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High fat feeding and dietary L-arginine supplementation differentially regulate gene expression in rat white adipose tissue

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Abstract Dietary L-arginine (Arg) supplementation reduces white-fat gain in diet-induced obese rats but the underlying mechanisms are unknown. This study tested the hypothesis that Arg treatment affects expression of genes related to lipid metabolism in adipose tissue. Four-week-old male Sprague–Dawley rats were fed a low-fat (LF) or high-fat (HF) diet for 15 weeks. Thereafter, lean or obese rats continued to be fed their same respective diets and received drinking water containing 1.51% Arg–HCl or 2.55% L-alanine (isonitrogenous control). After 12 weeks of Arg supplementation, rats were euthanized to obtain retroperitoneal adipose tissue for analyzing global changes in gene expression by microarray. The results were confirmed by RT-PCR analysis. HF feeding decreased mRNA levels for lipogenic enzymes, AMP-activated protein kinase, glucose transporters, heme oxygenase 3, glutathione synthetase, superoxide dismutase 3, peroxiredoxin 5, glutathione peroxidase 3, and stress-induced protein, while increasing expression of carboxypeptidase-A, peroxisome proliferator activated receptor (PPAR)-α, caspase 2, caveolin 3, and diacylglycerol kinase. In contrast, Arg supplementation reduced mRNA levels for fatty acid binding protein 1, glycogenin, protein phosphates 1B, caspases 1 and 2, and hepatic lipase, but increased expression of PPARγ, heme oxygenase 3, glutathione synthetase, insulin-like growth factor II, sphingosine-1-phosphate receptor, and stress-induced protein. Biochemical analysis revealed oxidative stress in white adipose tissue of HF-fed rats, which was prevented by Arg supplementation. Collectively, these results indicate that HF diet and Arg supplementation differentially regulate gene expression to affect energy-substrate oxidation, redox state, fat accretion, and adipocyte differentiation in adipose tissue. Our findings provide a molecular mechanism to explain a beneficial effect of Arg on ameliorating diet-induced obesity in mammals.

Abbreviations

AA	Amino acid
AMPK	AMP-activated protein kinase
Arg	L-Arginine
DIO	Diet-induced obese
HF	High fat
HO-3	Heme oxygenase 3
LF	Low fat
NO	Nitric oxide
PGC1α	PPARγ coactivator-1α
PPARγ	Peroxisome proliferator activator receptor γ
RP	Retroperitoneal
RT-PCR	Reverse transcriptase-polymerase chain reaction
SCD1	Stearoyl-CoA desaturase 1
ZDF	Zucker diabetic fatty

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Introduction

Over 300 million adults are obese and more than one billion are overweight worldwide (Hill et al. 2008). Obesity is a multisystem disease associated with an increased risk for insulin resistance, type II diabetes, fatty liver disease, atherosclerosis, stroke, hypertension, sleeping disorder, and certain types of cancer (Bray and Bellanger 2006). Consequently, this disease claims an increasing number of lives and contributes to tremendous costs of health care. In the US alone, about 300,000 people die of obesity-related diseases every year, the incidence of type II diabetes mellitus among children has increased tenfold over the past decade, and obesity accounts for 6–8% of all health care expenditures (Hill et al. 2008). Unfortunately, many pharmaceutical interventions to treat obesity have been largely ineffective in reducing excess fat and are associated with side effects (Bray and Bellanger 2006). Thus, identifying new therapeutic means to reduce body fat will be extremely beneficial for improving human health.

We recently reported that dietary supplementation with L-arginine (Arg) markedly reduced white-fat mass in Zucker diabetic fatty (ZDF) rats (a genetically obese animal model with a mutated leptin receptor) (Fu et al. 2005; Wu et al. 2007c) and diet-induced obese (DIO) rats (Jobgen et al. 2009). Using the microarray analysis as a powerful discovery tool (Guo and Xu 2008; Wang et al. 2008b), we found that Arg treatment markedly increased expression of NO synthase 1, heme oxygenase 3 (HO-3), AMP-activated protein kinase (AMPK), and peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC1α) in retroperitoneal (RP) adipose tissue of ZDF rats (Fu et al. 2005). To date, little is known about effects of Arg on gene expression in DIO rats. The present study was designed to test the hypothesis that Arg supplementation affects expression of key genes related to lipid metabolism in the white adipose tissue of DIO rats.

Materials and methods

Animals and diets

Male Sprague–Dawley rats (23-day-old, 80–100 g) were purchased from Harlan Laboratories (Indianapolis, IN) and were housed individually in carbonate cages in a temperature- and humidity-controlled room on a 12-h light:12-h dark cycle. After a 5-day period of adaptation during which rats were fed a regular non-purified rodent diet, they were randomly assigned to a low-fat (LF) or high-fat (HF) diet ($n = 16$/diet) obtained from Research Diets (New Brunswick, NJ), as we described (Jobgen et al. 2009). The LF (4.3% fat) and HF (23.6% fat) diets provided 10 and 40% of total energy as lipids (mainly lard), respectively. After 15-week HF or LF feeding (the phase of obesity induction), rats in the HF or LF group were divided randomly into two sub-groups, which continued to be fed their same respective diets and received drinking water containing either 1.51% Arg–HCl or 2.55% L-alanine (isonitrogenous control) ($n = 8$/sub-group). The drinking water was provided to rats daily. Alanine was chosen as an isonitrogenous control primarily because of its extensive catabolism in the body, its safety, and its inability to act as a precursor for endogenous synthesis of Arg (Kohli et al. 2004). Arg-supplemented rats within the LF or HF diet were individually pair-fed with alanine-supplemented rats on a body-weight basis to ensure similar intakes of all nutrients (except for Arg and alanine). After 12 weeks of the amino acid (AA) supplementation, rats were food-deprived for 5 h before being euthanized and RP adipose tissue was rapidly dissected (Jobgen et al. 2009). Approximately 3 g samples were immediately placed in RNAlater solution (Ambion, Austin, TX) and stored at $-80°C$ until analysis, whereas approximately 1 g samples were placed in liquid nitrogen for analysis of reduced glutathione and oxidized glutathione (Jobgen et al. 2009). This study was approved by the Institutional Animal Care and Use Committee of Texas A&M University.

RNA extraction

RP adipose tissue was minced on ice and homogenized in TRIzol (Invitrogen, Carlsbad, CA). Total RNA was isolated according to the manufacture's recommendation. RNA was precipitated with isopropanol, washed with 70% ethanol and resuspended in diethylpyrocarbonate-treated deionized water (Fermentas, Glen Burnie, MD). The quantity and quality of RNA were assessed by measuring absorbance at 280 and 260 nm, followed by electrophoresis on 1.25% agarose gels.

Microarray analysis of gene expression

Microarray analysis was performed using CodeLink^{TM} Rat Whole Genome Arrays that contain 35,000 rat gene probes (Applied Microarrays, Tempe, AZ), as described by Fu et al. (2005). Briefly, double-stranded cDNA was synthesized using 2 µg of total RNA, and cRNA was synthesized through an in vitro transcription reaction involving T7 RNA polymerase and biotin-11-UTP (Perkin-Elmer Corp, Walthan, MA). After purification on RNeasy columns, 10 µg cRNA was fragmented by heating at 94°C for 20 min in the presence of 1 mM MgCl$_2$. The fragmented cRNA was hybridized to Rat Whole Genome Arrays at 37°C overnight with shaking at 300 rpm. After hybridization, arrays were washed in 0.75× Tris buffer at 46°C for

1 h, and incubated with Streptavidin-Alexa 647 (Invitrogen) in the dark for 30 min at room temperature. Finally, the slides were dried by centrifugation, and scanned in the dark on the Axon GenePix Scanner using CodeLink Expression Scanning Software. Images were analyzed using the CodeLink Expression Analysis Software. Expression values were normalized to the median expression value of the whole array spots.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Real time RT-PCR analysis was performed using the SYBR Green method and the ABI 7900 Sequence Detection System (Applied Biosystems, University Park, IL), as we described (Fu et al. 2005). Briefly, first-strand cDNAs were synthesized from 1 µg of total RNA using oligo (deoxythymidine) primers, random hexamer primers, and superscript II reverse transcriptase. The thermal cycling parameters were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Primers were designed using Primer Express Software Version 1.5 (Applied Biosystems). Information about primer pairs for selected genes, their specific locations in cDNA sequences, and product sizes are summarized in Table 1. Values for cycle threshold were determined using the Applied Biosystems Software. All the data were normalized with the glyceraldehyde 3-phosphate dehydrogenase gene in the same samples and are expressed as the relative values to those of alanine-supplemented rats fed the HF diet.

Statistical analysis

Results are expressed as mean $\pm$ SEM. Statistical analysis was performed using two-way ANOVA to determine the significance of main treatment effects and interactions between diet and AA (SAS, Cary, NC). Microarray data were analyzed using the Procedure of PROC GLM in SAS, as described by Fu et al. (2005). Briefly, an ANOVA model was fitted to log-transformed gene expression intensities with covariates, including diet, AA treatment, rat ID, gene number, row and columns of gene on the array, as well as interaction between diet and AA. Statistical significance in levels of genes potentially expressed differentially among treatment groups was determined by a combination of two criteria: P-value < 0.05 and false discovery rate (FDR). The latter was calculated on the basis of 0.05-level significance and the order of genes with their P-values through a step-up procedure to adjust the multiplicity of hypothesis testing, because a large number of genes were examined simultaneously for statistical significance. With the step-up procedure, genes that had a P-value <FDR were regarded as differentially expressed. RT-PCR cycle

Table 1 Primers of genes for real-time PCR analysis

Gene	Accession number	Forward primer	Reverse primer	cDNA location	Size	CDS
AMP-activated protein kinase α-subunit	NM_023991	ACACCTCAGCGCTCCTGTTC	CTGTGCTGGAATCGACACT	1451–1527	67	8–1666
Slc2 (facilitated glucose transporter), member 4	NM_012751	TGTGGCCTTCTTTGAGATTGG	CTGAAGAGCTCGGCCACAA	1313–1375	69	141–1670
Fatty acid synthase	NM_017332	GGCCCTACCTTAACACGACTCA	GAACACAGTGATGGAACCTTCAAT	6745–6834	90	82–7599
Stearoyl-CoA desaturase 1	NM_139192	CCTCCTGCTCATGTGCTTCA	GGCTGTGCAGGAAAGTTTCG	762–835	74	103–1179
Peroxisome proliferator activator receptor γ	NM_013124	CATGACCAGGGAGGTTCCTCAA	AGCAAACTCAAACTTAGGCTCCAT	1173–1245	73	49–1566
Hormone-sensitive lipase	NM_012859	CCTCTACTCGTCACCCATAGTCAA	GGCAGGGTCTTCAGCATGA	3094–3171	78	194–3400
Lipoprotein lipase	NM_012598	CCCAGCTTTGTCATCGAGAAG	CCTGGCACAGAAGATGACCTTT	1444–1515	72	175–1599
Glyceraldehyde-3-phosphate dehydrogenase	NM_017008	GGGCAGCCCAGAACATCAT	CCAGTGAGCTTCCCGTTCAG	1445–1532	88	850–1851

Slc2 solute carrier family 2, *CDS* coding sequence

threshold values were analyzed using the generalized estimating equations model and the PROC GENMOD procedure of SAS, as described by Fu et al. (2006). P values ≤0.05 were taken to indicate statistical significance.

Results

Overall observations

HF feeding over a 12-week period increased the weight of RP adipose tissue by 56% compared to the LF diet, whereas dietary Arg supplementation reduced RP fat by 36% compared to alanine-supplemented rats (Jobgen et al. 2009). Expression of 1,102 genes was altered ($P < 0.05$) in the RP adipose tissue of HF-fed rats compared with LF-fed rats, whereas expression of 43 genes was affected ($P < 0.05$) in the fat pad of Arg-supplemented rats compared with alanine-supplemented controls. The interaction of diet and AA influenced ($P < 0.05$) expression of 125 genes in the RP fat pad. We identified 107 genes that are known to play an important role in lipid, glucose and AA metabolism (Tables 2, 3, 4). Genes whose mRNA levels were not altered in the RP adipose tissue of either HF-fed or Arg-supplemented rats but which encode proteins crucial for nutrient metabolism included: (1) arginases I and II, protein arginine N-methyltransferase 3, ornithine decarboxylase, S-adenosylmethionine synthetase, glutaminase, glutamine synthetase, transglutaminase, glutamate dehydrogenase, branched-chain amino acid transaminase, branched-chain α-ketoacid dehydrogenase, GTP cyclohydrolase I, as well as NO synthases 1 and 3; (2) hexokinase 4, glucose-6-phosphate dehydrogenase, phosphofructokinase 1, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase, pyruvate dehydrogenase, isocitrate dehydrogenase, and glycogen phosphorylase; (3) hormone-sensitive lipase, lipoprotein lipase, carnitine palmitoyltransferases I and II, and acyl-CoA synthase; and (4) angiotensin/vasopressin receptor and calcium/calmodulin-dependent protein kinase 1.

Altered expression of genes in the RP adipose tissue of HF-fed rats

The mRNA levels for the following genes were lower ($P < 0.05$) in RP adipose tissue of HF-fed rats than in LF-fed rats: fatty acid synthase (−69%), stearoyl-CoA desaturase-1 (SCD1 −58%), ATP citrate lyase (−63%), AMPK α-subunit (−36%), AMPK β-subunit (−44%), glucose transporter 4 (−45%), aldehyde dehydrogenase (−42%), acetyl-CoA carboxylase β-subunit (−42%), and acetyl-CoA acyltransferase 2 (−31%) (Table 2). HF feeding also reduced ($P < 0.05$) mRNA levels for pyruvate

dehydrogenase kinase 2, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2, glucose transporters 1, 8 and 13, phospholipase A2, phospholipase C, succinate dehydrogenase complex subunit D, steroid 5-α-reductase 2, sterol O-acyltransferase 2, glutamate decarboxylase, glutamate-pyruvate transaminase, sulfite oxidase, dihydrolipoamide succinyltransferase, glutathione S-transferase, peroxiredoxin 5, superoxide dismutase 3, NO synthase 2, asparagine synthetase, cathepsins B and D, protein tyrosine phosphatase, cAMP-responsive element binding protein, adenylyl cyclase type VI, integrin β, MAPK kinase, nuclear protein 1, and protein kinase C (Table 2). In contrast, mRNA levels for cytochrome P450 subfamily 2A-peptide 1, carboxypeptidase A1, peroxisome proliferator activated receptor (PPAR)-α, diacylglycerol kinase α subunit (80 kDa), and interleukin 1 receptor-like 1 were higher ($P < 0.05$) in the RP fat tissue of HF-fed rats, compared with LF-fed rats (Table 2).

Altered expression of genes in the RP adipose tissue of Arg-supplemented rats

Arg supplementation reduced mRNA levels for fatty acid binding protein (−77%), hepatic lipase (−49%), PPARγ coactivator-1α (PGC1α −41%), mitochondrial ATP synthase β subunit (−25%), glycogenin (−48%), testosterone 6-β-hydroxylase (−68%), caspase-1 (−35%), and protein phosphatase 1B (−42%) (Table 3). In contrast, the Arg treatment increased mRNA levels for PPARγ (+41%), cytochrome P450 2C39 subunit (+37%), serotonin receptor (+30%), and insulin-like growth factor II (+226%). In rats whose gene expression was not affected by HF feeding, the effects of Arg supplementation on expression of some genes depended on dietary fat intake (Table 3). For example, Arg provision enhanced ($P < 0.05$) adipose tissue mRNA levels for hexokinase 2, pyruvate carboxylase, and carboxyl ester lipase in LF-fed rats, while reducing ($P < 0.05$) their mRNA levels in HF-fed rats. Additionally, Arg supplementation increased ($P < 0.05$) adipose tissue mRNA levels for 3-hydroxy-3-methylglutaryl-CoA synthase 1, cytochrome P450 (2C39), and lipogenin in LF-fed rats but had no effect on the same genes in HF-fed rats.

Altered expression of genes in the RP adipose tissue of both HF-fed and Arg-supplemented rats

Expression of the following genes was reduced ($P < 0.05$) by HF feeding but increased ($P < 0.05$) by Arg supplementation: mitochondrial gycerol-3-phosphate acyltransferase, fatty acid transport protein (Slc27a1), HO-3, cytochrome P450 (subfamily 19), glutathione synthetase, stress-induced protein, flavin-containing monooxygenase, inositol 1,4,5-triphosphate 3-kinase, and sphingosine

Table 2 Altered expression of genes in the RP adipose tissue of high fat-fed rats

Genbank ID	Gene name	Low-fat diet		High-fat diet		P value		
		Alanine	Arginine	Alanine	Arginine	Diet	AA	Diet × AA
Decreased expression								
NM_017332.1	Fatty acid synthase	1.00 ± 0.09	1.45 ± 0.16	0.43 ± 0.06	0.32 ± 0.06	0.01	0.20	0.01
NM_016987.1	ATP citrate lyase	1.00 ± 0.12	1.25 ± 0.17	0.41 ± 0.04	0.43 ± 0.03	0.01	0.23	0.58
NM_139192.1	Stearoyl-CoA desaturase 1	1.00 ± 0.20	1.26 ± 0.25	0.48 ± 0.08	0.47 ± 0.07	0.01	0.65	0.13
NM_023991.1	AMP-activated protein kinase, α subunit	1.00 ± 0.06	1.10 ± 0.12	0.59 ± 0.07	0.75 ± 0.16	0.01	0.71	0.72
NM_031976.1	AMP-activated protein kinase, β1 subunit	1.00 ± 0.06	0.78 ± 0.09	0.48 ± 0.06	0.51 ± 0.07	0.01	0.14	0.48
NM_053922.1	Acetyl-CoA carboxylase β subunit	1.00 ± 0.09	0.73 ± 0.19	0.48 ± 0.14	0.37 ± 0.07	0.04	0.28	0.27
NM_130433.1	Acetyl-CoA acyltransferase 2	1.00 ± 0.01	1.19 ± 0.11	0.67 ± 0.15	0.85 ± 0.12	0.04	0.51	0.62
NM_138827.1	Slc2 (facilitated glucose transporter), member 1	1.00 ± 0.06	0.91 ± 0.03	0.78 ± 0.11	0.68 ± 0.04	0.02	0.37	0.78
NM_012751.1	Slc2 (facilitated glucose transporter), member 4	1.00 ± 0.07	1.20 ± 0.19	0.69 ± 0.12	0.51 ± 0.06	0.04	0.55	0.20
NM_053494.1	Slc2 (facilitated glucose transporter), member 8	1.00 ± 0.08	0.93 ± 0.07	0.63 ± 0.06	0.87 ± 0.17	0.01	0.79	0.10
NM_133611.1	Slc2 (facilitated glucose transporter), member 13	1.00 ± 0.04	0.89 ± 0.06	0.72 ± 0.07	0.81 ± 0.06	0.01	0.32	0.23
NM_199081.1	Galactose transporter	1.00 ± 0.05	0.97 ± 0.09	0.75 ± 0.09	0.76 ± 0.04	0.03	0.76	0.61
NM_153728.2	Sterol O-acyltransferase 2	1.00 ± 0.11	1.07 ± 0.09	0.58 ± 0.09	0.76 ± 0.08	0.01	0.68	0.36
NM_031972.1	Aldehyde dehydrogenase family 3, member A1	1.00 ± 0.04	0.76 ± 0.08	0.46 ± 0.04	0.56 ± 0.04	0.01	0.16	0.09
NM_198788.1	Succinate dehydrogenase complex, subunit D	1.00 ± 0.08	1.02 ± 0.04	0.76 ± 0.11	0.77 ± 0.06	0.04	0.82	0.96
NM_031000.2	α-Keto reductase family 1, member A1	1.00 ± 0.03	1.00 ± 0.11	0.80 ± 0.06	0.89 ± 0.05	0.04	0.87	0.41
M25148.1	Pospholipase A2	1.00 ± 0.09	0.80 ± 0.12	0.50 ± 0.07	0.50 ± 0.07	0.01	0.19	0.30
NM_022711.2	Steroid 5-α-reductase 2	1.00 ± 0.08	0.98 ± 0.14	0.59 ± 0.05	0.63 ± 0.06	0.01	0.80	0.24
NM_024353.1	Pospholipase C, β4	1.00 ± 0.06	1.11 ± 0.11	0.72 ± 0.06	0.78 ± 0.03	0.01	0.40	1.00
NM_013187.1	Phospholipase C, γ1	1.00 ± 0.07	1.04 ± 0.12	0.70 ± 0.09	0.82 ± 0.05	0.04	0.71	0.65
NM_031049.1	2,3-Oxidosqualene: lanosterol cyclase	1.00 ± 0.04	1.12 ± 0.09	0.74 ± 0.08	0.64 ± 0.03	0.01	0.42	0.21
NM_012847.1	Farnesyltransferase, CAAX box, α	1.00 ± 0.06	0.99 ± 0.04	0.76 ± 0.07	0.86 ± 0.06	0.01	0.98	0.36
NM_012563.1	Glutamate decarboxylase 2	1.00 ± 0.11	1.21 ± 0.10	0.72 ± 0.09	0.89 ± 0.13	0.02	0.77	0.33
NM_031039.1	Glutamate-pyruvate transaminase	1.00 ± 0.08	1.19 ± 0.09	0.66 ± 0.17	0.81 ± 0.07	0.02	0.47	0.52
D90401.1	Dihydrolipoamide succinyltransferase	1.00 ± 0.08	0.94 ± 0.06	0.74 ± 0.10	0.86 ± 0.09	0.03	0.71	0.26
NM_139256.1	Mannosidase, α subunit, class 2C, member 1	1.00 ± 0.04	0.98 ± 0.05	0.74 ± 0.05	0.89 ± 0.08	0.04	0.86	0.41
NM_138974.1	Glutathione S-transferase	1.00 ± 0.03	0.96 ± 0.07	0.75 ± 0.03	0.87 ± 0.08	0.03	0.74	0.23
NM_053724.1	Phosphotidylinositol transfer protein β	1.00 ± 0.12	0.89 ± 0.12	0.80 ± 0.15	0.84 ± 0.14	0.01	0.45	0.13
NM_012876.1	Ribosomal protein S29	1.00 ± 0.03	0.99 ± 0.05	0.75 ± 0.02	0.66 ± 0.08	0.01	0.76	0.57
AF391798.1	Transcription factor IIIA	1.00 ± 0.09	1.06 ± 0.06	0.68 ± 0.08	0.73 ± 0.05	0.01	0.56	0.32
NM_013079.1	Asparagine synthetase	1.00 ± 0.07	0.98 ± 0.09	0.66 ± 0.05	0.79 ± 0.04	0.01	0.34	0.41
NM_012654.1	Cathepsin B	1.00 ± 0.05	1.05 ± 0.07	0.70 ± 0.03	0.69 ± 0.04	0.01	0.39	0.01
NM_017019.1	Interleukin 1α	1.00 ± 0.11	1.09 ± 0.13	0.58 ± 0.04	0.57 ± 0.06	0.01	0.17	0.18

Table 2 continued

Genbank ID	Gene name	Low-fat diet		High-fat diet		P value		
		Alanine	Arginine	Alanine	Arginine	Diet	AA	Diet × AA
NM_022177.2	Chemokine ligand 12	1.00 ± 0.08	0.91 ± 0.07	0.62 ± 0.08	0.76 ± 0.04	0.01	0.49	0.10
NM_030872.1	Pyruvate dehydrogenase kinase 2	1.00 ± 0.06	0.93 ± 0.08	0.64 ± 0.04	0.72 ± 0.09	0.02	0.26	0.93
NM_053908.1	Protein tyrosine phosphatase	1.00 ± 0.12	0.91 ± 0.10	0.69 ± 0.05	0.65 ± 0.04	0.02	0.32	0.08
NM_134443.1	cAMP-responsive element binding protein 1	1.00 ± 0.06	0.96 ± 0.07	0.76 ± 0.07	0.73 ± 0.08	0.02	0.71	0.98
L01115.1	Adenylyl cyclase type VI	1.00 ± 0.05	1.03 ± 0.10	0.70 ± 0.05	0.79 ± 0.06	0.01	0.94	0.49
NM_031127.2	Sulfite oxidase	1.00 ± 0.09	1.24 ± 0.11	0.83 ± 0.06	0.93 ± 0.13	0.01	0.27	0.08
NM_012836.1	Carboxypeptidase D	1.00 ± 0.02	0.89 ± 0.12	0.66 ± 0.08	0.67 ± 0.03	0.01	0.29	0.21
NM_031145.1	Calcium and integrin-binding protein	1.00 ± 0.05	0.95 ± 0.06	0.78 ± 0.06	0.86 ± 0.06	0.01	0.55	0.24
NM_017155.1	Adenosine A1 receptor	1.00 ± 0.07	1.07 ± 0.12	0.72 ± 0.09	0.78 ± 0.06	0.01	0.69	0.75
NM_134334.2	Cathepsin D	1.00 ± 0.15	1.24 ± 0.13	0.73 ± 0.07	0.90 ± 0.12	0.01	0.26	0.17
NM_017016.1	Histidine decarboxylase	1.00 ± 0.07	1.02 ± 0.11	0.68 ± 0.10	0.73 ± 0.06	0.01	0.88	0.17
NM_013180.1	Integrin β	1.00 ± 0.05	0.99 ± 0.12	0.74 ± 0.09	0.72 ± 0.08	0.01	0.69	0.24
NM_022396.1	Guanine nucleotide binding protein γ subunit	1.00 ± 0.04	1.11 ± 0.14	0.77 ± 0.07	0.87 ± 0.08	0.01	0.62	0.37
NM_080904.1	ADP-ribosylation factor 3	1.00 ± 0.07	0.85 ± 0.10	0.64 ± 0.05	0.66 ± 0.06	0.01	0.25	0.21
NM_012880.1	Superoxide dismutase 3	1.00 ± 0.08	0.93 ± 0.12	0.71 ± 0.09	0.78 ± 0.05	0.01	0.38	0.22
NM_053586.1	Cytochrome C oxidase subunit 5b	1.00 ± 0.07	1.10 ± 0.09	0.72 ± 0.11	0.78 ± 0.09	0.02	0.51	0.99
NM_033236.1	Proteasome 26S subunit	1.00 ± 0.05	0.88 ± 0.07	0.75 ± 0.08	0.82 ± 0.08	0.02	0.34	0.19
D90401.1	Dihydrolipoamide succinyltransferase	1.00 ± 0.08	0.94 ± 0.06	0.74 ± 0.09	0.79 ± 0.07	0.03	0.71	0.26
NM_017246.1	MAPK kinase 5	1.00 ± 0.05	0.93 ± 0.06	0.82 ± 0.08	0.80 ± 0.03	0.03	0.32	0.18
NM_017055.1	Transferrin	1.00 ± 0.06	0.90 ± 0.08	0.72 ± 0.08	0.65 ± 0.05	0.03	0.52	0.72
NM_017065.1	GABA receptor, subunit β3	1.00 ± 0.12	1.02 ± 0.16	0.60 ± 0.07	0.68 ± 0.09	0.03	0.18	0.35
NM_053611.1	Nuclear protein 1	1.00 ± 0.15	0.73 ± 0.10	0.52 ± 0.04	0.47 ± 0.06	0.03	0.70	0.26
NM_080477.1	PFK/FB2	1.00 ± 0.05	1.10 ± 0.07	0.72 ± 0.03	0.77 ± 0.05	0.04	0.59	0.46
NM_199099.1	IMP dehydrogenase	1.00 ± 0.07	1.01 ± 0.05	0.82 ± 0.04	0.84 ± 0.04	0.04	0.83	0.21
XM_234108.2	Protein kinase C	1.00 ± 0.08	1.12 ± 0.10	0.75 ± 0.09	0.79 ± 0.07	0.04	0.48	0.90
NM_053610.1	Peroxiredoxin 5	1.00 ± 0.05	1.06 ± 0.05	0.79 ± 0.06	0.84 ± 0.07	0.05	0.68	0.81
NM_053831.1	Guanylate cyclase 2F	1.00 ± 0.08	1.02 ± 0.14	0.41 ± 0.02	0.47 ± 0.06	0.05	0.97	0.65
NM_012611.2	Nitric oxide synthase 2 (inducible)	1.00 ± 0.09	0.95 ± 0.07	0.38 ± 0.05	0.44 ± 0.06	0.01	0.63	0.54
Increased expression								
NM_012693.1	Cytochrome P450, subfamily 2A, peptide 1	1.00 ± 0.05	1.11 ± 0.06	1.18 ± 0.07	1.24 ± 0.09	0.04	0.58	1.00
NM_016998.2	Carboxypeptidase A1	1.00 ± 0.07	1.34 ± 0.15	2.43 ± 0.22	2.26 ± 0.13	0.01	0.44	0.55
NM_013196.1	Peroxisome proliferator activated receptor α	1.00 ± 0.06	1.09 ± 0.05	1.12 ± 0.05	1.30 ± 0.07	0.03	0.15	0.67
NM_080787.1	Diacylglycerol kinase, α subunit (80 kDa)	1.00 ± 0.02	1.03 ± 0.07	1.27 ± 0.07	1.19 ± 0.08	0.04	0.24	0.79
NM_013037.1	Interleukin 1 receptor-like 1	1.00 ± 0.12	0.91 ± 0.09	1.38 ± 0.08	1.41 ± 0.05	0.01	0.19	0.09

Values are mean ± SEM, $n = 6$ per treatment group. Data are expressed as the relative values to those of alanine-supplemented rats fed a LF diet

GABA γ-aminobutyrate, *MAPK* mitogen-activated protein kinase, *PFK/FB2* 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2, *Slc2* solute carrier family 2

Table 3 Altered expression of genes in the RP adipose tissue of Arg-supplemented rats

Genbank ID	Gene name	Low-fat diet		High-fat diet		P value		
		Alanine	Arginine	Alanine	Arginine	Diet	AA	Diet × AA
Decreased expression								
NM_012556.1	Fatty acid binding protein 1	1.00 ± 0.11	0.21 ± 0.02	0.88 ± 0.09	0.23 ± 0.03	0.11	0.01	0.31
NM_031347.1	PGC1α	1.00 ± 0.07	0.79 ± 0.05	1.13 ± 0.28	0.46 ± 0.04	0.40	0.01	0.37
M19044.1	Mitochondrial ATP synthase β subunit	1.00 ± 0.05	0.86 ± 0.06	0.91 ± 0.09	0.58 ± 0.05	0.09	0.04	0.82
NM_012597.1	Hepatic lipase	1.00 ± 0.13	0.46 ± 0.07	0.87 ± 0.04	0.50 ± 0.08	0.17	0.01	0.32
NM_012735.1	Hexokinase 2	1.00 ± 0.18	2.03 ± 0.20	1.47 ± 0.27	0.94 ± 0.12	0.09	0.03	0.01
NM_012744.2	Pyruvate carboxylase	1.00 ± 0.12	1.54 ± 0.23	0.83 ± 0.07	0.61 ± 0.05	0.89	0.03	0.04
NM_031043.1	Glycogenin	1.00 ± 0.07	0.43 ± 0.04	0.82 ± 0.10	0.51 ± 0.03	0.19	0.01	0.12
NM_153312.2	Testosterone 6-β-hydroxylase	1.00 ± 0.10	0.28 ± 0.05	0.79 ± 0.08	0.29 ± 0.04	0.30	0.02	0.19
NM_012762.2	Caspase-1	1.00 ± 0.14	0.55 ± 0.07	0.95 ± 0.09	0.71 ± 0.06	0.61	0.03	0.34
NM_033096.1	Protein phosphatase 1B	1.00 ± 0.08	0.63 ± 0.05	1.01 ± 0.13	0.53 ± 0.07	0.70	0.03	0.38
Increased expression								
NM_013124.1	Peroxisome proliferator activated receptor γ	1.00 ± 0.14	1.46 ± 0.10	0.98 ± 0.08	1.33 ± 0.11	0.14	0.01	0.32
NM_017158.1	Cytochrome P450, 2c39	1.00 ± 0.05	1.63 ± 0.13	1.05 ± 0.06	1.18 ± 0.05	0.99	0.01	0.20
NM_012692.1	Cytochrome P450 IIA1[a]	1.00 ± 0.04	1.53 ± 0.08	1.27 ± 0.07	1.59 ± 0.08	0.18	0.01	0.24
NM_017268.1	3-Hydroxy-3-methylglutaryl-CoA synthase 1	1.00 ± 0.07	1.42 ± 0.08	1.09 ± 0.14	1.03 ± 0.06	0.60	0.02	0.06
NM_016997.1	Carboxyl ester lipase	1.00 ± 0.09	1.77 ± 0.21	1.13 ± 0.11	0.94 ± 0.05	0.52	0.03	0.06
NM_145790.1	Lipogenin	1.00 ± 0.06	1.41 ± 0.11	1.27 ± 0.09	1.20 ± 0.06	0.51	0.04	0.12
NM_012853.1	Serotonin receptor 4	1.00 ± 0.05	1.24 ± 0.12	1.02 ± 0.10	1.38 ± 0.08	0.37	0.01	0.26
M17960.1	Insulin-like growth factor II	1.00 ± 0.07	3.04 ± 0.38	0.94 ± 0.12	3.28 ± 0.46	0.47	0.01	0.13
NM_017047.1	Solute carrier family 10, member 1	1.00 ± 0.06	1.03 ± 0.04	0.84 ± 0.07	1.07 ± 0.05	0.95	0.01	0.99
NM_012853.1	Serotonin receptor 4	1.00 ± 0.05	1.24 ± 0.12	1.02 ± 0.10	1.38 ± 0.08	0.37	0.01	0.28

Values are mean ± SEM, $n = 6$ per treatment group. Data are expressed as the relative values to those of alanine-supplemented rats fed a LF diet

[a] Also known as hepatic steroid hydroxylase IIA1

1-phosphate receptor (Table 4). In contrast, HF feeding increased but Arg supplementation decreased expression of the following genes ($P < 0.05$): caspase 2, GTP cyclohydrolase I feedback regulatory protein, N-acetylglucosamine galactosyltransferase, and caveolin 3 (Table 4). There were diet × AA interaction effects on expression of malic enzyme 1, casein kinase 1γ, protein kinase inhibitor α, adenosine deaminase, pyruvate dehydrogenase kinase 4, and glutamate receptor (Table 4). For example, dietary Arg supplementation increased and decreased, respectively, malic enzyme 1 expression in LF- and HF-fed rats ($P < 0.05$), and the opposite was observed for glutamate receptor. Additionally, Arg supplementation reduced ($P < 0.05$) expression of casein kinase 1γ, protein kinase inhibitor α, adenosine deaminase, and pyruvate dehydrogenase kinase 4 in LF-fed rats, but not in HF-fed rats.

Verification of microarray gene expression data using RT-PCR analysis

We used the RT-PCR technique to confirm a subset of differentially expressed genes identified by microarray analysis. A small set of genes was chosen for this purpose (Table 5), which included genes that were up- or down-regulated and those that were not affected by HF feeding or Arg supplementation. The results indicated that HF feeding reduced ($P < 0.05$) mRNA levels for AMPK α subunit (−27%), glucose transporter 4 (−45%), fatty acid synthase (−72%), SCD1 (−81%), whereas Arg supplementation increased ($P < 0.05$) the mRNA level for PPARγ (+50%), in RP adipose tissue (Table 5). The mRNA levels for hormone-sensitive lipase and lipoprotein lipase in the fat pad were not affected by either HF feeding or dietary Arg supplementation (Table 5). The RT-PCR results were consistent with the data obtained from the microarray analysis.

Ratios of oxidized glutathione:reduced glutathione in RP adipose tissue

HF feeding decreased ($P < 0.01$) concentrations of reduced glutathione in rat RP adipose tissue by 26% but increased ($P < 0.01$) those of oxidized glutathione by 43% (Table 6). In contrast, dietary Arg supplementation

Table 4 Altered expression of genes in the RP adipose tissue of both HF-fed and Arg-supplemented rats

Genbank ID	Gene name	Low-fat diet		High-fat diet		P value		
		Alanine	Arginine	Alanine	Arginine	Diet	AA	Diet × AA
NM_017274.1	Gycerol-3-phosphate acyltransferase, Mito	1.00 ± 0.04	1.80 ± 0.17	0.57 ± 0.02	0.93 ± 0.11	0.04	0.01	0.13
NM_053580.1	Fatty acid transport protein (Slc27a1)	1.00 ± 0.13	1.97 ± 0.15	0.67 ± 0.08	0.93 ± 0.05	0.05	0.01	0.32
AF058787.1	Heme oxygenase-3	1.00 ± 0.05	1.37 ± 0.06	0.56 ± 0.03	0.89 ± 0.07	0.02	0.03	0.68
NM_017085.1	Cytochrome P450, subfamily 19	1.00 ± 0.10	1.63 ± 0.21	0.86 ± 0.06	1.23 ± 0.14	0.03	0.05	0.40
NM_012962.1	Glutathione synthetase	1.00 ± 0.03	1.21 ± 0.08	0.60 ± 0.04	0.79 ± 0.07	0.01	0.04	0.78
NM_022525.2	Glutathione peroxidase 3	1.00 ± 0.05	0.78 ± 0.06	0.76 ± 0.09	0.75 ± 0.03	0.02	0.05	0.14
NM_181084.2	Stress-induced protein	1.00 ± 0.06	1.16 ± 0.10	0.43 ± 0.06	0.86 ± 0.07	0.01	0.03	0.29
NM_144737.1	Flavin-containing monooxygenase	1.00 ± 0.08	1.20 ± 0.07	0.64 ± 0.04	0.93 ± 0.07	0.01	0.03	0.25
NM_022522.2	Caspase 2	1.00 ± 0.07	0.71 ± 0.06	1.24 ± 0.09	1.01 ± 0.05	0.03	0.04	0.29
NM_133595.1	GTPCH feedback regulatory protein	1.00 ± 0.09	0.71 ± 0.06	1.51 ± 0.10	0.92 ± 0.08	0.02	0.03	0.35
AF102262.1	N-acetylglucosamine galactosyltransferase	1.00 ± 0.08	0.57 ± 0.07	1.24 ± 0.10	0.76 ± 0.09	0.03	0.01	0.19
NM_019155.1	Caveolin 3	1.00 ± 0.11	0.56 ± 0.03	1.22 ± 0.08	0.73 ± 0.10	0.02	0.01	0.23
NM_031045.1	Inositol 1,4,5-triphosphate 3-kinase	1.00 ± 0.06	1.53 ± 0.13	0.73 ± 0.08	1.16 ± 0.09	0.02	0.01	0.72
NM_022403.1	Tryptophan 2,3-dioxygenase	1.00 ± 0.12	0.44 ± 0.06	0.72 ± 0.09	0.51 ± 0.04	0.04	0.02	0.12
NM_021775.2	Sphingosine 1-phosphate receptor	1.00 ± 0.14	2.36 ± 0.30	0.90 ± 0.07	1.28 ± 0.16	0.01	0.03	0.18
NM_012600.1	Malic enzyme 1	1.00 ± 0.10	2.40 ± 0.19	0.60 ± 0.04	0.43 ± 0.04	0.01	0.01	0.01
NM_022288.1	Casein kinase 1γ	1.00 ± 0.07	0.70 ± 0.07	0.63 ± 0.03	0.65 ± 0.04	0.01	0.03	0.01
NM_053772.1	Protein kinase inhibitor α	1.00 ± 0.08	0.64 ± 0.06	0.48 ± 0.02	0.60 ± 0.03	0.01	0.03	0.01
NM_031006.1	Adenosine deaminase	1.00 ± 0.09	0.68 ± 0.04	0.56 ± 0.07	0.66 ± 0.06	0.01	0.04	0.01
NM_053551.1	Pyruvate dehydrogenase kinase 4	1.00 ± 0.14	0.46 ± 0.06	0.60 ± 0.06	0.55 ± 0.04	0.01	0.01	0.02
NM_181373.2	Glutamate receptor	1.00 ± 0.04	0.45 ± 0.06	0.71 ± 0.06	0.92 ± 0.07	0.04	0.01	0.01

Values are mean ± SEM, $n = 6$ per treatment group. Data are expressed as the relative values to those of alanine-supplemented rats fed a LF diet

GTPCH GTP cyclohydrolase I, *Mito* mitochondrial

increased ($P < 0.05$) tissue concentrations of reduced glutathione by 14% and decreased ($P < 0.01$) those of oxidized glutathione by 25%. Consequently, the ratio of oxidized glutathione to reduced glutathione in RP fat pad was 93% higher ($P < 0.01$) in HF- than in LF-fed rats, but was 37% lower ($P < 0.01$) in Arg- than in alanine-supplemented rats (Table 6).

Discussion

Obesity has emerged as a major concern in human health worldwide. Although eating less and exercising more appear to be an easy solution to this problem, the increasing proportion of the population who is overweight or obese in the past decade implies that these seemingly simple approaches have not been implemented effectively to prevent excess fat deposition. This is likely because of the complex biological mechanisms that regulate food intake, behavioral difficulties in lifestyle changes, and the development of metabolic disease (Hill et al. 2008).

Therefore, new alternative means are needed to reduce excess lipid deposition in mammals. Results of recent studies indicate that dietary Arg supplementation is an effective way to decrease white fat gain in both ZDF (Fu et al. 2005; Wu et al. 2007c) and DIO (Jobgen et al. 2009) rats, as well as growing-finishing pigs (He et al. 2008; Tan et al. 2008).

HF feeding has been shown to reduce expression of genes in rodent liver and white adipose tissue that encode key lipogenic enzymes (Kim et al. 2004; Li et al. 2008a; Nadler et al. 2000). Similar results were obtained from the current study with DIO rats (Tables 2, 3, 4), indicating the robust capability of microarray technology to detect changes in gene expression (Wang et al. 2008b). A novel observation of the current work was that HF diet markedly reduced expression of both α (catalytic) and β1 (regulatory) subunits of AMPK in the RP fat pad, compared to the LF diet (Table 2). Because AMPK acts as a sensor for cellular energy and regulates the oxidation of fatty acids and glucose (Ruderman and Prentki 2004), our findings help to explain the decreased oxidation of energy substrates and

Table 5 RT-PCR analysis of gene expression in the RP adipose tissue of rats fed a LF or HF diet supplemented with or without Arg

Genes	Low-fat diet		High-fat diet		P value		
	Alanine	Arginine	Alanine	Arginine	Diet	AA	Diet × AA
AMP-activated protein kinase α subunit	1.00 ± 0.08	1.11 ± 0.09	0.76 ± 0.08	0.79 ± 0.13	0.04	0.65	0.82
Slc2 (facilitated glucose transporter), member 4	1.00 ± 0.07	1.20 ± 0.19	0.69 ± 0.12	0.52 ± 0.06	0.03	0.55	0.20
Fatty acid synthase	1.00 ± 0.19	1.48 ± 0.16	0.39 ± 0.05	0.31 ± 0.06	0.01	0.14	0.04
Stearoyl-CoA desaturase 1	1.00 ± 0.12	1.29 ± 0.28	0.23 ± 0.04	0.20 ± 0.04	0.01	0.56	0.48
Peroxisome proliferator activated receptor γ	1.00 ± 0.09	1.59 ± 0.24	1.07 ± 0.22	1.51 ± 0.25	0.98	0.04	0.76
Lipoprotein lipase	1.00 ± 0.14	0.73 ± 0.19	0.80 ± 0.21	0.90 ± 0.29	0.97	0.69	0.39
Hormone-sensitive lipase	1.00 ± 0.09	0.88 ± 0.16	1.13 ± 0.26	1.04 ± 0.28	0.22	0.67	0.42

Values are mean ± SEM, $n = 8$ per treatment group. Data are expressed as the relative values to those of alanine-supplemented rats fed a LF diet

Slc2 solute carrier family 2

Table 6 Glutathione concentrations in the RP adipose tissue of rats fed a LF or HF diet supplemented with or without Arg

Glutathione concentration	Low-fat diet		High-fat diet		P value		
	Alanine	Arginine	Alanine	Arginine	Diet	AA	Diet × AA
Reduced glutathione, nmol/g tissue	708 ± 42	801 ± 57	516 ± 39	599 ± 45	0.01	0.03	0.77
Oxidized glutathione, nmol/g tissue	88.2 ± 5.6	64.7 ± 3.9	125 ± 9.2	93.8 ± 6.6	0.01	0.01	0.53
Oxidized glutathione/reduced glutathione	0.127 ± 0.010	0.081 ± 0.006	0.246 ± 0.017	0.155 ± 0.012	0.01	0.01	0.86

Values are mean ± SEM, $n = 8$

the increased assembly of triacylglycerols in the fat pad of HF-fed rats (Jobgen 2007). Compared with LF-fed rats, expression of a number of genes involved in the oxidative defensive system was reduced in the RP adipose tissue of HF-fed rats, including glutathione synthetase, glutathione S-transferase, glutathione peroxidase 3, superoxide dismutase 3, peroxiredoxin 5, HO-3, and stress-induced protein (Tables 2, 4). Reduced removal of NO and other reactive oxygen species would lead to the accumulation of oxidants, resulting in the nitration and oxidation of both proteins (Bartesaghi et al. 2007; Voss and Grune 2007) and lipids (Trostchansky and Rubbo 2007). Accordingly, the ratio of oxidized glutathione to reduced glutathione, which is an indicator of cellular redox state (Wu et al. 2004), was higher in the RP fat pad of HF-fed rats, compared with LF-fed rats (Table 6), as reported for other cell types (Higashida et al. 2009; Manna et al. 2008). Oxidative stress is likely a major mechanism for impaired insulin sensitivity in the adipose tissue of DIO rats (Jobgen et al. 2009; Zou and Shao 2008) and obese humans (Willoughby et al. 2007).

A novel and important observation of the present study is that adipose tissue gene expression differed between DIO rats with normoglycemia (present study) and ZDF rats with hyperglycemia (Fu et al. 2005) in response to dietary Arg supplementation. This may be explained, in part, by marked differences in plasma concentrations of metabolites and hormones between these two animal models (Clark et al. 1983; Fu et al. 2005; Jobgen et al. 2009), which may affect gene expression in mammalian cells (Flynn et al. 2008; Palii et al. 2008). Of particular note, Arg enhanced expression of key genes for fatty acid oxidation (AMPK, NO synthase-1, and PGC1α) in the RP adipose tissue of ZDF rats (Fu et al. 2005), but had no effect on AMPK or NO synthase 1 and even decreased PGC1α expression in DIO rats (Table 3). In contrast, Arg promoted expression of lipogenic genes (including malic enzyme 1 and PPARγ) and reduced expression of glycogenin (the physiological primer for glycogen synthesis) in the white fat pad of DIO rats (Table 3), but not in ZDF rats (Fu et al. 2005). It should be noted that there was no detectable conversion of D-[U-^{14}C] glucose into fatty acids in the RP adipose tissue of 19-week-old ZDF rats (Fu et al. 2005) or 31-week-old DIO rats (Jobgen 2007) due to the absence of acetyl-CoA carboxylase activity. Thus, in Arg-supplemented DIO rats, increased gene expression of malic enzyme 1, fatty acid synthase, and SCD1 may represent only a physiological response to dietary manipulation but would have little effect on de novo synthesis of fatty acids. However, increased expression of PPARγ (a transcription factor) likely plays an important role in adipose tissue metabolism and function.

PPARγ is known to regulate the differentiation of pre-adipocytes into mature adipocytes (Yoon et al., 2000). Recent studies have shown that PPARγ has an adipogenic

effect in fat pads and stimulates lipogenesis in both adipose tissue and liver (Kersten 2001). Consistent with the results of our in vivo study, Yan et al. (2002) reported that increasing extracellular concentrations of Arg in culture medium promoted the differentiation of rat white preadipocytes into mature adipocytes, enhanced the activity of lipoprotein lipase and glycerol-3-phosphate dehydrogenase, and increased triglyceride accumulation. This may explain increased mass of intramuscular fat in Arg-supplemented pigs (Tan et al. 2008). However, in adult-rat RP adipose tissue with limited de novo synthesis of fatty acids (Jobgen et al. 2006), increased expression of PPARγ may promote adipocyte differentiation, possibly affecting lipid droplet formation and lipolysis in adipose tissue. In support of this view, we found that Arg supplementation increased the release of glycerol as well as the oxidation of glucose and fatty acids in RP adipose tissue (Jobgen 2007), therefore reducing the size of adipocytes (Jobgen et al. 2009).

Another significant observation from this work is that dietary Arg supplementation increased expression of fatty acid transport protein but decreased expression of caspases 1 and 2 in RP adipose tissue (Tables 3, 4). Because high levels of fatty acids are toxic to cells, their transport by specific proteins is crucial for utilization by multiple tissues and minimizing the production of lipid peroxides (Pohl et al. 2004). Additionally, caspases are involved in activation of inflammatory processes and apoptosis in cells (Kim 2008; Li and Yuan 2008). Based on these findings, we suggest that Arg supplementation ameliorates oxidative stress in HF-fed rats (Fig. 1). Consistent with this view, hepatic concentrations of reduced glutathione and tetrahydrobiopterin [which is sensitive to destruction by oxidants (Shi et al. 2004)] were much greater in Arg- than in alanine-supplemented rats (Jobgen et al. 2009). Also, the ratio of oxidized glutathione to reduced glutathione in the RP fat pad was lower in Arg- than in alanine-supplemented rats (Table 6). Furthermore, Ma et al. (2008) reported that dietary Arg supplementation increased tissue anti-oxidative capacity in growing-finishing pigs. Thus, enhancement of the oxidative defense system may be a mechanism for improvements of both insulin sensitivity and metabolic profiles in DIO rats (Jobgen et al. 2009) and pigs (Han et al. 2008; He et al. 2008).

Despite the divergent expression of adipose tissue genes between ZDF and DIO rats, a common outcome in these two animal models in response to Arg supplementation is an enhanced expression of HO-3 in RP fat pad (Table 4). This enzyme catalyzes the oxidation of heme to form biliverdin and carbon monoxide (Muz et al. 2008; Olszanecki et al. 2008), which can activate guanylyl cyclase to generate cGMP (Maines 1997). The subsequent cGMP signaling results in enhancements of lipolysis and acetyl-CoA oxidation in adipose tissue (Jobgen et al. 2006). This may compensate for the reduced expression of PGC1α,
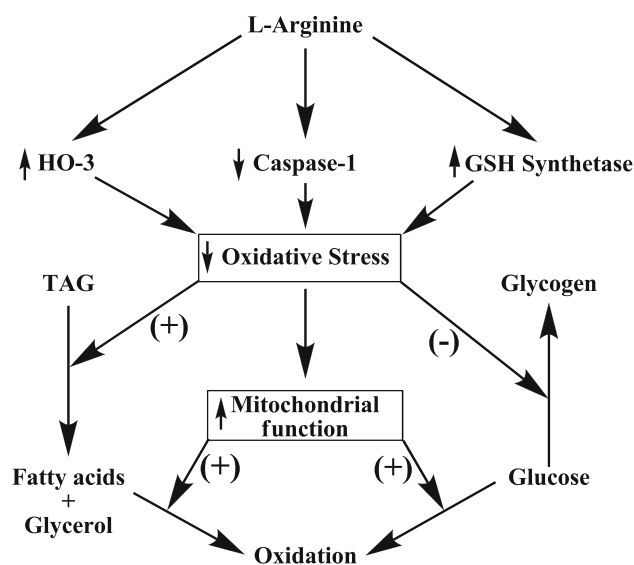

Fig. 1 Proposed mechanism for the beneficial effect of dietary Arg supplementation on reducing adiposity in the white adipose tissues of diet-induced obese rats. The signs (+) and (−) denote increase and decrease, respectively. *GSH* glutathione, *TAG* triacylglycerols

a master regulator of mitochondrial biogenesis and oxidative phosphorylation (Nisoli et al. 2003), in Arg-supplemented rats (Table 3). Free fatty acids released from fat pads are taken up by skeletal muscle (Frayn et al. 2003), where their oxidation is increased due to AMPK activation by Arg (Jobgen 2007). In support of this notion, we found that addition of a carbon monoxide donor to incubation medium stimulated the release of glycerol from RP fat pad, while enhancing the oxidation of both oleic acid and glucose in rat skeletal muscle and RP adipose tissue (W.S. Jobgen, P. Li and G. Wu, unpublished data). Additionally, Li et al. (2008b) recently reported that increasing endogenous synthesis of CO in obese diabetic mice through intraperitoneal administration of an HO inducer (once per week for 6 weeks) reduced visceral and subcutaneous adiposity, decreased plasma concentrations of glucose and proinflammatory cytokines (e.g., interleukins 6 and 1-α, and tumor necrosis factor α), and improved whole-body insulin sensitivity. Future studies are warranted to define a role for HO-3 in obesity development and treatment.

Arg can be synthesized from glutamine/glutamate and proline in most mammals, including humans (Hu et al. 2008a, b) and rats (Wu et al. 2008a, b). Therefore, Arg was traditionally classified as a nutritionally nonessential AA (Wang et al. 2008a; Wu et al. 2007a). However, there is rapid turnover of Arg in mammals (Wu et al. 2007b). In addition, Arg can be degraded by multiple pathways to generate NO (Montanez et al. 2008; Orlando et al. 2008), methylarginines (Sotgia et al. 2008), proline (Lupi et al. 2008; Phang et al. 2008), creatine (Bassit et al. 2008; Gualano et al. 2008), agmatine (Marques et al. 2008; Suenaga et al. 2008), and

polyamines (Dekaney et al. 2008; Wang 2007), with each having enormous physiological importance [including cellular signaling roles (Liao et al. 2008)]. Exogenous provision of Arg helps sustain elevated concentrations in plasma to meet optimal metabolic requirements (Wu et al. 2008b). Thus, Arg supplementation may be beneficial for ameliorating adiposity and the metabolic syndrome in obese subjects (Wu and Meininger 2009). Given the complexity of Arg metabolism in vivo (Wu and Morris 1998), studies involving cell cultures are necessary to determine whether Arg itself or its metabolite(s) regulate gene expression in adipose tissue.

In summary, results of this study indicate that HF feeding decreased expression of key genes for both lipogenic and anti-oxidative enzymes but increased expression of genes for triacylglycerol assembly and oxidative stress in RP adipose tissue. In contrast, dietary Arg supplementation enhanced expression of key genes that promotes lipolysis, the oxidation of energy substrates, and the removal of oxidants. These findings provide a molecular mechanism for the beneficial effect of Arg on reducing white-fat gain, as well as improving insulin sensitivity and anti-oxidative defense capacity in mammals.

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