shown in Supplementary Table S1. The purity of resveratrol was 98.1%, which was provided by Ci Yuan Biotechnology Co. Ltd. (Xi'an, Shanxi, China) and was extracted from *Polygonum cuspidatum*. Resveratrol was added to the standard diet at the expense of corn starch. Experimental diets were prepared weekly.

Animals were individually housed in 2.0- × 2.5-m pens with totally slatted concrete floors, and were exposed to an environmental temperature of 22 ± 1 °C and a natural day–night light cycle. Pigs had ad libitum access to water and feed. The experimental period lasted for 49 days. Feed intake and body weight were collected individually at the end of the experiment.

Blood samples were collected by jugular venipuncture using vacuum tubes at the end of the experiment after 12-h fasting, and then all pigs were electrically stunned and killed by exsanguination. After water bath for 30 min at 30 °C, blood samples were centrifuged at 3000 × *g* for 10 min at 4 °C for serum collection. Serum samples were stored at –80 °C until analysis. *Longissimus dorsi, psoas major* and *semitendinosus* muscle from the left side carcass, kidney, liver, heart and visceral adipose tissue were dissected and weighed. *Longissimus dorsi* muscle and subcutaneous adipose tissue samples at the 10th rib of the left side carcass were collected and immediately frozen in liquid nitrogen for RNA extraction and enzyme activity measurement.

2.2. Serum resveratrol and biochemical analysis

The activities of aspartate aminotransferase (GOT) and alanine aminotransferase (GPT), and the contents of triacylglycerol, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and leptin in serum were determined using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) according to the manufacturer's instructions. Serum resveratrol contents were analyzed as previously described by Muzzio et al. (2012) using an API 3000 MS/MS system (Applied Biosystems/MDS Sciex, Foster, CA, USA) equipped with an Agilent 1100 HPLC (Agilent Technologies, Wilmington, DE, USA).

2.3. RNA isolation, reverse transcription and real-time PCR

Tissue total RNA isolation, cDNA synthesis and real-time PCR analysis were conducted as previously described (Zhang et al., 2015). Primers used for real-time PCR analysis were synthesized commercially by TaKaRa Biotechnology (TaKaRa, Dalian, China), and are listed in Supplementary Table S2. The relative mRNA amount of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method according to a previous publication (Livak & Schmittgen, 2001). The 18S rRNA gene was used as the reference gene to normalize mRNA expression of target genes.

2.4. Enzyme activity analysis

About 1.2 g frozen sample was weighed and homogenized on ice in 4 mL 10 mM HEPES buffer containing 1 mM EDTA, 1 mM dithiothreitol and 0.25 M sucrose, and then centrifuged at 100,000 × *g* for 30 min at 4 °C. The supernatant was collected and used to measure enzyme activity in triplicate at appropriate dilution. Lipoprotein lipase (LPL) activity was determined using a commercial kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) according to the manufacturer's instructions. The activity of acetyl-CoA