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# A long-term high-fat, high-sucrose diet in Bama minipigs promotes lipid deposition and amyotrophy by up-regulating the myostatin pathway

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ABSTRACT

Skeletal muscle is as an important regulator of blood glucose and glycolipid metabolism and is closely related to motor ability. The underlying mechanisms by which dietary ectopic lipids in skeletal muscle prevents muscle growth remain elusive. We utilized miniature Bama swine as a model to mimic human obesity using prolonged dietary induction. After 23 months on a high-fat, high-sucrose diet, metabolic disorders were induced in the animals, which exhibited increased body weight, extensive lipid deposition in the skeletal muscle and amyotrophy. Microarray profiles demonstrated the up-regulation of genes related to fat deposition and muscle growth inhibition. We outline a clear potential pathway that in combination with increased 11β-hydroxysteroid dehydrogenase type 1, promotes expression of a major inhibitor, myostatin, by converting corticosterone to cortisol, which leads to the growth inhibition of skeletal muscle. This research provides new insights into the treatment of muscle diseases induced by obesity.



## 1. Introduction

Obesity and associated metabolic disorders are worldwide health problems and are becoming an increasing epidemic globally. Over 500 million individuals are overweight or obese (Akhmedov and Berdeaux, 2013), and most of these individuals suffer from associated metabolic abnormalities such as type 2 diabetes, insulin resistance, visceral obesity, dyslipidemia or hypertension. In obese individuals, lipids excessively accumulate in not only adipose tissue (AT) but also non-AT such as skeletal muscle (Vettor et al., 2009). Skeletal muscle serves as an important regulator of blood glucose and glycolipid metabolism and is closely related to motor ability (Ralt, 2007; Wells et al., 2008). Ectopic lipids in skeletal muscle affect many physiological functions, including muscle mass, muscle growth, and muscle maintenance. For example, obese children often have decreased muscle mass, and obesity decreases whole-body muscle mass in young women (Ralt, 2007; Wells et al., 2008). Furthermore, there are human diseases that include sarcopenic obesity (SO), which describes the copresence of both sarcopenia and obesity.

Many studies have examined the relationship between obesity and skeletal muscle and have demonstrated that myostatin (MSTN) expression is increased in the skeletal muscles of extremely obese women (Wells et al., 2008; Hittel et al., 2009). Researchers have identified increased ceramide pools from palmitate loading or stearoyl-CoA desaturase 1 (SCD1) silencing, which might lead to increased apoptosis in differentiated L6 and C2C12 muscle cells (Akhmedov and Berdeaux, 2013; Yuzefovych et al., 2010). However, the underlying mechanisms by which obesity affects skeletal muscle remain elusive. To explain these mechanisms and to combat the negative effects induced by obesity, several obese rodent

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models have been used, including leptin-deficient mice ("ob/ob" mice), leptin receptor-deficient mice ("db/db" mice) and obese Zucker rats (leptin receptor mutation), as well as mice and rats fed a high-fat diet (Wang et al., 2014). These models exhibit excessive weight, increased lipid levels and abnormal metabolic indices, which are similar to human metabolic syndrome and can be used to at least partially explain some mechanisms. For example, some studies have noted crosstalk between AT and skeletal muscle, and a variety of adipokines secreted by AT might act locally in an auto- or paracrine manner or exert systemic effects on metabolism, immunology and endocrinology (Mitrou et al., 2013; Raschke and Eckel, 2013; Argiles et al., 2005). However, the way obesity affects skeletal muscle, especially growth and maintenance, remains unclear.

Minipigs have similar organ sizes and physiological characteristics as humans and have been proven to be good models in several studies, and different types of minipigs have been fed a high-fat, high-sucrose, high-cholesterol or combination diet to generate a human obesity model. For example, Clark used the minipig as a model to study the effects of diet-induced obesity on skeletal muscle fiber types (Clark et al., 2011). However, these models are induced for only several weeks or months and cannot correctly simulate the process of human disease. Here, we chose the Bama minipig as our model to mimic human obesity and associated metabolic disorders using chronic dietary induction, allowing us to examine how the ectopic fat accumulation in skeletal muscle influenced muscle growth. Our work might facilitate the identification of a potential therapeutic method for muscle growth inhibition induced by obesity.

## 2. Materials and methods

### 2.1. Animal model

The Animal Care and Use Committee of the Germplasm Resource Center of Chinese Experimental Minipig Ethics statement: The animals used in this study received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences", and all procedures were approved by the Animal Care and Use Committee of the Germplasm Resource Center of Chinese Experimental Minipig (Permit Number: ACGRCM2008-012). All surgery was performed under sodium ketamine and xylazine, and all efforts were made to minimize suffering.

Male and female Bama minipigs, aged 6 months at the start of the study and of either sex, were divided into the following two groups for 23 months of treatment. The Bama minipig control group (CD group, N = 6) was fed standard pig chow. The experimental group (N = 6) was fed a high-fat, high-sucrose diet (included 37% sucrose, 53% control diet and 10% pork lard. Both groups of Bama swine were gonadally intact. All swine were fed twice per day on a restricted schedule and dietary dose [3% of body weight monthly; facility certification No.: SYXK (Beijing) 2008–007], and the swine were housed in individual pens with controlled conditions (temperature, 18–22 °C; relative air humidity, 30–70%). At the end of the experiment, the animals were fasted (16–20 h) and were weighed prior to dissection. They were euthanized with an overdose of ketamine and xylazine.

### 2.2. Measurements of serum parameters

Blood samples from both groups were collected at the beginning and end of the diet induction. The blood was collected from the anterior vena cava of the animals after 16–20 h of overnight fasting and centrifuged at 3500 rpm for 10 min at 4 °C. To confirm whether metabolic syndrome occurred in our model, the following indices were measured: blood glucose, insulin, triglycerides (TGs), cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

### 2.3. Tissue preparation

At the time of sacrifice, the loin eye muscle areas were measured, and the middle portions of several skeletal muscles (including the longissimus dorsi, quadriceps, gastrocnemius and soleus) located far from adipose tissue were immediately harvested. The samples were cut to 10 × 10 × 2 mm, and some samples were stored in −80 °C freezers. A portion of the sample was immediately frozen in liquid nitrogen for microarray, real-time PCR and Western blot analysis, and another portion was fixed in 4% paraformaldehyde phosphate buffer (pH 7.4), 75% ethanol and Bouin solution for histopathological observation.

### 2.4. Histological analysis

The skeletal muscle tissue fixed in 4% paraformaldehyde phosphate buffer (pH 7.4) was processed and embedded in paraffin for hematoxylin-eosin (H&E) staining and evaluated with a light microscope (Olympus). The longissimus dorsi tissue fixed in 75% ethanol and Bouin solution was processed and embedded in paraffin for PAS and Mallory trichrome staining. The longissimus dorsi tissue fixed in Bouin solution was processed and embedded in Optimal Cutting Temperature (OCT) compound for Sudan III staining.

### 2.5. Morphometric analysis

The muscle fiber cross-sectional areas (CSAs) and average fiber areas were measured in four types of skeletal muscle using Image-Pro Plus (IPP) software. The samples for measurements of fiber CSAs were obtained from the middle portions of the longissimus dorsi, quadriceps, gastrocnemius and soleus muscles. The samples were stained with H&E and subsequently imaged (Olympus, DP72). We randomly selected five horizons and measured 400 random muscle fiber CSAs with image analysis software (IPP) to calculate the muscle fiber area.

### 2.6. Determination of skeletal muscle glutathione peroxidase (GSH-PX), catalase (CAT), superoxide dismutase (SOD), total antioxide capacity (T-AOC) and malondialdehyde (MDA) levels

A skeletal muscle homogenate including longissimus dorsi, quadriceps, gastrocnemius and soleus muscles from 9 pigs, 6 from the HFHSD group and 3 from the CD group, was prepared. First, 9 times the muscle volume was added to skeletal muscle for normalization, and the homogenates were centrifuged at 3000 r/min for 10 min. Then, the supernatant was collected for detection of GSH-PX, SOD, T-AOC and MDA levels using the commercial kits A005, A001–3, A015, and A003-1, respectively (Jiancheng, Nanjing, China) using the chemical colorimetry method. The CAT level was detected using the A007-2 commercial kit (Jiancheng, Nanjing, China) with a UV-spectrophotometry method.

### 2.7. Cortisol and cortisone test

Homogenates of the longissimus dorsi mixed with 9 times the volume of saline from 9 pigs (6 from the HFHSD group and 3 from the CD group) were prepared for cortisol and cortisone testing using the Cortisol Radioimmunoassay Kit and CORT Elisa Kit, and the proportional amount of each was calculated (cortisol level/