

Beneficial effects of L-arginine on reducing obesity: potential mechanisms and important implications for human health

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Abstract Over the past 20 years, growing interest in the biochemistry, nutrition, and pharmacology of L-arginine has led to extensive studies to explore its nutritional and therapeutic roles in treating and preventing human metabolic disorders. Emerging evidence shows that dietary L-arginine supplementation reduces adiposity in genetically obese rats, diet-induced obese rats, finishing pigs, and obese human subjects with Type-2 diabetes mellitus. The mechanisms responsible for the beneficial effects of L-arginine are likely complex, but ultimately involve altering the balance of energy intake and expenditure in favor of fat loss or reduced growth of white adipose tissue. Recent studies indicate that L-arginine supplementation stimulates mitochondrial biogenesis and brown adipose tissue development possibly through the enhanced synthesis of cell-signaling molecules (e.g., nitric oxide, carbon monoxide, polyamines, cGMP, and cAMP) as well as the increased expression of genes that promote whole-body oxidation of energy substrates (e.g., glucose and fatty acids) Thus, L-arginine holds great

promise as a safe and cost-effective nutrient to reduce adiposity, increase muscle mass, and improve the metabolic profile in animals and humans.

Keywords Arginine · Fat metabolism · Brown adipose tissue · NO

Abbreviations

ACC	Acetyl-CoA carboxylase
AMPK	AMP-activated protein kinase
Arg	L-Arginine
BAT	Brown adipose tissue
CPT-1	Carnitine palmitoyl transferase-1
DIO	Diet-induced obese
GC	Guanylyl cyclase
LCFA	Long-chain fatty acid
NO	Nitric oxide
NOS	Nitric oxide synthase
PGC-1 α	Peroxisome proliferator-activated receptor γ coactivator-1 α
UPC1	Uncoupling protein-1
WAT	White adipose tissue
ZDF	Zucker diabetic fatty

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Introduction

Obesity is a major health problem in North America, Europe, and many developing nations (CDC 2009; WHO 2009). For example, nearly 1 billion adults worldwide are overweight and at least 300 million are obese (WHO 2009). In the United States alone, 35% of the population is obese, and approximately two-thirds of the population is

overweight (Flegal et al. 2010). Excess body fat is stored primarily in white adipose tissue (WAT). Although obesity is recognized as a leading risk factor for insulin resistance, Type-2 diabetes, atherosclerosis, stroke, hypertension, and some types of cancer (including colon and breast cancers), there are few medications for treating this chronic disease (Pi-Sunyer 2003).

Growing evidence from animal studies indicates that physiological levels of L-arginine (Arg) promote the oxidation of glucose and long-chain fatty acids (LCFA), while decreasing de novo synthesis of glucose and triacylglycerols (Jobgen et al. 2009a; Wu et al. 2009). Additionally, Arg supplementation increases lipolysis and inhibits lipogenesis by modulating the expression and function of key enzymes involved in anti-oxidative response and fat metabolism in insulin-sensitive tissues (Jobgen et al. 2009b). Nitric oxide (NO), which is synthesized from Arg by NO synthase (NOS) (Wu and Morris 1998), participates in multiple cell-signaling pathways and, therefore, regulates the metabolism of energy substrates in a cell- and tissue-dependent manner (Jobgen et al. 2006).

In view of the important progress in Arg research, the objectives of this review are to (1) highlight recent major findings from animal models and human subjects regarding anti-obesity effects of Arg; and (2) propose cellular and molecular mechanisms mediating Arg effects. These mechanisms may include the stimulation of mitochondrial biogenesis and brown adipose tissue (BAT) development, as well as the regulation of gene expression and metabolic pathways.

Anti-obesity effects of Arg on animals and humans

Studies with rats

A novel anti-obesity effect of Arg was observed by Fu et al. (2005) in a seminal study involving chronic Arg supplementation to prevent endothelial dysfunction in adult Zucker diabetic fatty (ZDF) rats (an animal model of Type-2 diabetes mellitus). Beginning on week 4 of supplementation, Arg-treated rats (which received drinking water containing 1.51% L-Arg-HCl) began to lose white fat mass when compared with alanine-supplemented rats (isonitrogenous control) despite similar food intake between the two groups of obese animals. At the end of a 10-week period of supplementation, epididymal and retroperitoneal fat weights in Arg-treated rats were 28 and 46% lower, respectively, when compared with the control group (Fu et al. 2005). Similarly, dietary supplementation with either Arg (0.24% L-Arg-HCl in drinking water) or watermelon juice (0.2% L-citrulline plus Arg in drinking water) for 4 weeks reduced adiposity and improved endothelium-

dependent relaxation in adult ZDF rats (Wu et al. 2007b). More recently, Jobgen et al. (2009a) reported that Arg supplementation (free access to drinking water containing 1.51% Arg-HCl) for 12 weeks substantially reduced WAT in diet-induced obese (DIO) rats (Fig. 1) without altering intramuscular lipid content. Interestingly, Arg supplementation decreased the size of white adipocytes (Fig. 2) but had no effect on their numbers (Jobgen et al. 2009a). Additionally, the long-term Arg treatment increased skeletal muscle-weight by 12% and the whole-body disposal of glucose by 14% without affecting serum levels of insulin, adiponectin, growth hormone, corticosterone, or thyroid hormones (Jobgen et al. 2009a). Furthermore, Arg enhanced BAT mass in both ZDF rats (Wu et al. 2007b) and DIO rats (Fig. 1), underscoring a novel role for Arg in BAT growth and development.

Studies with pigs

The pig is both a useful animal model in biomedical research and an agriculturally important species for meat production (Deng et al. 2009; Elango et al. 2009; Suryawan et al. 2009; Yin et al. 2009). A major issue in swine production is that excessive amounts of subcutaneous WAT (e.g., backfat) are naturally deposited in market-weight pigs fed a conventional finishing diet (Mersmann and Smith 2005). Tan et al. (2009) reported that supplementing 1% Arg to the diet of 110-day-old barrows for 60 days reduced serum triglyceride levels by 20% and whole-body fat content by 11%, while increasing whole-body skeletal-muscle content by 5.5%. The effects of Arg on reducing lipids and improving the efficiency of protein deposition in finishing pigs were also detected by the metabolomic analysis of serum samples (He et al. 2009). Unexpectedly, intramuscular lipid content was 70% greater in Arg-supplemented than in control pigs (Tan et al. 2009), indicating that lipid metabolism and its regulation vary with the anatomical location of WAT. Because muscle lipid represents <3% of carcass fats, it has little impact on whole-body lipid content in Arg-supplemented pigs (Tan et al. 2009). Thus, supplementing Arg to finishing pigs favorably reduced overall body white fat accretion, enhanced muscle gain, and improved the metabolic profile.

Studies with humans

Multiple studies have evaluated the effect of Arg supplementation on endothelial function in adult human subjects, which have been summarized by us (Wu and Meininger 2000) and others (Yongyi et al. 2009). However, only one clinical trial has been published regarding the specific effect of Arg on adiposity in humans (Lucotti et al. 2006). This was a 21-day randomized, placebo-controlled trial in

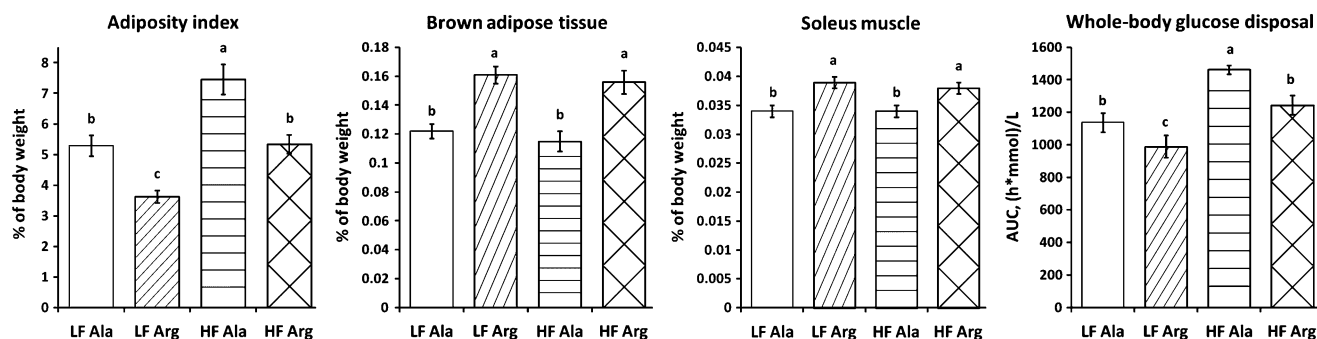


Fig. 1 Beneficial effects of dietary L-arginine supplementation on diet-induced obese rats. Data (mean ± SEM *n* = 8) are adapted from Jobgen et al. (2009a). After a 15-week period of low-fat or high-fat feeding, rats continued to receive their respective diets and either 1.51% L-arginine-HCl or 2.55% L-alanine (isonitrogenous control) in drinking water for 12 weeks. Whole-body glucose disposal was

assessed by oral glucose tolerance test at the end of 10-week supplementation, and area under the curve (AUC) was calculated. All other variables were determined at the end of 12 week supplementation. Adiposity index is the sum of major fat pads (retroperitoneal, mesenteric, epididymal, and subcutaneous adipose tissues) divided by body weight

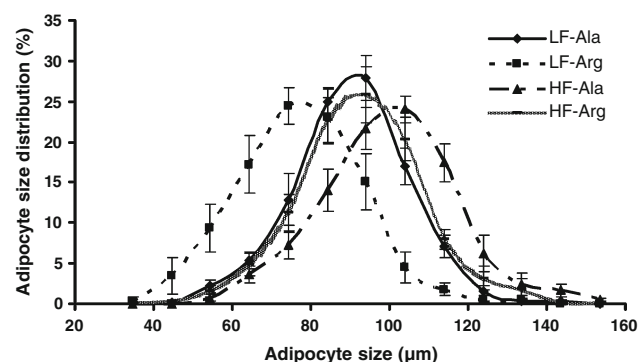


Fig. 2 Size distribution of adipocytes from retroperitoneal adipose tissue (white fat) of rats. After a 15-week period of low-fat (LF) or high-fat (HF) feeding, rats continued to receive their respective diets and either 1.51% L-arginine-HCl (Arg) or 2.55% L-alanine (Ala, isonitrogenous control) in drinking water for 12 weeks (Jobgen et al. 2009a). At the end of the 12-week period of L-arginine/L-alanine supplementation, retroperitoneal adipose tissue was dissected to determine adipocyte size (Jobgen et al. 2009a). Data are expressed as mean ± SEM (*n* = 8)

P-value	P values for treatment effects										
	Diameter of adipocytes (µm)										
	45	55	65	75	85	95	105	115	125	135	145
Diet	0.13	0.005	0.002	0.001	0.052	0.51	0.001	0.0005	0.015	0.086	0.13
AA	0.13	0.020	0.003	0.004	0.23	0.16	0.001	0.0005	0.14	0.62	0.021
Diet x AA	0.19	0.056	0.016	0.14	0.052	0.011	0.050	0.17	0.60	0.80	0.21

exhibited reductions in (1) body weight (3.0 vs. 3.7 kg); (2) fat mass (3.0 vs. 2.1 kg); (3) waist circumference (8.3 vs. 3.2 cm); and (4) circulating levels of glucose (3.2 vs. 1.8 mmol/L), fructosamine (54 vs. 23 µmol/L), and insulin (8.2 vs. 3.0 mU/L). Moreover, increases in antioxidant capacity and circulating levels of adiponectin were observed for these patients. Importantly, all improvements were significantly greater (*P* values <0.0001 for most variables) in the Arg group than in the placebo group. Additionally, over the 3-week period of study, fat-free mass was maintained in the arginine group but reduced by 1.6 kg in the placebo group. Notably, fat mass accounted for 100% of the weight loss in the Arg group (without any loss of fat-free mass) whereas the loss of fat-free mass accounted for 43% of the total weight loss in the placebo group. Thus, Arg supplementation to obese subjects promoted fat reduction and spared lean body mass during weight loss. Pediatric obesity is of particular concern in the healthcare system, yet no studies to date have evaluated the use of Arg for weight management in this population.

Potential mechanisms for Arg to reduce adiposity

Expression of genes

It is now known that amino acids can regulate expression of genes in diverse cell types, including myocytes, adipocytes, and hepatocytes (Palii et al. 2009; Tan et al. 2010; Wang et al. 2009a, b, c). Using the microarray analysis technique and the DIO rat model, Jobgen et al. (2009b) reported that dietary Arg supplementation affected expression of many genes in WAT. Of particular interest, high-fat feeding decreased mRNA levels for lipogenic enzymes, AMP-activated protein kinase (AMPK), glucose transporters, heme oxygenase 3, glutathione synthetase,

33 hospitalized middle-aged, obese (mean body mass index = 39.1 ± 0.5 kg/m²) subjects with diet-controlled Type-2 diabetes mellitus. During the study period, each patient received a low-calorie diet (1,000 kcal/day) and a regular exercise-training program (45 min twice a day for 5 days/week). They were randomized to 8.3 g Arg/day (approximately 80 mg/kg body weight per day) or placebo. This dose of Arg is equivalent to that for DIO rats (Jobgen et al. 2009a) and finishing pigs (Tan et al. 2009). As expected from the hypocaloric diet, both groups of subjects

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 phosphatase 1B, caspases 1 and 2, and hepatic lipase, but increased expression of PPAR γ , heme oxygenase 3, glutathione synthetase, insulin-like growth factor II, sphingosine-1-phosphate receptor, and stress-induced protein. Biochemical analysis revealed that Arg supplementation prevented oxidative stress in WAT, skeletal muscle and livers of obese rats (Jobgen et al. 2009b) and finishing pigs (Ma et al. 2010). Collectively, these results indicate that Arg beneficially modulates gene expression to enhance energy-substrate oxidation and reduce white fat accretion in insulin-sensitive tissues.

Gene expression in WAT differed between DIO rats (Jobgen et al. 2009b) and ZDF rats (Fu et al. 2005) in response to dietary Arg supplementation. This may be explained, in part, by marked differences in plasma concentrations of metabolites (e.g., amino acids, glucose and fatty acids) and hormones (e.g., insulin and leptin) between these two animal models, which may affect gene expression in mammalian cells (Flynn et al. 2009; Palii et al. 2009). Interestingly, Arg enhanced expression of key genes for fatty acid oxidation [AMPK, NOS-1, and PPAR γ coactivator-1 α (PGC1 α)] in WAT of ZDF rats (Fu et al. 2005), but had no effect on AMPK or NOS-1 and even decreased PGC1 α expression in WAT of DIO rats (Jobgen et al. 2009b). In contrast, Arg promoted expression of lipogenic genes (including malic enzyme 1 and PPAR γ) and reduced expression of glycogenin (the physiological primer for glycogen synthesis) in WAT of DIO rats, but not in ZDF rats. Because there is little synthesis of fatty acids in WAT of adult rats due to the absence of acetyl-CoA carboxylase (ACC) activity (Jobgen et al. 2006), the increased expression of malic enzyme 1 and fatty acid synthase in WAT of DIO rats may represent only a physiological response to dietary manipulation. However, increased expression of PPAR γ , which stimulates differentiation and proliferation of preadipocytes (Chung et al. 2005), may enhance lipogenesis in intramuscular adipose tissue and, therefore, intramuscular lipids (Tan et al. 2009).

Mitochondrial biogenesis

The mitochondrion is the major organelle for complete oxidation of energy substrates in all cells except for mammalian red blood cells (Jobgen et al. 2006). Approximately 20% of cellular proteins regulate mitochondrial formation and growth (Nisoli et al. 2008). It is now known that PGC-1 α is the master regulator of mitochondrial

biogenesis (Lehman et al. 2000; Puigserver et al. 1998; Wu et al. 1999) and its expression is influenced by NO (Fu et al. 2005). In turn, PGC-1 α stimulates expression of key genes involved in mitochondrial biogenesis, including nuclear respiratory factors 1 and 2, as well as PPAR α (Table 1). Studies with eNOS knockout mice and cell cultures have shown that endogenous NO has an obligatory role in the mitochondrial biogenesis, oxidation, and remodeling of animal cells through the generation of cGMP (Nisoli et al. 2003, 2004). This conclusion is supported by growing evidence from work with cold-acclimated rodents (Petrović et al. 2008a, b, 2010), exercising rats (Wadley and McConell 2007), and cultured cells (McConell et al. 2009). Furthermore, Arg-derived polyamines are necessary for cell proliferation and differentiation as well as mitochondrial function and integrity (Flynn et al. 2009; Wu et al. 2009).

BAT development and thermogenesis

Brown adipose tissue is responsible primarily for non-shivering thermogenesis in mammals (e.g., sheep, rats, and humans) (Himmshagen 1990). Distinct from other tissues, BAT contains very high quantities of uncoupling protein-1 (UCP1). Mitochondrial UCP1, which is localized exclusively in brown adipocytes of BAT (Nisoli et al. 2003), uncouples ATP synthesis from the oxidative process to generate heat (Cannon and Nedergaard 2004). Of particular interest, BAT produces 150–300 times more heat per kg tissue than non-BAT organs (Power 1989). Excitingly, new evidence shows that functional BAT exists in adult humans (Cypess et al. 2009; Virtanen et al. 2009). In addition, BAT activity is reduced in adult overweight or obese humans and is positively correlated with resting metabolic rate (van Marken Lichtenbelt et al. 2009). Furthermore, under certain conditions (e.g., increases in NO production and PGC-1 α expression), white adipocytes may be converted into brown adipocytes that express high levels of UCP-1 (Nisoli and Carruba 2004).

NO has long been known to play an important role in heat production and thermoregulation in mammals (Scammell et al. 1996). It is now clear that protein kinase G, a target protein for NO, controls BAT cell differentiation and mitochondrial biogenesis (Haas et al. 2009). Thus, inhibition of NO synthesis reduces blood flow to BAT (De Luca et al. 1995), BAT development (Petrović et al. 2005, 2008a, b; Saha et al. 1996), and cold-induced thermogenesis in rats (De Luca et al. 1995; Kamerling et al. 2003; Saha et al. 1996). The recent discovery that Arg supplementation increased amounts of BAT in fetal lambs (Satterfield et al. 2009), DIO rats (Jobgen et al. 2009b), ZDF rats (Wu et al. 2007a, b), and cold-acclimated rats (Petrović et al. 2010) raises an expectation that this nutritional

Table 1 Key proteins in mitochondrial biogenesis, brown adipocyte development, and metabolism of energy substrates in animal cells and tissues

Gene	Functions
NO and CO synthesis	
NOS-1, 2, 3	NOS-1 (nNOS) is a constitutive isoform of NOS that synthesizes NO from Arg in skeletal muscle, white adipose tissue, BAT, and liver. NOS-2 (iNOS) is an inducible isoform of NOS that synthesizes NO from Arg in response to cytokines and other inflammatory agents. NOS-3 (eNOS) is a constitutive isoform of NOS that synthesizes NO from Arg in endothelial cells, skeletal muscle, heart, white adipose tissue, BAT, and liver. Among animal tissues, NOS-3 is most abundant in BAT
HO-1, 2, 3	Heme oxygenase (HO) generates CO from heme. HO-1 is highly inducible by oxidants, hypoxia and inflammatory cytokines in diverse cell types. HO-2 is constitutively expressed in diverse cell types. HO-3 is expressed in white adipose tissue and skeletal muscle where CO regulates oxidation of fatty acids and glucose
Mitochondrial biogenesis and BAT development	
PGC-1 α	A master regulator of mitochondrial biogenesis and BAT development
NRF-1, 2, 3	Regulators and markers of mitochondrial biogenesis
PPAR α	A transcription factor that regulates cellular differentiation, development, and metabolism. It is highly expressed in hepatocytes and skeletal muscle
mtTFA	A mitochondrial transcription factor and a marker of mitochondrial biogenesis
Cytochrome <i>c</i>	An enzyme of the mitochondrial respiratory chain and a marker of mitochondrial biogenesis
UCP-1	A unique protein in mitochondria of BAT which uncouples ATP synthesis from substrate oxidation
VEGF	A key regulator of endothelial cell proliferation and angiogenesis in blood vessels and BAT
Metabolism of fatty acids and glucose	
AMPK α	A key regulator of (a) oxidation of energy substrates, (b) gluconeogenesis, and (c) fat synthesis
FAS	An enzyme involved in the synthesis of fatty acids from glucose and amino acids
ACC-1, 2	ACC-1 is a major isoform of ACC in lipogenic tissues where it converts acetyl-CoA into malonyl-CoA in fatty acid synthesis. ACC-2 is the predominant isoform of ACC in oxidative tissues (e.g., liver, muscle, and BAT) where it converts acetyl-CoA into malonyl-CoA, an inhibitor of LCFA-CoA transport from cytoplasm into mitochondria. Phosphorylation of ACC reduces its activity
SREBP-1c	A key regulator of fatty acid and glucose synthesis in liver
HSL	A key regulator of lipolysis in tissues, particularly white adipose tissue, BAT, and muscle
GAPDH	An enzyme of the glycolysis pathway. It is often used to normalize gene expression in cells

Expression of the listed genes in cells and tissues may be altered by dietary L-arginine supplementation

ACC acetyl-CoA carboxylase, AMPK AMP-activated protein kinase, BAT brown adipose tissue, FAS fatty acid synthase, GAPDH glyceraldehyde-3-phosphate dehydrogenase, HSL hormone-sensitive lipase, mtTFA mitochondrial transcription factor A, NOS nitric oxide synthase, NRF nuclear respiration factor, PGC-1 α peroxisome proliferator activator receptor γ coactivator-1 α , PPAR α peroxisome proliferator activator receptor- α , SREBP-1c sterol regulatory element-binding protein-1c, UCP uncoupling protein, VEGF vascular endothelial growth factor

approach may provide a new means to stimulate physiological thermogenesis and reduce white fat accretion in animals and humans.

Cell signaling and metabolism

Effects of Arg on cell metabolism appear to be mediated partially by NO and other metabolites (Eklou-Lawson et al. 2009; Gaudiot et al. 1998; Gouill et al. 2007). The conversion of Arg to NO by NOS requires NADPH, thereby creating another possible explanation for how Arg increases glucose utilization through the pentose pathway and cellular redox state (Phang et al. 2008). NO stimulates blood flow to organs (e.g., the brain, skeletal muscle, cardiac tissue, small intestine, and kidney), which allows for greater uptake of energy substrates (e.g., glucose and LCFA) by for oxidation to CO₂ and water (Jobgen et al. 2006). Additionally,

physiological levels of NO up-regulate the activity of carnitine palmitoyl transferase-1 (CPT-1; the mitochondrial transporter of LCFA) and the expression of glucose transporter 4 in hepatocytes (Garcia-Villafranca et al. 2003) and skeletal muscle (Lira et al. 2007), respectively. This would result in increased whole-body oxidation of both LCFA and glucose via the mitochondrial Krebs cycle and electron transport system. Furthermore, spermine and spermidine (formed from Arg) may increase oxidation of LCFA and glucose by maintaining mitochondrial function and integrity in cells (Madsen et al. 1996).

Physiological levels of Arg increase the production of not only NO but also carbon monoxide (CO) from diverse cell types (Li et al. 2009). These two gaseous molecules activate guanylyl cyclase, thereby increasing the production of cGMP. The cGMP-dependent protein kinase phosphorylates ACC, thereby reducing the conversion of acetyl-CoA to

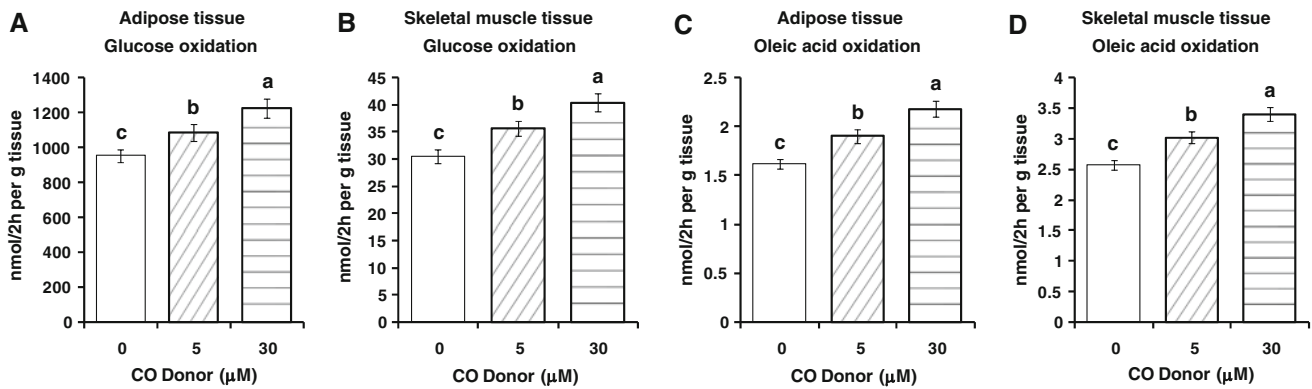


Fig. 3 Effect of CO donor on glucose and fatty acid oxidation in muscle and white adipose tissue. Data are means \pm SEM $n = 30$. Means with different letters (a–c) differed ($P < 0.05$). Tissue (~ 100 mg) was incubated at 37°C for 2 h in 1 ml Krebs buffer

malonyl-CoA, which is both an intermediate of the fatty acid synthesis pathway and an inhibitor of CPT-I (Jobgen et al. 2006). By decreasing malonyl-CoA concentration, fatty acid synthesis is inhibited while CPT-1 remains active to promote the transport of LCFA from cytoplasm into mitochondria for oxidation. Additionally, NO enhances AMPK activity by both increasing gene expression and AMPK phosphorylation to (1) decrease glycerol-6-phosphate acyltransferase activity and thus triacylglycerol synthesis; (2) down-regulate sterol regulatory element-binding protein 1c (SREBP-1c) and thus fatty acid synthesis; and (3) stimulate the conversion of glucose to pyruvate and the subsequent complete oxidation of pyruvate via the Krebs cycle (Jobgen et al. 2006; Lira et al. 2007). Thus, physiological levels of NO and CO stimulate the oxidation of both LCFA and glucose in insulin-sensitive tissues (Jobgen et al. 2006; Fig. 3). Additionally, we found that dietary Arg supplementation increased cAMP concentrations in WAT, BAT, skeletal muscle, and livers of adult rats without altering AMP:ATP ratios (Table 2). Because cAMP activates hormone-sensitive lipase (Kersten 2001), an increase in cAMP is another mediator of enhanced lipolysis in the adipose tissue of Arg-supplemented rats.

Implications of Arg supplementation for human health

Obesity in humans and animals results from a chronic imbalance between energy intake and expenditure. Results of both animal and human studies indicate that Arg supplementation may be a novel therapy for obesity and the metabolic syndrome, acting via decreased plasma levels of glucose, homocysteine, fatty acids, dimethylarginines, and triglycerides, as well as improved whole-body insulin sensitivity (Jobgen et al. 2009a; Kohli et al. 2004; Lucotti et al. 2006; Mendez and Balderas 2001; Wu et al. 2007b).

containing either 5 mM D-glucose plus D-[^{14}C]glucose or 0.2 mM oleic acid plus [^{14}C]oleic acid. The medium also contained 0, 5, or 30 μM CO donor, [Ru(CO) $_3$ Cl] $_2$. Data are taken from published work (Li et al. 2009)

Notably, an anabolic effect of Arg on muscle gain in adult rats (Jobgen et al. 2009a) and finishing pigs (Tan et al. 2009) is achieved independent of changes in serum concentrations of insulin or growth hormone. This finding indicates that dietary Arg supplementation enhances insulin sensitivity and amplifies its signaling mechanisms on protein synthesis (Yao et al. 2008) as well as the metabolism of glucose and fatty acids (Jobgen et al. 2006). Thus, Arg supplementation regulates the repartitioning of dietary energy to favor muscle over fat gain in the body. Based on the fact that protein intake by adult humans (0.8 g/kg body weight per day) is approximately 13% of that for adult rats and finishing pigs (6.2 and 6.0 g/kg body weight per day, respectively) (Wu et al. 2007a), supplemental Arg doses of 950 and 365 mg/kg body weight per day for adult rats (Jobgen et al. 2009a) and finishing pigs (Tan et al. 2009) are equivalent to 85 (50–120) mg Arg/kg body weight per day for adult humans. These amounts of Arg are physiologically attainable when the human diet is supplemented with synthetic Arg.

Arg is stable under sterilization conditions (e.g., high temperature and high pressure) and is not toxic to mammalian cells (Wu 2009). Therefore, multiple studies in both animals and humans conclude that there are no safety concerns regarding Arg supplementation at an appropriate dose and chemical form (Böger and Bode-Böger 2001; Mendez and Balderas 2001; Wu et al. 2009). Results of the third National Health and Nutrition Examination Survey, a program of studies designed to assess the health and nutritional status of adults and children in the United States, indicate that mean Arg intake for the US adult population is 4.4 g/day, with 25, 20, and 10% of people consuming <2.6 (suboptimal), 5–7.5, and >7.5 g/day, respectively (King et al. 2008). Thus, large numbers of adults do not have adequate Arg intake from diets to maintain optimal metabolic pathways (e.g., syntheses of

Table 2 Concentrations of adenylyl purines in insulin-sensitive tissues of lean and obese rats supplemented with or without L-arginine

Tissue	Variable	LF-Ala	LF-Arg	HF-Ala	HF-Arg	P value		
						Diet	AA	Diet × AA
G. muscle	Arginine	1.31 ± 0.10 ^d	2.09 ± 0.14 ^b	1.67 ± 0.12 ^c	2.65 ± 0.16 ^a	0.01	0.01	0.62
	ATP	5.05 ± 0.32	5.24 ± 0.36	4.95 ± 0.29	5.01 ± 0.31	0.39	0.24	0.94
	ADP	0.68 ± 0.05	0.60 ± 0.04	0.66 ± 0.06	0.63 ± 0.05	0.72	0.19	0.85
	AMP	0.30 ± 0.02 ^{bc}	0.27 ± 0.01 ^c	0.37 ± 0.02 ^a	0.32 ± 0.02 ^{ab}	0.01	0.01	0.41
	Adenosine	0.26 ± 0.02	0.23 ± 0.01	0.24 ± 0.02	0.22 ± 0.02	0.64	0.42	0.88
	cAMP	1.06 ± 0.07 ^b	1.35 ± 0.09 ^a	0.78 ± 0.05 ^c	1.10 ± 0.06 ^b	0.01	0.01	0.53
	AMP/ATP	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.38	0.18	0.76
WAT	Arginine	0.11 ± 0.01 ^d	0.18 ± 0.01 ^b	0.14 ± 0.01 ^c	0.23 ± 0.01 ^a	0.01	0.01	0.25
	ATP	0.44 ± 0.03	0.46 ± 0.05	0.42 ± 0.04	0.48 ± 0.03	0.58	0.37	0.42
	ADP	0.16 ± 0.01	0.15 ± 0.02	0.14 ± 0.02	0.17 ± 0.02	0.63	0.82	0.91
	AMP	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	0.44	0.69	0.83
	Adenosine	0.26 ± 0.03	0.23 ± 0.02	0.24 ± 0.02	0.22 ± 0.02	0.62	0.14	0.38
	cAMP	0.41 ± 0.03 ^a	0.53 ± 0.03 ^a	0.36 ± 0.02 ^b	0.48 ± 0.03 ^b	0.01	0.01	0.55
	AMP/ATP	0.19 ± 0.02	0.17 ± 0.02	0.21 ± 0.02	0.20 ± 0.02	0.47	0.59	0.80
BAT	Arginine	1.59 ± 0.09 ^d	2.34 ± 0.16 ^b	1.96 ± 0.13 ^c	2.84 ± 0.18 ^a	0.01	0.01	0.27
	ATP	3.28 ± 0.17	3.41 ± 0.22	3.18 ± 0.20	3.02 ± 0.25	0.76	0.18	0.52
	ADP	1.10 ± 0.09	1.29 ± 0.11	1.22 ± 0.10	1.06 ± 0.12	0.81	0.90	0.34
	AMP	0.48 ± 0.03	0.44 ± 0.02	0.53 ± 0.04	0.50 ± 0.03	0.72	0.66	0.49
	Adenosine	0.21 ± 0.02	0.19 ± 0.01	0.20 ± 0.02	0.18 ± 0.02	0.55	0.47	0.91
	cAMP	0.66 ± 0.04 ^b	0.81 ± 0.06 ^a	0.50 ± 0.04 ^c	0.64 ± 0.05 ^b	0.01	0.01	0.20
	AMP/ATP	0.15 ± 0.02	0.13 ± 0.01	0.17 ± 0.02	0.16 ± 0.01	0.40	0.86	0.95
Liver	Arginine	0.05 ± 0.01 ^b	0.08 ± 0.01 ^a	0.06 ± 0.01 ^b	0.09 ± 0.01 ^a	0.16	0.01	0.74
	ATP	4.02 ± 0.38	4.28 ± 0.30	3.85 ± 0.27	3.71 ± 0.34	0.72	0.65	0.91
	ADP	1.32 ± 0.14	1.17 ± 0.16	1.05 ± 0.12	1.20 ± 0.15	0.85	0.72	0.96
	AMP	0.42 ± 0.04	0.39 ± 0.03	0.37 ± 0.03	0.41 ± 0.05	0.46	0.78	0.50
	Adenosine	0.28 ± 0.03	0.31 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.70	0.59	0.87
	cAMP	1.21 ± 0.10 ^b	1.64 ± 0.13 ^a	0.89 ± 0.07 ^c	1.14 ± 0.08 ^b	0.01	0.01	0.46
	AMP/ATP	0.12 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.94	0.81	0.88

Values are mean ± SEM, $n = 8$, and expressed as nmol/g fresh tissue for cAMP and $\mu\text{mol/g}$ fresh tissue for other variables (arginine, ATP, ADP, AMP, and adenosine). Data were analyzed by two-way analysis of variance and the Tukey multiple comparison test (SAS, Cary, NC, USA). After a 15-week period of low-fat (LF) or high-fat (HF) feeding, rats continued to receive their respective diets and either 1.51% L-arginine-HCl (Arg) or 2.55% L-alanine (Ala, isonitrogenous control) in drinking water for 12 weeks (Jobgen et al. 2009a). At the end of the 12-week period of L-arginine/L-alanine supplementation, various tissues were rapidly obtained and frozen in liquid nitrogen for analysis of adenylyl purines using high performance liquid chromatography (Haynes et al. 2009)

AA amino acid, *G. muscle* gastrocnemius muscle, *WAT* white adipose tissue (retroperitoneal adipose tissue), *BAT* interscapular brown adipose tissue

^{a-d} Means in a row with different superscript letters differ ($P < 0.05$)

NO, creatine and polyamines, as well as ammonia detoxification via the urea cycle) or physiological functions (e.g., endothelium-dependent relaxation, vascular integrity, and oxidation of energy substrates) and would potentially benefit from Arg supplements for weight management. As noted above, Arg regulates gene expression, mitochondrial biogenesis, BAT development, and cellular signaling

transduction pathways. Thus, based on the results of animal studies (Jobgen et al. 2009a; Tan et al. 2009), increasing Arg provision beyond the need for the maintenance of body protein may also be beneficial for preventing and treating obesity in humans who have an average intake of Arg from diets. As with any other nutrients, improper use of Arg (e.g., high dose and imbalance among basic amino acids in

the diet) may yield an undesirable effect and should be avoided in dietary supplementation and clinical therapy (Baker 2009; Elango et al. 2009; Stipanuk et al. 2009; Rhoads and Wu 2009). It is advisable that Arg be taken in divided doses (e.g., up to 3×3 g/day for a 70-kg person) on each day of supplementation to (1) prevent gastrointestinal tract discomfort due to abrupt production of large amounts of NO; (2) increase the availability of circulating Arg over a longer period of time; and (3) avoid a potential imbalance among dietary amino acids (Wu et al. 2009). A distinct advantage of Arg over drugs (e.g., metformin and thiazolidinediones) is that dietary Arg supplementation reduces adiposity while improving insulin sensitivity (Fu et al. 2005; Jobgen et al. 2009a; Wu et al. 2007b). Therefore, Arg holds great promise in preventing and treating obesity in both animals and humans.

Conclusion

Over the past decade, landmark studies have shown that Arg supplementation is beneficial in reducing adiposity and improving insulin sensitivity in multiple animal models and in a limited number of human subjects. The underlying mechanisms are likely complex at molecular, cellular, and whole-body levels, but may include the stimulation of mitochondrial biogenesis and BAT development, as well as the regulation of gene expression and cellular metabolic pathways. Arg is expected to play an important role in fighting the current global obesity epidemic.

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