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Dietary L-arginine supplementation differentially regulates expression of lipid-metabolic genes in porcine adipose tissue and skeletal muscle

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Abstract

Obesity is a major health crisis worldwide and new treatments are needed to fight this epidemic. Using the swine model, we recently reported that dietary L-arginine (Arg) supplementation promotes muscle gain and reduces body-fat accretion. The present study tested the hypothesis that Arg regulates expression of key genes involved in lipid metabolism in skeletal muscle and white adipose tissue. Sixteen 110-day-old barrows were fed for 60 days a corn- and soybean-meal-based diet supplemented with 1.0% Arg or 2.05% L-alanine (isonitrogenous control). Blood samples, longissimus dorsi muscle and overlying subcutaneous adipose tissue were obtained from 170-day-old pigs for biochemical studies. Serum concentrations of leptin, alanine and glutamine were lower, but those for Arg and proline were higher in Arg-supplemented pigs than in control pigs. The percentage of oleic acid was higher but that of stearic acid and linoleic acid was lower in muscle of Arg-supplemented pigs, compared with control pigs. Dietary Arg supplementation increased mRNA levels for fatty acid synthase in muscle, while decreasing those for lipoprotein lipase, glucose transporter-4, and acetyl-coenzyme A carboxylase-α in adipose tissue. Additionally, mRNA levels for hormone sensitive lipase were higher in adipose tissue of Arg-supplemented pigs compared with control pigs. These results indicate that Arg differentially regulates expression of fat-metabolic genes in skeletal muscle and white adipose tissue, therefore favoring lipogenesis in muscle but lipolysis in adipose tissue. Our novel findings provide a biochemical basis for explaining the beneficial effect of Arg in improving the metabolic profile in mammals (including obese humans).

Keywords: Arginine; Fat metabolism; Gene expression; Pig; Muscle; Adipose tissue

1. Introduction

Obesity is a growing epidemic worldwide and livestock species also exhibit excessive amounts of subcutaneous adipose tissue at market weight [1–3]. Therefore, identifying new means to promote skeletal muscle growth and reduce excess fat accretion is important for both human health and animal production [4–7]. Studies with obese rats have demonstrated that oral administration of L-arginine (Arg) effectively reduces carcass white fat and enhances whole-body insulin sensitivity [2,3]. Using the swine model, we previously reported that dietary supplementation with Arg reduced white adipose tissue mass [8] and improved the metabolic profile [9] in the whole body. Interestingly, we and others observed that intramuscular fat content was increased in Arg-supplemented pigs [8,10]. These results indicate that white adipose tissue and skeletal muscle respond differentially to Arg treatment possibly due to tissue-specific regulation of the expression of lipogenic and lipolytic enzymes by this nutrient.

Adipose tissue accumulation is determined by the balance between lipogenesis and lipolysis/fatty acid oxidation. Lower accretion of body fat may result from increased lipid catabolism or diminished synthesis of fatty acids or both processes. Fatty acid synthesis is regulated by key enzymes, including acetyl coenzyme A (CoA) carboxylase (ACC) and fatty acid synthase (FAS), whereas hydrolysis of triacylglycerols in adipose tissue is catalyzed by hormone-sensitive lipase (HSL) [11,12]. Partitioning of fatty acids between storage in white adipose tissue and oxidation in skeletal muscle can be modulated by lipoprotein lipase (LPL) [13–15]. Although protein phosphorylation and allosteric regulation of key enzymes provide a rapid mechanism for the regulation of lipolysis and fatty acid oxidation via changes in their specific activities, gene expression affects long-term regulation of these events through alterations in the amounts of enzyme proteins [2].

Arg is the physiological precursor for the synthesis of nitric oxide (NO), which stimulates the oxidation of fatty acids and glucose in a

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cell-specific manner [2]. In addition, Arg may affect multiple metabolic pathways involving fatty acid and glucose syntheses, amino acid degradation, and cellular redox state [3–5]. Growing evidence shows that Arg plays an important role in regulating the metabolism of energy substrates and, therefore, nutrient partitioning in mammals [2,6,7]. The major objective of the present study tested the hypothesis that dietary Arg supplementation differentially regulates expression of key lipid-metabolic genes in adipose tissue and skeletal muscle.

2. Materials and methods

2.1. Animals and experimental treatment

Sixteen barrows (Duroc×Large White×Landrace) were fed a corn- and soybean-meal-based diet (Table 1) that met National Research Council (1998) [16] requirements of growing-finishing swine. At 110 days of age, pigs with an average body weight of 41.4 kg were assigned randomly into two treatments, representing supplementation with 1.0% Arg or 2.05% L-alanine (isonitrogenous control) to the basal diet. L-Alanine is an appropriate isonitrogenous control because it is extensively catabolized to CO_2 plus water [5] and does not affect circulating levels of glucose or insulin in growing-finishing pigs (e.g., Refs. [8–10]). The contents of dry matter (90.6%), crude protein (16.5%), Arg (0.97%), L-lysine (0.91%), and gross energy (4270 kcal/kg) in the basal diet were determined according to Association of Official Analytical Chemists (1996) methods and our published study [17]. Arg or L-alanine (Ajinomoto, Tokyo, Japan) was added to the basal diet at the expense of corn starch. 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. During the entire 2-month trial, feed intake did not differ between control and Arg-supplemented pigs [8] and they consumed 40 g dry matter, 155 kcal gross energy, 6.0 g protein and 352 mg Arg per kg body weight per day from the basal diet. The supplemental dose of Arg was 363 mg/kg body weight per day.

After a 60-day period of Arg supplementation, blood samples were obtained from the jugular vein of pigs at 12 h after the last feeding and 1 h after consumption of supplemental Arg or Ala. The animals were then slaughtered, as we previously described [18]. Samples of longissimus dorsi muscle (skeletal muscle) and overlying subcutaneous adipose tissue (white adipose tissue) were collected immediately, snap-frozen in liquid nitrogen, and stored at −80°C for analysis. 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.

2.2. Fatty acids compositions of intramuscular fat and backfat lipids analysis

Fatty acids compositions of intramuscular fat and backfat lipids were determined by capillary gas chromatography [19]. Briefly, lipids were extracted from longissimus dorsi muscle and subcutaneous adipose tissue using petroleum ether/anhydrous diethyl ether (1:1, v/v). Methyl esters of lipids were obtained via saponification with a solution of KOH:methanol (4 mol:1 L) and the organic layer was aspirated for the analysis of fatty acids by gas chromatography using a capillary column (HP-INOWAX) (30 m×2.5 mm×2.5 μm). The gas chromatograph program temperature was as follows: initial temperature of 150°C for 3 min, 8°C/min to 200°C; 1 min at this temperature and, thereafter, 15°C–250°C per minute and maintained at 250°C for 4 min. Injector and detector temperatures were 240°C and 260°C, respectively. Carrier gas was hydrogen at a flow rate of 40 ml/min. Individual fatty acid peaks were identified by comparison of their retention times with those of standards (Sigma Chemicals, St. Louis, MO, USA). Results were expressed as grams per 100 g of total identified fatty acids.

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	3416

[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 D_3, 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 B_2, 3.9 mg; calcium pantothenate, 15.4 mg; niacin, 23 mg; and vitamin B_{12}, 15.4 μg.

[b] The analyzed 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.

Table 2
Primers used for real-time RT-PCR

Gene	Accession no.	Primers
18 s	DQ_437859	S: 5′- AAT TCC GAT AAC GAA CGA GAC T -3′ A: 5′- GGA CAT CTA AGG GCA TCA CAG -3′
LPL	AY_686761	S: 5′- CAA ACT TGT GGC TGC CCT AT -3′ A: 5′- GTG GAC ATT GTT GGG AGG AT -3′
PPARγ	AB_097926	S: 5′- CGC TGA CCA AAG CAA AGG C -3′ A: 5′- CCA CGG AGC GAA ACT GAC AC -3′
FAS	EF_589048	S: 5′- GTC CTG CTG AAG CCT AAC TC -3′ A: 5′- TCC TTG GAA CCG TCT GTG -3′
HSL	AY_686758	S: 5′- TCC TTG GAA CCG TCT GTG -3′ A: 5′- GTA AGG CTC GTG GGA TTT GG -3′
GLUT4	NM_012751	S: 5′- CGA GGC AGG ACG TTT GAC C -3′ A: 5′- CTC CAG TTC TGT GCT GGG TTT C -3′
ACCα	EF_618729	S: 5′- TCC CAG TGC AAG CAG TAT G -3′ A: 5′- TGC CAA TCC ACA CGA AGA C -3′

18s, 18s ribosomal.

2.3. Analysis of serum amino acids and leptin concentrations

Amino acids in serum were analyzed by high-performance liquid chromatography [17]. Authentic standards (Sigma Chemicals) were used to quantify amino acids in samples. The concentration of serum leptin was determined by radioimmunoassays using kits from Tianjin Nine Tripods Biomedical Engineering (Tianjin, China).

2.4. LPL activity in skeletal muscle and adipose tissue analysis

Lipoprotein lipase activities in muscle and adipose tissue were determined using kits from Nanjing Jianchen Bioengineering Institute (Nanjing, China).

2.5. Real-time quantitative reverse transcriptase-polymerase chain reaction analysis

Total RNA was extracted from tissues using Trizol-reagent (Invitrogen) according to the manufacturer's instructions and quantified by measuring optical density at 260 and 280 nm. The cDNA was reverse-transcribed from 0.2 μg of eluted RNA using the first strand cDNA synthesis kit (Fermentas) according to the manufacturer's instructions. The real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for LPL, peroxisome proliferator-activated receptor γ (PPARγ), FAS, HSL, glucose transporter 4 (GLUT4), ACCα and 18s RNA (internal control) was performed with 50 ng of reverse-transcribed RNA, as well as both sense and antisense primers (Table 2, 500 nM for each gene), in a final volume of 20 μl, using SYBR Premix Ex Taq kit (Takara, Japan) in the Rotor-Gene Multi-filter system Rotor-Gene 3000 instrument (Corbett Research, Sydney, Australia). Forty cycles of amplification were performed, with each cycle consisting of pre-denaturation at 95°C for 10 s; denaturation at 95°C for 5 s and annealing and extension at 60°C for 20 s. Cycle threshold (Ct) values are means of triplicate measurements. The comparative Ct value method was employed to determine expression levels for target genes relative to those for 18s RNA, as described by Fu et al. [20], and data are expressed as the relative values to those for control pigs.

2.6. Statistical analysis

Values are expressed as mean±S.E.M. Results were statistically analyzed by the unpaired t-test using SPSS 13.0 (SPSS, Chicago, IL, USA). $P<.05$ was taken to indicate significance.

3. Results

3.1. Fatty acid compositions of intramuscular fat and backfat lipids

Among total identified fatty acids, the percentage (g/100 g of total identified fatty acids) of oleic acid (C18:1n-9) was 25% higher ($P<.01$) but the percentages of stearic acid (C18:0) and linoleic acid (C18:2n-6) were 12–25% lower ($P<.05$) in skeletal muscle of Arg-

Table 3
Fatty acid composition of intramuscular lipid and subcutaneous lipids in growing-finishing pigs fed diets supplemented with Arg or L-alanine (isonitrogenous control) for 60 days

Fatty acid composition	L-Alanine	Arg	P-Value
Intramuscular lipids			
C16:0	28.7±4.24	27.2±0.34	.528
C16:1n-7	1.21±0.13	1.49±0.19	.319
C18:0	16.8±0.89	14.8±0.58	.043
C18:1n-9	33.0±1.78	41.2±2.27	.001
C18:2n-6	20.3±3.45	15.3±1.28	.021
C16:1n-7/C16:0	0.042±0.002	0.056±0.003	.001
C18:1n-9/C18:0	1.93±0.06	2.81±0.10	.001
Subcutaneous adipose tissue lipids			
C16:0	23.9±0.70	25.6±0.74	.146
C16:1n-7	1.77±0.04	1.84±0.04	.257
C18:0	14.9±0.58	16.1±0.20	.102
C18:1n-9	41.1±0.31	37.9±0.42	.001
C18:2n-6	18.4±0.38	18.6±0.36	.619
C16:1n-7/C16:0	0.073±0.004	0.072±0.003	.853
C18:1n-9/C18:0	2.80±0.08	2.31±0.06	.001

Data are means±S.E.M., n=8. Values are expressed as g/100 g of total identified fatty acids or C16:1n-7/C16:0 and C18:1n-9/C18:0 ratios.

supplemented pigs than in control pigs (Table 3). The percentage of palmitic acid (C16:0) or palmitoleic acid (C16:1n-7) did not differ (P>.05) between the two groups of pigs. In subcutaneous adipose tissue, dietary Arg supplementation decreased (P<.01) the percentage of oleic acid and had no effect (P>.05) on the percentages of other four identified fatty acids. C16:1n-7/C16:0 and C18:1n-9/C18:0 ratios were higher (P<.01) in skeletal muscle of Arg-supplemented pigs than in the control group whereas the opposite was observed for the C18:1n-9/C18:0 ratio in subcutaneous adipose tissue.

3.2. Concentrations of leptin and amino acids in serum

The concentration of leptin in serum was 13% lower (P<.05) in Arg-supplemented pigs than in control pigs (Table 4). Dietary supplementation with 1% Arg increased (P<.01) serum concentrations of Arg and proline but reduced (P<.01) those for glutamine (Table 4). In response to L-alanine supplementation (isonitrogenous control), the concentration of alanine in serum was greater (P<.01) in the control group, compared with Arg-supplemented pigs. The Arg treatment had no effects on concentrations of other measured amino acids (including lysine and histidine) (data not shown).

3.3. LPL activity in skeletal muscle and white adipose tissue

Supplementing Arg to the diet increased (P<.05) LPL activity by 52% in skeletal muscle (Table 5). Compared with the control group, LPL activity in subcutaneous adipose tissue tended to be lower (P=.091) in Arg-supplemented pigs (Table 5). Arg supplementation reduced (P<.01) the ratio of LPL activity in subcutaneous adipose tissue over skeletal muscle.

Table 4
Concentrations of leptin and amino acids in serum of growing-finishing pigs fed diets supplemented with Arg or L-alanine (isonitrogenous control) for 60 days

	L-Alanine	Arg	P
Leptin (ng/ml)	2.69±0.13	2.35±0.08	.046
Glutamine (μmol/L)	528±28	383±12	.003
Alanine (μmol/L)	498±68	290±30	.028
Proline (μmol/L)	203±7	272±10	.002
Arginine (μmol/L)	166±5	253±16	.002

Data are means±S.E.M., n=8.

Table 5
LPL activities in skeletal muscle and subcutaneous adipose tissue in growing-finishing pigs fed diets supplemented with Arg or L-alanine (isonitrogenous control) for 60 days

	L-Alanine	Arg	P-Value
LPL activity in adipose tissue (U/mg protein)	1.14±0.15	0.84±0.04	.091
LPL activity in skeletal muscle (U/mg protein)	0.98±0.10	1.49±0.13	.019
Ratio of LPL activity in adipose tissue over muscle	1.16±0.10	0.58±0.05	.002

Data are means±S.E.M., n=8.

3.4. Expression of fat-metabolic gene levels in white adipose tissue and skeletal muscle

Dietary Arg supplementation increased (P<.01) mRNA levels for HSL by 467%, while reducing (P<.05) mRNA levels for LPL (−49%), GLUT4 (−51%) and ACCα (−38%) in subcutaneous adipose tissue of growing-finishing pig (Table 6). Arg treatment had no effect (P>.05) on mRNA levels for PPARγ or FAS in the white adipose tissue.

The mRNA levels for FAS were 128% higher (P<.05) in skeletal muscle of Arg-supplemented pigs, compared with control pigs (Table 7). However, mRNA levels for HSL, LPL or GLUT4 did not differ in skeletal muscle between these two groups of pigs (Table 7).

4. Discussion

Recent studies demonstrated that dietary supplementation with Arg reduced body-fat mass but increased intramuscular fat content in growing-finishing pigs [8]. These results indicate that lipid distribution between muscle and adipose tissue might be regulated by Arg. The new findings of the current work provide for important clues about possible biochemical mechanisms responsible for the differential effects of Arg on lipid accretion in skeletal muscle and white adipose tissue.

Lipogenesis and lipolysis are major factors affecting lipid accumulation in tissues. Dietary Arg supplementation decreased whole-body fat mass [8] and serum levels of very low-density lipoprotein in growing-finishing pigs [9], which was consistent with a reduction in the circulating level of leptin (Table 4), a hormone secreted exclusively by white adipose tissue [21]. Similarly, Stingl et al. [22] reported that Arg infusion transiently decreased plasma concentrations of leptin both in insulin-deficient and hyperinsulinemic diabetic patients. Intriguingly, in contrast to fat content in the whole body, total intramuscular lipid concentrations were higher in Arg-supplemented than in control pigs [8], indicating enhanced lipogenesis in skeletal muscle. The enhancement of muscle lipids, which represented <3% of the body fat [8], did not contribute to an increase of carcass lipid content in Arg-supplemented pigs. There is evidence that addition of Arg or nitric oxide (NO) donors to culture medium increased PPARγ expression in preadipocytes [23,24]. Because PPARγ

Table 6
mRNA levels for fat-metabolic genes in subcutaneous adipose tissue of growing-finishing pigs fed diets supplemented with Arg or L-alanine (isonitrogenous control) for 60 days

Genes	L-Alanine	Arg	P
LPL	1.00±0.19	0.51±0.03	.046
PPARγ	1.00±0.34	1.47±0.32	.395
FAS	1.00±0.28	1.60±0.34	.231
HSL	1.00±0.39	5.67±0.59	<.001
FAS/HSL	1.00±0.23	0.30±0.05	.017
GLUT4	1.00±0.12	0.49±0.15	.017
ACCα	1.00±0.05	0.62±0.02	.001

Data are means±S.E.M., n=8. mRNA levels were determined by real-time RT-PCR. The comparative Ct value method was employed to determine expression levels for target genes relative to those for 18s RNA. Results are expressed as the relative values to those of control pigs.

Table 7
mRNA levels for fat-metabolic genes in skeletal muscle of growing-finishing pigs fed diets supplemented with Arg or L-alanine (isonitrogenous control) for 60 days

Genes	L-Alanine	Arg	P
FAS	1.00±0.14	2.28±0.28	.016
HSL	1.00±0.16	0.62±0.12	.142
LPL	1.00±0.15	1.30±0.07	.083
GLUT4	1.00±0.12	0.69±0.06	.163
FAS/HSL	1.00±0.14	3.65±0.46	.005

Data are means±S.E.M., $n=8$. mRNA levels were determined by real-time RT-PCR. The comparative Ct value method was employed to determine expression levels for target genes relative to those for 18s RNA. Results are expressed as the relative values to those of control pigs.

stimulates differentiation and proliferation of porcine adipocytes [25], an increase in its expression would lead to enhanced lipogenesis in intramuscular adipose tissue. This action of Arg is analogous to that of PPARγ agonist drugs that are currently used to improve insulin sensitivity in obese subjects [2] and explains why dietary Arg supplementation enhances intramuscular fat content in growing-finishing pigs [8].

The substrates needed for triacylglycerol biosynthesis in skeletal muscle may be derived from the hydrolysis of circulating lipoproteins by LPL, whose activity in muscle was substantially increased in response to dietary Arg supplementation (Table 5). LPL is considered as a key factor affecting the partitioning of dietary lipids among tissues, and, therefore, functions as a 'metabolic gatekeeper' [13–15]. Indeed, LPL activity correlates well with the uptake of triglyceride-derived fatty acids by skeletal muscle [26,27]. Interestingly, mRNA levels for LPL in skeletal muscle did not differ between control and Arg-supplemented pigs (Table 7). Thus, it is possible that Arg increased the translation of the muscle LPL mRNA, but this possibility could not be examined due to the lack of an antibody against the porcine LPL protein. Alternatively, Arg or NO may regulate the phosphorylation of muscle LPL protein, therefore increasing its enzymatic activity.

Another novel and interesting finding from the present study is that Arg supplementation increased the percentage of oleic acid (C18:1n-9) in skeletal muscle (Table 3). This result may be explained by Arg- or NO-dependent activation of stearoyl CoA desaturase (SCD-1), a key enzyme in the formation of oleic acid through the desaturation of stearate [28]. As an indirect measure of SCD-1 activity, 16:1n-7/16:0 and 18:1n-9/18:0 ratios were higher in skeletal muscle of Arg-supplemented pigs than in the control group (Table 3). Such a physiological effect of Arg is beneficial for humans, because oleic acid stimulates glucose uptake by skeletal muscle for oxidation [29], thereby potentially reducing the availability of circulating glucose for fatty acid synthesis in other tissues [2]. Additionally, palmitoleate (C16:1n7) has recently been identified as a lipokine to enhance muscle insulin action and suppress hepatosteatosis in mice [30]. Furthermore, the findings of the current work may have important implications for meat production, because oleic acid is positively correlated with, but stearic acid and linoleic acid are negatively correlated with, pork quality [31–33].

In contrast to skeletal muscle, supplementing Arg to the diet of growing-finishing pigs reduced the mass of subcutaneous adipose tissue [8], the major fat depot in swine [1]. This observation may be explained by (1) reduced uptake of lipids from the circulation, (2) reduced availability of glucose for the provision of the glycerol backbone, (3) reduced de novo synthesis of fatty acids and (4) enhanced hydrolysis of triacylglycerols. Such explanations are supported further by the following lines of evidence. First, mRNA levels and activity of LPL were lower in subcutaneous adipose tissue of Arg-supplemented pigs (Tables 5 and 6), therefore suppressing the entry of fatty acid moieties in blood lipoprotein into adipocytes. Second, Arg supplementation attenuated GLUT4 expression in adipose tissue (Table 6) and, therefore, glycolysis to provide glycerol-3-phosphate for triglyceride formation. Third, ACCα (the major form of ACC in lipogenic tissues), which catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, is a key regulatory enzyme in fatty acid synthesis [34]. A decrease in ACCα expression (Table 6) likely results in low rates of this synthetic pathway. Fourth, a marked increase in HSL expression is expected to promote the hydrolysis of triacylglycerol in subcutaneous adipose tissue of Arg-supplemented pigs (Table 6), leading to reduced fat mass. Finally, as an indirect indicator of SCD-1 activity, the 18:1n-9/18:0 ratio was lower in the adipose tissue of Arg-supplemented pigs than in the control group (Table 3).

Pharmacological doses of Arg [e.g., 10 to 20 times the supplemental dosage used for the present study with pigs (364 mg/kg body weight per day)] are known to increase circulating levels of insulin animals and humans [3]. We [8] and others [10] have reported that dietary supplementation with 1% Arg for 60 or 80 days did not affect insulin concentrations in the plasma of growing-finishing pigs. Thus, the effects of Arg supplementation on gene expression in swine tissues (Tables 6 and 7), fatty acid composition (Table 3) and the metabolic profile (Table 4) appear to be independent of changes in circulating insulin. However, it is possible that dietary Arg supplementation augments insulin sensitivity and amplifies its signaling mechanisms on the metabolism of fatty acids, glucose and amino acids [3].

This study is relevant to fighting human obesity [35]. First, our findings provide much-needed animal data to propose clinical investigations to determine effects of dietary supplementation with Arg on reducing white-fat mass in overweight or obese subjects. Second, based on food dry-matter intake between finishing pigs and adult humans (40 and 5.5 g/kg body weight per day, respectively) [36], the present work helps guide the design of future studies involving the content of basic amino acids in the basal diet (e.g., 1% lysine, on a dry-matter basis) and an equivalent dose of supplemental Arg for adult humans (e.g., 50 mg Arg/kg body weight per day). This amount of Arg is physiologically attainable when the human diet is supplemented with synthetic Arg [3]. Of particular interest, Lucotti et al. [37] recently reported that oral administration of Arg (approximately 80 mg/kg body weight per day) for 21 days reduced fat mass by 0.9 kg and improved the metabolic profile in obese patients with Type 2 diabetes mellitus, compared with the placebo group. Third, results from animal models (including swine [8–10] and rats [38–42]) aid in elucidating the biochemical mechanisms responsible for the beneficial effect of Arg supplementation on overweight and obese subjects.

In conclusion, results of the present study demonstrate that dietary Arg supplementation up-regulates expression of lipogenic genes in skeletal muscle. In contrast, the Arg treatment down-regulates expression of lipogenic genes and increases expression of lipolytic genes in white adipose tissue. Such changes in expression of lipid-metabolic genes favor the storage of lipids in skeletal muscle but the loss of fat from subcutaneous adipose tissue in growing-finishing pigs. These novel findings provide a biochemical basis for explaining the beneficial effect of Arg in improving the metabolic profile in mammals (including obese humans).

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