Chapter 35
Obese Subjects and Supplemental L-Arginine

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Key Points

- L-arginine influences metabolism in obesity.
- The action of L-arginine in adiposity is related to fat reduction, glucose and fatty acid oxidation, increases in lipolysis, inhibition of lipogenic processes, and changes in fat tissue endocrine secretion.
- L-arginine supplementation improves insulin sensitivity in obese patients.
- Nitric oxide signaling, mitochondrial biogenesis, the growth of brown adipose tissue, and the regulation of fat metabolic gene expression are the main mechanisms underlying the beneficial effect of L-arginine in obesity.
- L-arginine can play a crucial role in preventing and treating obesity and metabolic syndrome.

Keywords L-Arginine • Obesity • Fat metabolism • Brown adipose tissue • White adipose tissue • Insulin sensitivity

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Abbreviations

ACCα	Acetyl-CoA carboxylase α
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
Arg	L-arginine
ATP	Adenosine triphosphate
cGMP	Cyclic guanosine monophosphate
DIO	Diet-induced obese
GLUT4	Glucose transporter type 4
GSS	Glutathione synthetase
HO-3	Heme oxygenase-3
HSL	Hormone-sensitive lipase
NO	Nitric oxide
NOS	NO synthase
NOS-1	NO synthase-1
PGC-1α	Peroxisome proliferator-activated receptor γ coactivator-1α
PPAR-α	Peroxisome proliferator-activated receptor-α
TAS	Total antioxidant status
ZDF	Zucker diabetic fatty

Introduction

Obesity and overweight are constantly growing health problems throughout the world. According to the World Health Organization, nearly one billion adults are overweight, and 300 million people are obese [1]. Although it is widely accepted that obesity is associated with dyslipidemia, hypertension, atherosclerosis, stroke, insulin resistance, and some types of cancers, pharmacological treatment for this chronic disease is limited. The alarming increase in the occurrence of obesity and overweight has compelled researchers to seek new forms of drug therapy. Emerging evidence from both experimental and clinical studies shows that L-arginine (Arg) holds great promise for the prevention and treatment of adiposity and associated metabolic disorders in humans and animals. An anti-obesity effect of Arg is yet to be demonstrated, and the mechanisms responsible for the beneficial effects of Arg are very complex and involve nitric oxide (NO) signaling, enhancing of mitochondrial biogenesis, growth of brown adipose tissue and stimulation of thermogenesis, regulation of fat metabolic gene expression, and changes in fat tissue endocrine secretion.

Crosstalk Between L-Arginine and Metabolism in Obesity

There are several mechanisms that may be involved in the biochemical changes responsible for the effect of Arg treatment on overweight and obese subjects. In experimental studies, it has been found that dietary Arg supplementation decreases adipose cell size without leading to a reduction in adipose cell numbers. It can be suggested that Arg reduces abdominal fat by decreasing triglycerides deposition in adipose cell and not through any influence on the differentiation or proliferation of adipose cells [2]. It has been demonstrated in clinical studies that the use of Arg in central obesity results in a significant decrease in waist circumference.

Table 35.1 Possible mechanisms of L-arginine influence on fat mass loss

Mechanism	Effect
Increases brown adipose tissue	Increases energy expenditure through enhanced oxidation
Increases blood flow to organs	of glucose and fatty acids
Modulates gene expression to enhance energy substrate oxidation	Reduces white fat accretion in insulin-sensitive tissues
Regulates fat metabolic genes in skeletal muscle and white adipose tissue; increases lipogenesis in muscle and lipolysis in adipose tissue	Regulates lipid distribution between muscle tissue and adipose tissue
Increases activity of lipoprotein lipase in skeletal muscle	
Increases content of oleic acid in skeletal muscle	Stimulates glucose uptake by skeletal muscle for oxidation and reduces glucose availability for fatty acid synthesis in other tissues

Arg regulates the metabolism of energy substrates—such as fatty acids, amino acids, and glucose—partly through the production of NO. This mechanism may be involved in the decreases in fat deposition and the increase in muscle growth and protein gain in the body [3]. In experimental studies, it has been shown that enhancing Arg availability stimulates muscle protein synthesis. In animal studies, increases in the proportional weight of skeletal muscle have been observed following Arg supplementation [4]. The growth of muscle tissue in conjunction with the decrease in body fat constitutes the main beneficial effect of Arg, especially in obese people whose muscle mass is relatively reduced. It seems possible that Arg may regulate lipid distribution between muscle and adipose tissue. We suggest that enhanced concentration of Arg upregulates the expression of lipogenic genes in skeletal muscle, while in white adipose tissue, Arg supplementation downregulates the expression of lipogenic genes and increases the expression of lipolytic genes. This idea is supported by the increased activity of lipoprotein lipase observed in skeletal muscle after Arg supplementation. Muscle lipoprotein lipase phosphorylation is regulated by Arg or NO. This enzyme activity provides a substrate for fat biosynthesis in skeletal muscle [5]. Another possible mechanism of Arg's effect on fat content is the increase in the concentration of oleic acid in skeletal muscle. It is known that oleic acid stimulates glucose uptake by skeletal muscles for oxidation and potentially reduces the availability of glucose for fatty acid synthesis in other tissues [5].

It has been found that the anabolic effect of Arg is independent of the insulin level in the blood. In experiments on pigs, Arg supplementation increased muscle mass and reduced white fat content without affecting the body mass. This suggests that Arg may regulate intracellular protein turnover, contributing to the accumulation of protein in muscle tissue. Through this, Arg—as a biological precursor of NO—may increase insulin sensitivity in muscle cells and amplify the signaling mechanisms to enhance protein deposition in skeletal muscle [2].

Arg may also reduce body mass by increasing the content of brown adipose tissue or by increasing blood flows. The main mechanisms through which Arg might reduce fat mass in the body are summarized in Table 35.1.

Experimental and Clinical Studies Concerning L-Arginine Supplementation in Obesity

Animal Studies

The first report on the role of dietary Arg supplementation in reducing fat mass in obese animals with non-insulin-dependent diabetes mellitus emerged in 2005, when Fu et al. observed a loss of weight and adipose tissue mass in supplemented Zucker diabetic fatty (ZDF) rats. They noted

that 10 weeks of oral administration of Arg (1.25 % in drinking water) was highly effective in enhancing NO production, lipolysis, and oxidation of glucose in the abdominal and epididymal adipose tissues. Compared to the placebo group, at the end of the supplementation period, the weight of the epididymal and retroperitoneal adipose tissue in the Arg-treated ZDF rats was 25 % and 45 % lower, respectively. The results of this study indicate that Arg supplementation reduced the serum levels of glucose, free fatty acids, triglycerides, homocysteine, and leptin. The expression of the key genes responsible for fatty acid and glucose oxidation in adipose tissue—among them NO synthase-1 (NOS-1), heme oxygenase-3 (HO-3), adenosine monophosphate (AMP)-activated protein kinase (AMPK), and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α)—was upregulated in the study [4]. In another study, the effect of Arg was observed after 4 weeks supplementation of watermelon pomace juice using 0.2 % L-citrulline, which is converted into Arg in the animals. ZDF rats supplemented with watermelon pomace juice exhibited increased serum concentration of Arg and increased brown adipose tissue mass, along with reduced excess white fat mass and enhanced NO-dependent vessel reactivity [6]. Studies conducted on diet-induced obese (DIO) rats have shown that 12 weeks supplementation with 1.51 % L-arginine–HCl in drinking water reduced the white fat gain, increased the skeletal muscle mass, and decreased the serum concentration of glucose and triglycerides. Improvements in insulin sensitivity were also observed [2].

In studies conducted on growing–finishing pigs, it was found that supplementation with 1.0 % Arg reduced fat accretion and promoted muscle gains. Following 60 days of supplementation, Arg was seen to increase the average daily weight gain and carcass skeletal muscle content by 6.5 % and 5.5 %, respectively, and to decrease carcass fat content by 11 %, compared with the control group. In the Arg-supplemented pigs, serum triglyceride concentration was 20 % lower, while the glucagon level was 36 % greater than in the control pigs [7]. The improvement in metabolic profile in the growing pigs was also detected by metabolomic analysis of serum samples [8]. Interestingly, in a more recent study, Go et al. were unable to demonstrate the depressing lipid synthesis effect of Arg in either subcutaneous or retroperitoneal adipose tissue in growing–finishing pigs. This effect was supported by the dramatic increase in adipocyte volumes. The net effect of supplementation with 1.0 % Arg was an increase in backfat thickness, leading to fatter carcasses [9].

Human Studies

Although the effect of Arg supplementation in human subjects has been dealt with in multiple studies, it seems to be limited mainly to its effect on endothelial function. Bai et al. summarized 13 previously randomized placebo-controlled trials and concluded that short-term oral Arg supplementation is effective at improving the fasting endothelial function [10].

The data from the literature show that the reduction in body weight in clinical studies is not as spectacular as in the experimental studies (Table 35.1). It seems that the greatest advantage of using Arg in obese people is associated with the fact that this compound promotes fat reduction and spares lean body mass during weight loss. In few studies, it was noted that supplementation with Arg in an amino acid mixture led to a reduction in white adipose tissue in both children and adults [11, 12]. However, only one clinical trial regarding the specific effect of Arg alone on adiposity in humans has been conducted. In that trial, a long-term oral Arg treatment was added to a hypocaloric diet and exercise training program in obese, insulin-resistant type 2 diabetes patients. Thirty-three patients with type 2 diabetes were treated with Arg (8.3 g/day) or placebo for 21 days. During the study, each patient received a low-calorie diet (1000 kcal/day) and took part in a regular exercise training program (45 min twice a day for 5 days per week). In both groups, the authors observed a decrease in body weight, waist circumference, and daily

Table 35.2 Body mass and fat content due to L-arginine supplementation in obesity

References	Study group	Dose of L-arginine	Duration of the supplementation	Body mass loss (BMI)	Fat mass loss
Clinical studies					
Lucotti et al. [13]	Obese, insulin-resistant type 2 diabetes adults	L-arginine 8.3 g (oral treatment with low-calorie diet and a regular exercise training program)	21 days	Body mass ↓ 2.8 % (NS)	Fat mass ↓ 6.1 % ($p<0.05$)
Monti et al. [14]	Subjects with impaired glucose tolerance and metabolic syndrome	L-arginine 6.6 g (oral treatment)	14 days	Body mass ↓ 2.4 % ($p<0.05$)	Fat mass ↓ 6.7 % ($p<0.05$)
Bogdanski et al. [15]	Obese adults	L-arginine 9 g (oral treatment)	3 months	BMI ↓ 0.8 % (NS)	Fat content ↓ 0.7 % (NS)
Suliburska et al. [16]	Obese adults	L-arginine 9 g (oral treatment)	6 months	BMI ↓ 2 % (NS)	Fat content ↓ 1.4 % (NS)
Hurt et al. [17]	Obese adults	L-arginine 9 g (oral treatment)	12 weeks	Body mass ↓ 2.9 % ($p<0.05$)	
Experimental studies					
Fu et al. [4]	Zucker diabetic fatty rats[a]	L-Arginine–HCl (1.51 %) in drinking water	10 weeks	Body weight ↓ 16.0 % ($p<0.05$)	Abdominal fat ↓ 44.5 % ($p<0.05$)
Jobgen et al. [2]	Sprague–Dawley rats	L-Arginine–HCl (1.51 %) in drinking water	12 weeks	Low-fat diet: Body weight ↓ 68.1 % ($p<0.05$) High-fat diet: Body weight ↓ 40.7 % ($p<0.05$)	Low-fat diet: White fat ↓ 64.8 % ($p<0.05$) High-fat diet: White fat ↓ 63.4 % ($p<0.05$)
Suliburska et al. [18]	Wistar male rats	L-arginine 20 g/kg diet (with high-fat diet)	6 weeks	Body weight ↓ 2.9 % (NS)	

NS not significant

[a]Animal model for human type 2 diabetes mellitus with obesity

glucose profiles, while an improvement was seen in insulin sensitivity; however, in the Arg-supplemented group, the improvement was significantly greater (*p* values <0.0001 for most variables) [13].

The results of selected experimental and clinical studies are presented in Table 35.2.

The Effect of L-Arginine Supplementation on Insulin Sensitivity

Arg may affect the endocrine system. It was observed that elevated plasma levels of Arg correlate with alteration in the secretion of numerous cytokines and hormones. Those alterations, in turn, may affect insulin sensitivity and glucose and lipid metabolism [19].

The possible effect of Arg on insulin resistance is presently under discussion, and the results of previous clinical and experimental studies are not clear. The study performed by Wascher et al. indicated an improvement in insulin sensitivity during Arg supplementation. The study group consisted of seven healthy subjects, nine patients with obesity, and nine non-insulin-dependent diabetes mellitus

individuals. In order to assess insulin sensitivity, the authors measured insulin-mediated vasodilatation by venous occlusion plethysmography during the insulin suppression test. Experiments were performed twice on each subject in the presence or absence of a concomitant infusion of Arg (0.52 mg/ kg/min). The authors concluded that insulin sensitivity was improved significantly in all three groups by the infusion of Arg [20]. During the long-term administration of Arg (9 g/day), improvements in peripheral and hepatic insulin sensitivity, acting through a normalization of the NO/cGMP pathway, have also been observed in diabetic patients [21]. Amelioration of insulin sensitivity was also emphasized by Luccotti et al. [13]. Other studies have shown that, in patients with cardiovascular disease, supplementation with Arg (6.4 g/day) serves to increase the insulin sensitivity index and adiponectin level. Following 6 months of treatment, a decrease in interleukin-6 and monocyte chemoattractant protein-1 levels was observed [22]. Suliburska et al. also provided evidence of the impact of Arg on insulin sensitivity in obese patients. Arg treatment (9 g/day) resulted in a significant increase in insulin sensitivity [16]. The beneficial effect of Arg on insulin sensitivity in patients with obesity was confirmed by Bogdanski et al. After 6 months of Arg supplementation (9 g/day), significant increases in NO, total antioxidant status (TAS), and insulin sensitivity level were noticed [23]. Interestingly, there are also studies in which improvement in insulin sensitivity during Arg supplementation was not observed [24]. Hormones released from white adipose tissue play a pivotal role in energy partitioning and influence insulin sensitivity [25]. Some clinical observations support the idea that rational manipulation of adipocytokines is a promising avenue for the therapy of obesity and associated metabolic abnormalities [26].

Mechanisms of the Favorable Effects of L-Arginine in Obesity

The mechanism responsible for the beneficial effects of Arg involves NO signaling, mitochondrial biogenesis, the growth of brown adipose tissue, and the regulation of fat metabolic gene expression (Fig. 35.1).

The NO Pathway in Lipid Metabolism

There is growing interest in NO, which is synthesized from Arg in almost all mammalian cells by NO synthase (NOS), in order to regulate energy and lipid metabolism [27]. Studies with knockout endothelial NOS mice have shown the importance of synthase in body fat accumulation [28]. Khedara et al., after feeding rats with the NOS inhibitor L-*N*(omega)nitroarginine for 8 weeks, observed a reduced combustion of body fat, leading to an increase in total body fat. The inhibition of NOS in this study was reversed by the addition of 4 % Arg to the rodent diet [29]. These findings providing evidence that NO may affect adiposity were also confirmed by Fu et al., who indicated that the increase in NO availability by Arg can improve lipolysis, as well as fatty acid and glucose oxidation [4]. It is believed that physiological levels of NO have a beneficial influence on the homeostasis of energy substrates involving fatty acid and glucose oxidation and affect energy metabolism in the whole body [30]. The underlying mechanism behind the stimulation of oxidation of the energy substrates may involve multiple cyclic guanosine monophosphate (cGMP)-dependent pathways in insulin-sensitive tissues [31]. Dai et al. highlighted the following functions of NO [30]. NO stimulates the phosphorylation of AMPK, which acts as a sensor of cellular energy. AMPK is activated by a rise in the intracellular AMP/adenosine triphosphate (ATP) ratio within the cell [32] and influences glucose transport by increasing GLUT4 translocation. It also decreases concentrations of malonyl-CoA (thus increasing the transport of long-chain fatty acids into mitochondria) and decreases the expression of genes related
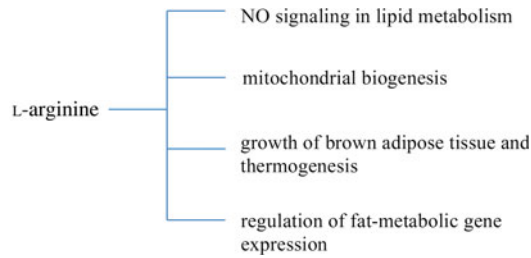
Fig. 35.1 Mechanism of the effects of L-arginine in obesity

to lipogenesis and gluconeogenesis. NO plays an important role in increasing the phosphorylation of hormone-sensitive lipase (HSL). HSL, which is considered to be a regulatory enzyme in lipolysis [33], is activated by cGMP-dependent protein kinase (PKG). This activation results in the translocation of the lipase to neutral lipid droplets and the stimulation of lipolysis in white adipose tissue [31]. NO is also a modulator of PGC-1α. The activation of PGC-1α by NO increases mitochondrial markers, demonstrating the induction of biosynthesis of functional mitochondria able to generate ATP via oxidative phosphorylation [34, 35]. The systemic role of NO (including interorgan cooperation) is to stimulate blood flow (enhancing the transport of fatty acids, glucose, and oxygen to insulin-sensitive tissues), promoting substrate uptake and product removal via circulation and improving mitochondrial oxidation of energy substrates [31, 36]. Arg increases blood flow to organs and thus enhances the uptake of energy substrates for oxidation and ATP production in various tissues in the body. This hemodynamic improvement is at least partly via NO-mediated mechanism. NO causes vasodilatation in blood vessels, and consequently blood pressure decreases and blood flow is improved. Moreover, hemodynamics may lead to improved exercise capacity, and it enables increased physical activity, which is an important aspect of the nonpharmacological treatment of obesity [12].

Mitochondrial Biogenesis

Mitochondria are sources of energy in their role producing ATP for cell metabolism. They are the major organelles for the complete oxidation of energy substrates and play a pivotal role in modifying adipocyte lipid metabolism and adipogenesis [31, 37]. It is known that PGC-1α, whose expression is influenced by NO [4], can induce mitochondrial biogenesis [38, 39]. During in vitro studies and experimental studies conducted on eNOS knockout mice, researchers have emphasized the crucial role of the NO-cGMP-dependent pathway for mitochondrial biogenesis and body energy balance [28, 35]. It is worth noting that Arg may upregulate nuclear transcription factors 1 and 2 expression and thereby enhance mitochondrial biogenesis [40]. An analysis of previous studies provides compelling evidence that Arg can stimulate mitochondrial biogenesis and brown adipose tissue development, while improving whole-body energy metabolism [19, 41] (Fig. 35.2).

Brown Adipose Tissue and Thermogenesis

Brown adipose tissue plays a crucial role in the oxidation of glucose, fatty acids, and some amino acids. It is also responsible for nonshivering thermogenesis in mammals. In contrast to white adipose tissue, brown adipose tissue consists of small brown adipocytes, containing a much greater number of
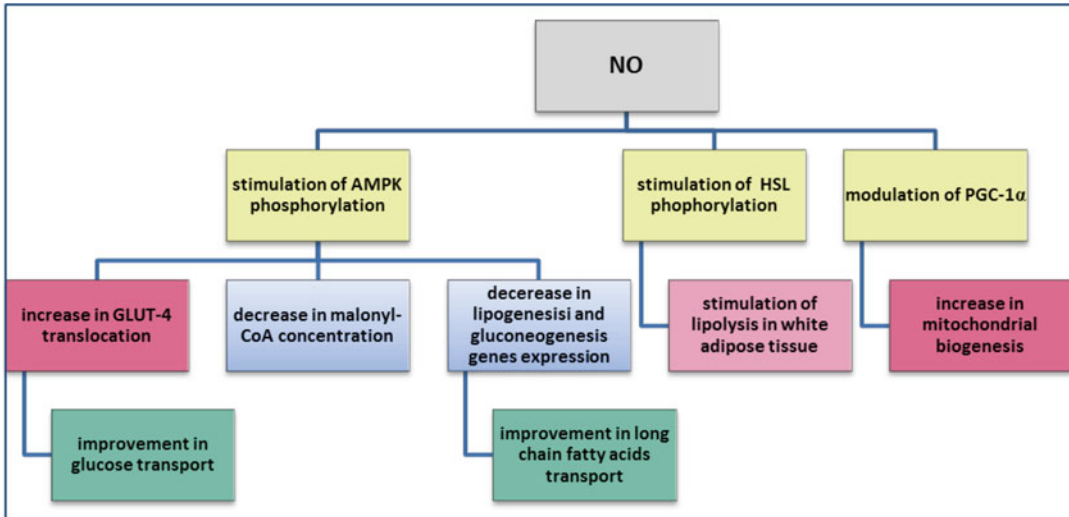
Fig. 35.2 Mechanism of NO to regulate energy, lipid, and glucose metabolism

mitochondria than in white adipose tissue. Mitochondria are also greater in size in brown adipose tissue [42, 43]. Brown adipose tissue is highly vascularized with blood vessels and can produce more heat than adipose tissue and other organs [44]. Interestingly, current evidence shows that brown adipose tissue exists in adult humans and can also play an important role in overall energy expenditure and heat production in adults, not just in neonates [45]. It has also been observed that brown adipose tissue activity is reduced in overweight and obese humans and is positively correlated with resting metabolic rate [46].

It has been observed in experimental and clinical studies that dietary Arg supplementation increases brown adipose tissue mass and mitochondrial biogenesis and thus ameliorates obesity. Observation of ZDF and DIO rats indicates that Arg supplementation significantly reduces white adipose tissue and increases brown adipose tissue mass [2, 4, 6]. These results are consistent with those obtained using growing–finishing pigs. During this study, it was indicated that lipid metabolism varies with the anatomical location of white adipose tissue [7]. Studies on animal models and studies with type 2 diabetes patients indicated the role of Arg in brown adipose tissue alteration [13]. Emerging reports propose that dietary supplementation with Arg stimulates brown adipose tissue growth and development through enhanced syntheses of NO, polyamines, and cyclic AMP [41].

Regulation of Fat Metabolic Gene Expression

A growing body of evidence emphasizes the role of Arg in the regulation of expression of various genes. Jobgen et al. analyzed global changes in gene expression by microarray in DIO rats supplemented with Arg. After 12 weeks of supplementation, they found that high-fat feeding decreased mRNA levels for lipogenic enzymes, AMPK, glucose transporters, HO-3, glutathione synthetase (GSS), superoxide dismutase 3, peroxiredoxin 5, glutathione peroxidase 3, and stress-induced protein. Conversely, the transcripts for carboxypeptidase A, peroxisome proliferator-activated receptor (PPAR)-α, caspase 2, caveolin 3, and diacylglycerol kinase were found to be upregulated in this study. Administration of Arg reduced mRNA levels for fatty acid-binding protein 1, glycogenin, protein

phosphatase 1B, caspases 1 and 2, and hepatic lipase but increased expression of PPARγ, HO-3, GSS, insulin-like growth factor 2, sphingosine-1-phosphate receptor, and stress-induced protein [40]. Changes in lipid metabolic gene expression have also been observed in studies conducted on pigs. Dietary Arg supplementation increased mRNA levels for HSL and decreased mRNA levels for lipoprotein lipase, GLUT4, and ACCα in subcutaneous adipose tissue. There were higher mRNA levels for fatty acid synthase in the skeletal muscle of Arg-supplemented pigs, compared to the control. These findings reveal an upregulation of lipogenic gene expression in skeletal muscle and a downregulation of lipogenic genes and an increase in lipolytic gene expression in white adipose tissue [5]. Together, these results indicate that Arg beneficially modulates gene expression to enhance energy substrate oxidation and to reduce white fat accretion in tissues [3].

Conclusions and Future Perspectives

Arg supplementation may represent a safe and efficient nutritional treatment for obesity. It has been demonstrated that Arg is stable under sterilization conditions and is not toxic to mammalian cells. A level of 85 mg/kg body mass of Arg is physiologically attainable when the human diet is supplemented with this amino acid. The Arg supplementation should be taken in divided doses each day to prevent gastrointestinal tract discomfort through the production of large amounts of NO, to increase the availability of circulating Arg over a longer period of time, and to avoid a potential imbalance among dietary amino acids [3].

Observations from recent studies indicate that Arg supplementation markedly reduces obesity in humans and animals. It beneficially alters hemodynamics in white adipose tissue and brown adipose tissue by increasing the oxidation of energy substrates. Studies have shown that Arg improves insulin sensitivity. Although a number of biochemical and molecular mechanisms have been proposed to explain the role of Arg in metabolism, the likely mechanisms are stimulation of NO signaling, mitochondrial biogenesis, growth of brown adipose tissue, regulation of fat metabolic gene expression, and fat tissue endocrine secretion. There are very promising reports, especially considering the good tolerance of Arg, its availability, and the low cost of treatment. Arg is inexpensive and readily available from natural foods. It seems that careful modulation of the Arg metabolic pathway through dietary supplementation may be beneficial in preventing and treating obesity, a problem that is currently growing worldwide. Although the present findings are very promising, further studies are necessary on the potential therapeutic role of Arg in obesity (Fig. 35.3).
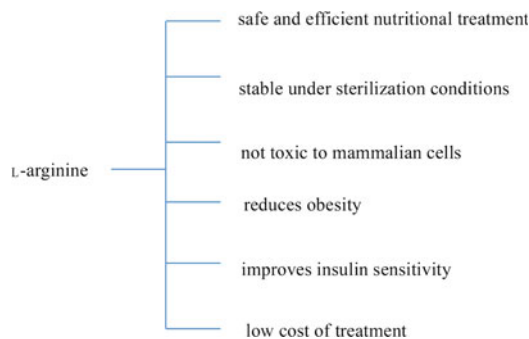
Fig. 35.3 Benefits to use L-arginine

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