

Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing-finishing pigs

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Received: 20 June 2008 / Accepted: 12 July 2008 / Published online: 6 August 2008
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Abstract Obesity in humans is a major public health crisis worldwide. In addition, livestock species exhibit excessive subcutaneous fat at market weight. However, there are currently few means of reducing adiposity in mammals. This study was conducted with a swine model to test the hypothesis that dietary L-arginine supplementation may increase muscle gain and decrease fat deposition. Twenty-four 110-day-old barrows were assigned randomly into two treatments, representing supplementation with 1.0% L-arginine or 2.05% L-alanine (isonitrogenous control) to a corn- and soybean meal-based diet. Growth performance was measured based on weight gain and food intake. After a 60-day period of supplementation, carcass and muscle composition were measured. Serum triglyceride concentration was 20% lower ($P < 0.01$) but glucagon

level was 36% greater ($P < 0.05$) in arginine-supplemented than in control pigs. Compared with the control, arginine supplementation increased ($P < 0.05$) body weight gain by 6.5% and carcass skeletal-muscle content by 5.5%, while decreasing ($P < 0.01$) carcass fat content by 11%. The arginine treatment enhanced ($P < 0.05$) longissimus dorsi muscle protein, glycogen, and fat contents by 4.8, 42, and 70%, respectively, as well as muscle pH at 45 min post-mortem by 0.32, while reducing muscle lactate content by 37%. These results support our hypothesis that dietary arginine supplementation beneficially promotes muscle gain and reduces body fat accretion in growing-finishing pigs. The findings have a positive impact on development of novel therapeutics to treat human obesity and enhance swine lean-tissue growth.

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Keywords Arginine · Growth · Muscle · Fat ·
Meat quality

Abbreviations

ACC Acetyl-CoA carboxylase
ADG Average daily gain
DM Dry matter
LD Longissimus dorsi
NO Nitric oxide
SM Semitendinosus

Introduction

Obesity in humans is a major public health crisis worldwide (Hill et al. 2008) and is a leading risk factor for insulin resistance, type II diabetes, atherosclerosis, stroke, hypertension, and some types of cancer (including colon

and breast cancers) (Zou and Shao 2008). Unfortunately, clinicians have few tools to fight the obesity epidemic, because current anti-obesity drugs are not highly effective and are fraught with side effects (Hill et al. 2008). Similarly, livestock species exhibit excessive amounts of subcutaneous adipose tissue at market weight, and there are few means of reducing white adipose tissue that are acceptable to consumers (Mersmann and Smith 2005). Interestingly, pigs start to accumulate large amounts of body fat beginning at 45 kg body weight and that fat content increases disproportionately tenfold between 45 and 110 kg body weights (approximately between 110 and 180 days of age) (Etherton and Walton 1986). In market-weight (~110 kg) pigs, the subcutaneous adipose tissue represents 70% of the carcass lipid (Etherton and Walton 1986). Because of similarities in anatomy and physiology between pigs and humans, the pig is an established model for studying human nutrition and metabolism (Bergen and Mersmann 2005; Ou et al. 2007; Tan et al. 2008).

In addition to serving as a biomedical model, the pig is an economically important species in animal agriculture. A major goal of pork production is to increase skeletal muscle growth and reduce excess fat accretion. However, reductions in carcass lipid through breeding improvements have resulted in low levels of intramuscular fat and reduced pork palatability (Henckel et al. 2000). Therefore, new technologies should be developed to enhance muscle gain and reduce carcass fat without affecting meat quality. Recent studies have demonstrated that dietary arginine supplementation stimulates protein synthesis and accretion in skeletal muscle of young pigs (Kim and Wu 2004; Yao et al. 2008), while reducing white adipose tissue in obese rats (Fu et al. 2005; Wu et al. 2007c). Growing evidence shows that arginine plays an important role in regulating metabolism of energy substrates, and, therefore, nutrient partitioning in mammals (Frank et al. 2007; Jobgen et al. 2006; Wu et al. 2007a). Based on these findings, we hypothesized that dietary arginine supplementation may increase muscle gain and reduce adiposity in growing-finishing pigs.

Materials and methods

Animals and experimental treatment

Twenty-four barrows (Duroc × Large White × Landrace) were fed a corn- and soybean meal-based diet (Table 1) that met NRC (1998) requirements of growing-finishing swine. The contents of dry matter (DM), crude protein, and gross energy in the diet were determined according to AOAC (1996) methods. Amino acids in the diet were analyzed by HPLC (Wu et al. 1997). At 110 days of age,

Table 1 Composition and nutrient levels of the basal diet, as-fed basis

Items	Content
Ingredients	
Corn grain (%)	62.93
Soybean meal (44% crude protein) (%)	24.35
Wheat bran (%)	4.50
Soya oil (%)	3.17
Premix ^a (%)	3.00
Cornstarch (%)	2.05
Chemical composition	
Dry matter (%)	90.6
Crude protein ^b (%)	16.5
Digestible energy (kcal/kg)	3,416

^a Premix provided for 1 kg of complete diet: Cu as copper sulfate, 10 mg; Fe as iron sulfate, 100 mg; Se as sodium selenite, 0.30 mg; Zn as zinc oxide, 100 mg; Mn as manganese oxide, 10 mg; vitamin D₃, 386 IU; vitamin A as retinyl acetate, 3086 IU; vitamin E as D- α -tocopherol, 15.4 IU; vitamin K as menadione sodium bisulfate, 2.3 mg; vitamin B₂, 3.9 mg; calcium pantothenate, 15.4 mg; niacin, 23 mg; and vitamin B₁₂, 15.4 μ g

^b The composition of amino acids (%; as-fed basis) in the basal diet was as follows: alanine, 0.88; arginine, 0.97; aspartate plus asparagine, 1.60; cysteine, 0.38; glutamate plus glutamine, 3.30; glycine, 0.64; histidine, 0.40; isoleucine, 0.65; leucine, 1.54; lysine, 0.91; methionine, 0.33; phenylalanine, 0.90; proline, 1.36; serine, 0.80; threonine, 0.62; tryptophan, 0.16; tyrosine, 0.43; and valine, 0.74

pigs with an average body weight of 41.4 kg were assigned randomly into two treatments (12 pigs/treatment), representing supplementation with 1.0% L-arginine or 2.05% L-alanine (isonitrogenous control) to the basal diet (Table 1). Arginine or alanine was added to the basal diet at the expense of corn starch. The amino acids were obtained from Ajinomoto Inc. (Tokyo, Japan). The pigs were housed individually in an environmentally controlled facility with hard plastic slatted flooring, and had free access to drinking water and their respective diets. Growth performance was measured based on average daily gain (ADG), daily feed intake, and gain:feed ratio (Yin et al. 2004). After a 60-day period of arginine supplementation, eight pigs were selected randomly from each treatment group to obtain jugular vein blood samples at 12 h after feeding. Blood samples were immediately centrifuged at 3,000g for 10 min to obtain sera, which were stored at −20°C until analysis within 1 week.

When blood sampling was completed, pigs were immediately anesthetized with sodium pentobarbital (50 mg/kg body weight) and killed by jugular puncture (Deng et al. 2008). Samples of longissimus dorsi (LD) muscle and semitendinosus (SM) muscle were immediately obtained after slaughter, weighed, and stored at −20°C for chemical analyses (including crude protein, lipid, dry

matter, glycogen, and lactate) within 48 h. LD muscle on the same (right) side of the carcass was removed, and sections of ~2.5 cm thicknesses were cut from the anterior end for assessment of meat quality before and after 24 h chilling of the carcass. The experiment was carried out in accordance with the Chinese guidelines for animal welfare and experimental protocol, and approved by the Animal Care and Use Committee of The Chinese Academy of Sciences.

Analysis of serum metabolites and hormones

Triglycerides, cholesterol, glucose, low-density lipoprotein, and high-density lipoprotein were measured using Biochemical Analytical Instrument (Beckman CX4) and commercial kits (Sino-German Beijing Leadman Biotech Ltd, Beijing, China). Tumor necrosis factor- α , insulin, growth hormone, insulin-like growth factor-I, and glucagon were determined by radioimmunoassays using kits from Tianjin Nine Tripods Biomedical Engineering Inc. (Tianjin, China).

Determination of carcass composition

Carcass weight, carcass length, skin thickness at the sixth to seventh rib, backfat thickness, and lion-eye area at the tenth rib were measured immediately post-mortem according to the Chinese Guidelines on *Performance Measurement Technology and Regulations for Pigs* (GB8467-87, 1988). Backfat thickness was the average of measurements at three points: the first rib, the last rib, and the last lumbar vertebra. Carcass length was measured from the first rib to the end of the pubic bone. The left half of the carcass was dissected into skeletal muscle, fat, skin, and bone, which were weighed separately, and then their double weights were used to calculate total percentages of these components in the carcass. Slaughter yield was calculated as carcass weight divided by live body weight.

Assessment of meat quality

Meat quality was estimated using marbling score, drip loss, pH value, and loin muscle color. Initial pH (pH_{45min}) was measured at the last rib position at 45 min after slaughter using a hand-held pH meter (Russell CD700, Russell pH Limited). The final pH (pH_{24h}) was measured at 24 h after slaughter. Drip loss was estimated without freezing on the day of slaughter according to the procedures of Honikel (1998). Briefly, approximately 100 g fresh LD muscle was weighed and placed in a Whirl-Pak bag, suspended in a 4°C cooler for 24 h, reweighed, and drip loss was recorded. Muscle color (1 = pale gray to 5 = dark purplish-red) and marbling (1 = devoid to practically devoid to

5 = moderately abundant or greater) scores were assigned to LD muscle at the tenth rib interface (Wilborn et al. 2004). Percentage of cooking loss was measured by determining the weight loss of LD muscle during cooking. The muscle sample was weighed and covered in a container before cooking. Immediately after a 45-min period of cooking, the sample was removed from the container, patted dry with a paper towel, and weighed. Cooking loss was expressed as the percentage decrease of the sample weight at the end of cooking.

Chemical analysis of skeletal muscle

Analysis of chemical composition in skeletal muscle was performed in duplicate according to AOAC methods (1996). Dry matter contents of LD and SM muscles were determined gravimetrically using oven drying at 110°C for 24 h. Protein and lipid contents were measured using Kjeldahl and Soxhlet extraction methods, respectively. Muscle glycogen and lactate contents were analyzed using kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Statistical analysis

Results are expressed as mean $\pm$ SEM. The statistical analysis was performed by unpaired t-test using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). *P* values <0.05 were taken to indicate significance.

Results

Growth performance and carcass quality

There were no differences ($P > 0.05$) in initial or final body weights, or feed intakes between control and arginine-supplemented pigs (Table 2). Supplementing arginine to the diet increased ($P < 0.05$) the ADG of pigs by 6.5% and had a modest effect ($P = 0.081$) on the gain:feed ratio. Slaughter yield, bone percentage, skin thickness, carcass length, or lion-eye area did not differ ($P > 0.05$) between control and arginine-supplemented pigs. However, arginine supplementation increased skeletal muscle content in the carcass by 5.5% ($P < 0.05$) and decreased carcass fat content by 11% ($P < 0.01$), compared with the control group.

Muscle chemical analysis

Dry matter content was 11% greater ($P < 0.01$) in SM muscle of arginine-supplemented pigs, compared with control pigs (Table 3). However, muscle lipid and crude

Table 2 Effects of dietary supplementation of arginine on growth performance and carcass quality of growing-finishing pigs

	Alanine	Arginine	<i>P</i> value
Growth performance (<i>n</i> = 12)			
Initial body weight (kg)	41.7 ± 1.0	41.2 ± 1.1	0.765
Final body weight (kg)	89.1 ± 1.2	91.7 ± 1.8	0.672
Average daily gain (g/day)	790 ± 16	841 ± 10	0.012
Daily feed intake (g/day)	2334 ± 44	2359 ± 53	0.722
Gain:feed ratio (g/g)	0.337 ± 0.005	0.360 ± 0.012	0.081
Carcass quality (<i>n</i> = 8)			
Slaughter yield (%)	76.0 ± 0.80	74.6 ± 0.75	0.214
Total skeletal muscle (%)	57.9 ± 0.61	61.1 ± 0.93	0.011
Total bone (%)	11.5 ± 0.54	10.7 ± 0.46	0.296
Total fat (%)	23.1 ± 0.35	20.5 ± 0.60	0.002
Total skin (%)	7.49 ± 0.28	7.66 ± 0.27	0.662
Average backfat thickness (cm)	2.23 ± 0.06	2.21 ± 0.07	0.798
Skin thickness (cm)	0.26 ± 0.02	0.29 ± 0.02	0.394
Carcass length (cm)	94.2 ± 1.2	96.7 ± 3.0	0.461
Lion-eye area (cm ²)	33.6 ± 1.9	35.1 ± 1.7	0.574

Values are mean ± SEM

Table 3 Effects of dietary supplementation of arginine on muscle chemical composition in growing-finishing pigs

	Alanine	Arginine	<i>P</i> value
Semitendinosus muscle			
Dry matter (g/100 g)	26.1 ± 0.44	28.9 ± 0.50	0.001
Lipid content (g/100 g)	5.82 ± 0.26	6.16 ± 0.31	0.399
Crude protein (g/100 g)	18.9 ± 0.55	18.7 ± 0.30	0.229
Longissimus dorsi muscle			
Dry matter (g/100 g)	23.5 ± 0.42	24.5 ± 0.43	0.095
Lipid content (g/100 g)	1.81 ± 0.17	3.08 ± 0.48	0.029
Crude protein (g/100 g)	18.9 ± 0.18	19.8 ± 0.21	0.004
Lactate (μg/g)	185 ± 6.7	116 ± 7.4	< 0.001
Glycogen (mg/g)	1.27 ± 0.04	1.80 ± 0.15	0.024

Values are mean ± SEM, *n* = 8. Muscles were obtained and stored at −20°C immediately post-mortem. Fresh tissue weight was used for data expression

protein contents in the SM muscle did not differ ($P > 0.05$) between these two groups of pigs. In LD muscle, dietary arginine supplementation had a modest effect ($P = 0.095$) on DM content, while increasing muscle contents of protein ($P < 0.01$) and lipid ($P < 0.05$) by 4.8 and 70%, respectively, compared with the control group. Muscle content of lactate was 37% lower ($P < 0.001$) but that of glycogen was 42% greater ($P < 0.05$) in arginine-supplemented than in control pigs.

Table 4 Effects of dietary supplementation of arginine on the meat quality of longissimus dorsi muscle from growing-finishing pigs

	Alanine	Arginine	<i>P</i> value
Meat color score	3.18 ± 0.16	3.09 ± 0.18	0.717
Muscle marbling score	2.40 ± 0.25	2.40 ± 0.19	1.000
Drip loss (%)	3.46 ± 0.09	3.50 ± 0.07	0.729
Cooking loss (%)	33.9 ± 1.27	33.6 ± 0.93	0.925
Muscle pH (at 45 min after slaughter)	6.08 ± 0.11	6.40 ± 0.09	0.039
Muscle pH (at 24 h after slaughter)	5.62 ± 0.07	5.69 ± 0.08	0.505

Values are mean ± SEM, *n* = 8

Table 5 Effects of dietary supplementation of arginine on serum concentrations of metabolites and hormones in growing-finishing pigs

	Alanine	Arginine	<i>P</i> value
Low density lipoprotein (mmol/L)	0.73 ± 0.02	0.76 ± 0.04	0.534
High density lipoprotein (mmol/L)	1.44 ± 0.06	1.38 ± 0.05	0.458
Glucose (mmol/L)	6.91 ± 0.56	6.30 ± 0.36	0.402
Triglyceride (mmol/L)	0.46 ± 0.03	0.37 ± 0.01	0.005
Cholesterol (mmol/L)	2.49 ± 0.07	2.50 ± 0.10	0.898
Insulin (μIU/mL)	8.19 ± 0.65	8.34 ± 1.38	0.887
Growth hormone (ng/mL)	0.97 ± 0.05	1.02 ± 0.05	0.491
Insulin-like growth factor-I (ng/mL)	22.1 ± 3.7	22.5 ± 1.63	0.920
Glucagon (pg/mL)	99.7 ± 10.6	135.6 ± 11.4	0.042
Tumor necrosis factor-α (ng/mL)	0.41 ± 0.08	0.45 ± 0.04	0.691

Values are mean ± SEM, *n* = 8

Meat quality

Meat color score, LD muscle marbling score, drip loss, cooking loss, and muscle pH at 24 h after slaughter did not differ ($P > 0.05$) between control and arginine-supplemented pigs (Table 4). Interestingly, dietary arginine supplementation substantially increased ($P < 0.05$) muscle pH at 45 min after slaughter by 0.32.

Concentrations of serum metabolites and hormones

Serum concentration of triglycerides was 20% lower ($P < 0.01$) and that of glucagon was 36% greater ($P < 0.05$) in arginine-supplemented than in control pigs (Table 5). Dietary arginine supplementation did not affect ($P > 0.05$) serum concentrations of low-density lipoprotein, high-density lipoprotein, glucose, tumor necrosis factor-α, insulin, growth hormone, insulin-like growth factor-I, or cholesterol (Table 5).

Discussion

Nutritional manipulation of protein and lipid metabolism remains a focus of growth biology research (Bergen and Mersmann 2005). At present, little is known about a role for arginine in regulating the body composition of any mammalian species between weaning and adult life. The major findings from the present study are that dietary arginine supplementation increased ADG, as well as protein and glycogen contents of skeletal muscle, while reducing white fat content in the carcass of growing-finishing pigs. Additionally, the arginine treatment enhanced LD intramuscular lipid concentration without altering pork quality. To our knowledge, this study is the first to demonstrate that dietary arginine supplementation can beneficially increase protein gain and reduce body fat accretion in pigs.

There is growing evidence that arginine regulates the metabolism of energy substrates (fatty acids, glucose, and amino acids) partly through the production of nitric oxide (NO) (Jobgen et al. 2006). NO is synthesized from L-arginine by NO synthase in virtually all cell types (Wu and Morris 1998). As a signaling molecule, physiological levels of NO stimulate glucose uptake, as well as glucose and fatty-acid oxidation in skeletal muscle, heart, liver, and adipose tissue, inhibit the synthesis of glucose, glycogen and lipid in target tissues (e.g., liver and adipose), and enhance lipolysis in subcutaneous adipocytes (Jobgen et al. 2006; Montanez et al. 2007; Nikolic et al. 2007). Thus, dietary arginine supplementation reduces fat mass in obese rats (Fu et al. 2005; Wu et al. 2007c) and humans (Lucotti et al. 2006). The possible underlying mechanisms may involve multiple cGMP-dependent pathways. First, NO stimulates the phosphorylation of AMP-activated protein kinase (Zou et al. 2004). This results in a decreased level of malonyl-CoA via inhibition of acetyl-CoA carboxylase and activation of malonyl-CoA decarboxylase. Second, by enhancing the phosphorylation of hormone-sensitive lipase and perilipins (Jobgen et al. 2006), NO facilitates the translocation of the lipase to the neutral lipid droplets and promotes lipolysis. Third, NO increases the expression of peroxisome proliferator-activated receptor γ coactivator-1 α , a master regulator of mitochondrial biogenesis and oxidative phosphorylation (Fu et al. 2005). Fourth, through its effect on vascular smooth muscle cells, NO increases blood flow to insulin-sensitive tissues (skeletal muscle, heart, adipose tissue, and liver) (Wu and Meininger 2000), therefore augmenting the uptake of energy substrates and the removal of their products via the circulation. Collectively, these mechanisms contribute to reductions of both blood triglycerides and body fat in growing-finishing pigs.

Insulin, growth hormone, and glucagon are known to regulate fat metabolism via cAMP-dependent mechanisms in animals (Mersmann and Smith 2005). At the supplemental

dosage, arginine treatment increased substantially circulating levels of arginine (Wu et al. 2007b) but had no effect on serum concentrations of insulin or growth hormone in growing-finishing pigs (Table 5). Importantly, the arginine supplementation increased serum concentrations of glucagon by 36%, compared with the control group (Table 2). Glucagon activates adenylyl cyclase to generate cAMP, which stimulates protein kinase A (Mersmann and Smith 2005). Protein kinase A phosphorylates hormone-sensitive lipase, which hydrolyzes triacylglycerides to free fatty acids plus glycerol. Fatty acids are then oxidized in multiple tissues (including skeletal muscle, heart, adipose tissue, and liver) via the mitochondrial β -oxidation pathway (Jobgen et al. 2006). In this regard, it is noteworthy that NO promotes mitochondrial biogenesis and oxidative phosphorylation in diverse cell types (Nisoli et al. 2003).

Lipogenesis also controls fat balance in animals (Azain 2004). In pigs, this synthetic pathway occurs primarily in adipose tissue (Smith et al. 1999). Acetyl-CoA carboxylase (ACC), which catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, is a key regulatory enzyme in fatty acid synthesis (Munday 2002). In lipogenic tissues, ACC α (ACC1) is the major form of ACC and malonyl-CoA is the immediate precursor for fatty acid synthesis. However, in oxidative tissues such as liver and muscle, ACC β (ACC2) predominates, where malonyl-CoA inhibits carnitine palmitoyltransferase activity and thus the entry of long-chain fatty acyl-CoA from the cytosol into mitochondria for oxidation (Jobgen et al. 2006). Recent evidence indicates that cAMP-dependent protein kinase A phosphorylates and inactivates ACC1 and ACC2, thereby reducing the production of malonyl-CoA (Munday 2002). A rise in serum glucagon, which would augment cAMP production by cells, can contribute to reduced lipogenesis and increased fatty acid oxidation in pigs. The net outcome is reduced fat mass in the whole body.

Fat metabolism and its regulation may vary with the anatomical location of white adipose tissue (Mersmann and Smith 2005). An intriguing finding from this study is that lipid content in LD muscle was 70% greater in arginine-supplemented than in control pigs (Table 3), indicating increased de novo fatty acid synthesis or lipid deposition in intramuscular adipose tissue. The enhancement of muscle lipid, which represented <3% of the body fat (Tables 2, 3), did not contribute to an increase of carcass lipid content in arginine-supplemented pigs. Recent studies indicate that addition of arginine or NO donors to culture medium increased PPAR γ expression in preadipocytes (Yan et al. 2002; Chung et al. 2005). Because PPAR γ stimulates differentiation and proliferation of porcine adipocytes (Ding et al. 2000), an increase in its expression would lead to enhanced lipogenesis in intramuscular adipose tissue.

Intramuscular lipid content has been considered as a determinant of meat quality and marbling (2–4%

intramuscular fat) as being ideal for pork flavor, tenderness and juiciness (Henckel et al. 2000). However, recent studies have reported that intramuscular fat content did not affect the eating quality of fresh pork loins (Rincker et al. 2008). Consistent with this notion, we found that dietary supplementation with arginine increased the lipid content of LD muscle from 1.81 to 3.08% (Table 3), without affecting meat color score, marbling score, drip loss, cooking loss, or muscle pH at 24 h post-mortem (Table 4).

Previous work has shown that rates of muscle glycolysis affect the extent of post-mortem pH decline (Henckel et al. 2000) and its water-holding capacity (Rosenvold et al. 2001). Notably, a rapid pH decline in muscle during the first 60 min post-mortem can lead to the pale-soft-exudative pork condition (Bendall and Swatland 1988). Results of the present study demonstrate that increasing arginine provision enhanced glycogen content by 42% and reduced lactate content by 37% in LD muscle (indicating reduced rates of glycogenolysis and glycolysis, respectively), and substantially increased muscle pH at 45 min post-mortem by 0.32 (Table 4). This is a novel and very important finding, because dietary arginine supplementation may be a new effective means to ameliorate the deleterious effects of rapid pH decline in post-mortem pork muscle early post-mortem. Future studies are warranted to determine whether arginine and its product NO regulate the postmortem conversion of glycogen into lactate in muscle.

In conclusion, dietary L-arginine supplementation enhanced protein deposition, while reducing serum levels of triglycerides and body fat mass in growing-finishing pigs. The arginine treatment increased muscle protein and fat contents, as well as muscle pH at 45 min post-mortem, while reducing muscle lactate content. These findings indicate that arginine supplementation is beneficial for promoting skeletal muscle gain, reducing whole-body fat accretion, and improving carcass quality in growing-finishing pigs. Modulation of the arginine-NO pathway may regulate nutrient partitioning and favorably reduce adiposity in mammals (including humans and pigs).

Acknowledgments This work was supported by the National Basic Research Program of China (No. 2004CB117502), NSFC (30528006, 30671517, 30700581, 30771558, 30371038), the Outstanding Overseas Chinese Scholars Fund of The Chinese Academy of Sciences (No. 2005-1-4), Texas AgriLife Research (No. H-8200), and National Research Initiative Competitive Grant (No. 2008-35206-18762) from the USDA Cooperative State Research, Education, and Extension Service Hunan Project C2007FJ1003.

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