



# The effect of L-arginine and ascorbic acid on the visceral fat and the concentrations of metalloproteinases 2 and 9 in high-fat-diet rats

Wpływ L-argininy i kwasu askorbinowego na zawartość tłuszczu trzewnego oraz stężenia metaloproteinaz 2 i 9 u szczurów karmionych dietą wysokotłuszczową

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## Abstract

**Introduction:** L-arginine (L-arg) and vitamin C supplementation may decrease fat accumulation and have a favourable effect on carbohydrate metabolism. This is partly caused by matrix metalloproteinases (MMPs), which are involved in adipocyte development and remodelling. Our study evaluated the effects of L-arg and vitamin C supplementation on the content of visceral fat (VF%), activity of MMPs, and insulin resistance (IR) in rats fed a high-fat diet (HFD).

**Material and methods:** The experiment was performed using 48 Wistar rats divided into four groups: Group 1 was fed a standard diet, Group 2 a HFD, Group 3 a HFD supplemented with L-arg (A), and Group 4 a HFD supplemented with L-arg and vitamin C (AC). The animals were euthanized after six weeks. The concentrations of serum glucose, insulin, MMP-2, and MMP-9, as well as IR by Homeostatic Model Assessment (HOMA) and VF% were measured.

**Results:** Statistically significant increases in VF%, MMP-2, MMP-9, insulin, and HOMA-IR levels were observed in the HFD group when compared to the control group. A smaller increase in VF%, insulin, and HOMA-IR was seen in Group 3 (A) and 4 (AC). L-arg supplementation protected against increases in MMP-2 and MMP-9 in Group 3 (A) and 4 (AC).

### Conclusions:

1. L-arginine could protect from an increase in visceral fat through a change in the activity of MMPs and amelioration of insulin sensitivity in rats fed a HFD.
2. The addition of vitamin C did not improve the effects of L-arginine supplementation. (*Endokrynol Pol* 2015; 66 (6): 526–532)

**Key words:** L-arginin; visceral fat; metalloproteinases; rats; high-fat diet

## Streszczenie

**Wstęp:** Suplementacja L-argininy (L-arg) i witaminy C może obniżać gromadzenie tłuszczu trzewnego i korzystnie wpływać na metabolizm węglowodanów. Jednym z potencjalnych mechanizmów jest działanie metaloproteinaz (MMP), które uczestniczą w rozwoju i remodelingu tkanki tłuszczowej. Badanie zaprojektowano w celu oceny ochronnego wpływu suplementacji L-arg i witaminy C na zawartość wisceralnej tkanki tłuszczowej, aktywność MMP oraz insulinooporność (IR) u szczurów żywionych dietą bogatotłuszczową.

**Materiał i metody:** 48 szczurów rasy Wistar rats przydzielono do 4 grup: żywionych dietą standardową (kontrola), bogatotłuszczową (HFD), HFD i L-arg (A), HFD, L-arg i witaminą C (AC). Po 6 tygodniach zwierzęta usypiano. Oceniono względną masę tłuszczu trzewnego (VF%), a także oznaczano stężenia glukozy, insuliny, MMP-2 i MMP-9 w osoczu. IR określono przy użyciu HOMA.

**Wyniki:** W grupie HFD obserwowano największy wzrost VF%, wskaźnika HOMA-IR, stężeń insuliny, MMP-2 i MMP-9 w stosunku do grupy kontrolnej, istotnie mniejszy wzrost VF% i stężenia insuliny i HOMA-IR stwierdzono w grupach A i AC. W grupach żywionych dietą wysokotłuszczową z dodatkiem L-arg (A i AC) nie stwierdzono wzrostu stężeń MMP-2 i MMP-9.

### Wnioski:

1. Suplementacja L-argininą może chronić szczury żywione dietą bogatotłuszczową przed gromadzeniem tłuszczu trzewnego poprzez wpływ na aktywność metaloproteinaz oraz poprawę insulinooporności.
2. Dodatek witaminy C nie poprawia efektu suplementacji L-argininy. (*Endokrynol Pol* 2015; 66 (6): 526–532)

**Słowa kluczowe:** L-arginina; tłuszcz trzewny; metaloproteinazy; szczury; dieta wysokotłuszczowa



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## Abbreviations

ANOVA — analysis of variance  
 HFD — high-fat diet  
 HOMA — homeostasis model assessment  
 IR — insulin resistance  
 IGF-1 — insulin-like growth factor 1  
 IVGTT — intravenous glucose tolerance test  
 L-arg — L-arginine  
 L-NAME — L-NG-Nitroarginine Methyl Ester  
 mRNA — messenger ribonucleic acid  
 MMP — metalloproteinase  
 NO — nitric oxide  
 NOS — nitric oxide synthase  
 OLETF rats — Otsuka Long-Evans Tokushima Fatty rats  
 VF% — percentage of visceral fat  
 PI3kinase — phosphoinositide-3-kinase  
 SD — standard diet  
 SEM — standard error of measurement  
 TIMP — tissue inhibitor metalloproteinase

## Introduction

It has been suggested that impaired adipocyte differentiation plays an important role in the pathogenesis of obesity [1], and may be influenced by several potential factors, such as: proinflammatory cytokines, chemokines, hormones, and matricellular proteins.

Increased gene expression of paracrine regulators could be involved in adipocyte differentiation within the stromal vascular fraction of adipose tissue, and may play an important role in obesity.

Matrix metalloproteinases (MMPs) are a family of molecules associated with the breakdown of the components of the extracellular matrix; therefore, MMPs are involved in the development and remodelling of adipose tissue. They are differentially expressed in adipose tissue; in the two genetic models of obesity (*ob/ob* and *db/db* mice) and in the diet-induced model of obesity. The mRNA levels for MMP-2, MMP-3, MMP-12, MMP-14, MMP-19, and tissue inhibitor metalloproteinase-1 (TIMP-1) were strongly increased in adipocytes of obese animals, in comparison with lean tissue. In contrast, MMP-7 and TIMP-3 mRNAs are markedly decreased in obesity [2].

Gelatinases (MMP-2 and MMP-9) are produced by many different vascular cells, including; endothelial cells, podocytes, pericytes, fibroblasts, and macrophages [3]. MMP-2 (gelatinase A, type IV collagenase) is an endopeptidase that degrades the basement membrane of adipocytes. In obesity, it may be responsible for the hypertrophic development of adipocytes and the formation of adipocyte clusters [4]. An elevated expression of MMP-2 has been found in adipose tissue in mouse

models of obesity [5]. In subjects with type-2 diabetes, larger adipocytes were observed than in the control group, and despite their similar adiposity, adipocytes showed a higher expression of matricellular MMP-2 [6]. MMP-9 is secreted by various cells in response to growth factors and cytokines [7]. Plasma levels of MMP-2 and MMP-9 are increased in obese patients [8]. Galardin, an MMP inhibitor administered to mice fed a high-fat (HF) diet, has been found to markedly reduce subcutaneous and gonadal fat deposits [9]. Increased plasma levels of MMP-2, MMP-9, TIMP-1, and TIMP-2 have been reported in type-2 diabetes and acute coronary syndrome [10]; the highest levels of these parameters have been observed in patients with both type-2 diabetes and acute coronary syndrome, reflecting abnormal extracellular matrix metabolism in these diseases [11]. Patients with (untreated) mild hyperlipidaemia have also been found to have increased levels of MMP-2, MMP-9, TIMP-1, and TIMP-2 when compared to the control group [12].

Nitric oxide (NO) is an important biological agent with a number of roles, exerting its effects in neurons, the cardiovascular system, and immune modulation [13]. NO is synthesised from L-arginine (L-arg) through the use of NO synthase. It has been observed that L-arg may decrease the accumulation of fat and favourably affect carbohydrate and lipid metabolism [14, 15].

Vitamin C is an essential dietary nutrient taking part in the biosynthesis of collagen and is a cofactor in the biosynthesis of many important substances, such as cholesterol, catecholamines, amino acids, and certain peptide hormones. Vitamin C is a potent antioxidative agent, it influences mitochondrial function by decreasing the generation of reactive oxygen species (ROS) through stimulation of the activity of superoxide dismutase and glutathione peroxidase and alteration of the activity of the electron transport chain [16]. Vitamin C also has anti-inflammatory properties [17]. Some data indicate that, in rodents, dietary supplementation with vitamin C could prevent fat deposition induced by a HF diet.

Currently there is a lack of research assessing the potential influence of L-arg (with or without the supplementation of vitamin C) on MMP activity in the presence of metabolic disorders caused by HF diets. Our study was designed to examine the influence of L-arg and vitamin C on the content of visceral fat, the serum concentration of MMPs, and insulin resistance in rats fed a high-fat diet.

## Material and methods

The protocol of the study was approved by the local bioethical commission in Poznań (approval no. 20/2011) and was

carried out in accordance with the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education issued by the New York Academy of Sciences Ad Hoc Committee on Animal Research.

### Laboratory animals

Male and female Wistar rats (six weeks old, weighing  $160 \pm 12$  g) were purchased from the Department of Toxicology at the Medical University of Poznań, Poland. All rats were housed individually in carbonate cages in a temperature and humidity-controlled room on a 12-hour light and 12-hour dark cycle, designed for the purpose of the study. The temperature inside the rat cages was  $21 \pm 2^\circ\text{C}$ , and the relative humidity was  $60 \pm 5\%$ .

### Experimental design

The experiment was performed using 48 Wistar rats. After a five-day period of adaptation to the laboratory conditions, the rats were randomly divided into four equal groups (male-to-female ratio 1:1):

- Group 1 (control, SD;  $n = 12$ ): untreated rats, were allowed free access to a standard diet;
- Group 2 (high-fat diet, F;  $n = 12$ ): were fed a high-fat diet;
- Group 3 (arginine group, A;  $n = 12$ ): were fed a high-fat diet supplemented with L-arginine at a dose of 20 g/kg diet;
- Group 4 (arginine and vitamin C group, AC;  $n = 12$ ): were fed a high-fat diet supplemented with L-arginine at a dose of 20 g/kg diet and vitamin C at 100 mg/kg diet.

The animals were fed a standard semisynthetic diet based on the AIN-93M diet [18] or a high-fat diet with modified amounts of fat and sodium chloride. The components of the diet were obtained partly from Sigma-Aldrich Sp. z o. o. and Merck Sp. z o. o. (vitamins, minerals and sodium chloride), Hortimex Plus Sp. z o. o. (casein and starch), and a local market (lard, sunflower oil, sucrose). The full composition of both diets is presented in Table I. All rats were provided ad libitum diet and distilled water for 42 days. Food intake was measured daily and weight changes were monitored weekly. The rats were under veterinary supervision during the whole course of the experiment.

### Dose, form, and route of administration of L-arg and vitamin C

The diet was supplemented with either L-arg or L-arg combined with vitamin C for six weeks. L-arg was administered at a dose of 20 g/kg diet (Curtis Healthcare, Warsaw, Poland) and vitamin C at a dose of 100 mg/kg diet (Biofarm, Poznań, Poland). In this type of experimental study, these are considered to be low standard doses of L-arginine and vitamin C. The appropriate

**Table I. Ingredient and nutrient composition of the diets (grams per kilogram diet)**

**Tabela I. Składniki odżywcze i skład diety (gramy na kilogram diety)**

Ingredient	Standard diet	High-fat diet
Casein	140	140
Wheat starch	625	430
Sucrose	100	100
Potato starch	50	50
Vitamin mixture*	10	10
Mineral mixture**	35	35
Sunflower oil	40	40
Lard	–	160
Sodium chloride	–	35
Total energy (kcal/100g diet)	420	515
Total protein (% of energy)	18	18
Total fat (% of energy)	9	39

\*composition of vitamin mixture (g/kg mix): nicotinic acid (3), Ca pantothenate (1.6), pyridoxine (0.7), thiamine (0.6), riboflavin (0.6), folic acid (0.2), biotin (0.02), vitamin B12 (0.003); vitamin E (500 IU/g), vitamin A (500,000 IU/g), vitamin D3 (400,000 IU/g), vitamin K1 (0.08), choline bitartrate (200), powdered sucrose (777.15); \*\*composition of mineral mixture (g/kg mix): calcium carbonate (357), potassium phosphate monobasic (250), potassium citrate (28), sodium chloride (74), potassium sulphate (46.6), magnesium oxide (24), ferric citrate (6.06), zinc carbonate (1.65), sodium meta-silicate  $9\text{H}_2\text{O}$  (1.45), manganese carbonate (0.64), cupric carbonate (0.30), chromium chloride  $6\text{H}_2\text{O}$  (0.0578), boric acid (0.0816), sodium fluoride (0.0635), nickel chloride  $6\text{H}_2\text{O}$  (0.0578), lithium sulfate  $\text{H}_2\text{O}$  (0.0263), sodium selenate anhydrous (0.0103), potassium iodate (0.010), ammonium paramolybdate  $4\text{H}_2\text{O}$  (0.0795), ammonium vanadate (0.066), powdered sucrose (209.758)

value of wheat starch was removed from the diets, 20 g in the case of L-arginine supplementation and 100 mg for the diet supplemented with vitamin C.

### Collection of material

At the end of the experiment (day 42), after 16 hours of fasting, the rats were weighed, anaesthetised by an intraperitoneal injection of thiopental (40 mg/kg body mass), and euthanised by cardiac puncture. The blood samples were collected in serum separation tubes, and subsequently used for biochemical studies. The coagulated blood was left to clot at room temperature for 30 minutes; then it was centrifuged for 15 minutes at 2500 r.p.m. at a temperature of  $4^\circ\text{C}$ ; and finally the supernatant fluid was separated. The serum samples used for analysis were stored at  $-70^\circ\text{C}$ . Visceral perirenal fat was dissected, weighed, and frozen rapidly at  $-70^\circ\text{C}$ .

### Examined parameters

In all animals, weight gain and the absolute and relative weights of visceral fat were determined. The relative weight of visceral fat was defined as the percentage of the body weight.

**Table II.** Diet intake, initial body mass, and effects of dietary L-arginine with and without vitamin C supplementation on weight gain and visceral fat tissue**Tabela II.** Wpływ suplementacji L-argininą i witaminą C na przyrost masy ciała i zawartość tkanki tłuszczowej trzewnej u szczurów, żywionych dietą bogatotłuszczową

Groups		Parameters				
		Number of rats	Diet intake [g/day]	Initial body mass [g]	Weight gain [g]	Visceral fat/percentage of body mass (%)
Group 1	Control	12	18.9 ± 1.2	160.1 ± 14.2	115.7 ± 12.0	0.95 <sup>a</sup>
Group 2	High fat diet	12	18.3 ± 1.0	159.5 ± 12.8	116.6 ± 13.2	1.23 <sup>b</sup>
Group 3	L-arginine	12	18.4 ± 1.1	162.4 ± 12.5	111.5 ± 15.1	1.10 <sup>ab</sup>
Group 4	L-arginine and vitamin C	12	18.4 ± 1.3	161.3 ± 13.1	105.6 ± 18.3	1.07 <sup>ab</sup>

Values are means ± SEM for twelve rats, a, b significant differences between all groups (Anova test,  $p < 0.05$ )

### Laboratory measurements

Fasting serum glucose concentration was analysed by an enzymatic method involving hexokinase and glucose-6-phosphate dehydrogenase (Siemens Healthcare Diagnostics, Erlangen, Germany).

The plasma concentration of insulin was determined by an enzyme-linked immunoassay, in strict accordance with the manufacturer's instructions (Demeditec Diagnostic GmbH, Kiel-Wellsee, Germany).

Insulin resistance was estimated by Homeostasis Model Assessment (HOMA), according to the formula: Insulin resistance index = fasting insulin ( $\mu\text{g/L}$ )  $\times$  fasting glucose ( $\text{mg/dL}$ )/405.

Serum MMP-2 and MMP-9 protein levels were determined using commercially available quantitative sandwich ELISA kits obtained from EIAab (Wuhan, China).

### Statistical analysis

Results are shown as mean  $\pm$  SEM. Continuous variables were assessed for normal distribution with the use of the Shapiro-Wilk test. Differences between means were analysed by ANOVA, followed by Tukey's post-hoc test. A P value of less than 0.05 was regarded as statistically significant. All calculations and demographics were performed with Statistica software (version 6.0 for Windows).

### Results

The average dietary intake, initial body weight, and weight gain were comparable in all groups (Table II). The relative mass of visceral fat (as a percentage of body mass) was significantly higher in rats fed a high-fat diet, as compared to the control group. Rats fed a HF diet supplemented with L-arg or a combination of L-arg and vitamin C showed smaller increases in relative visceral fat.

The results presented in Table III demonstrate a statistically significant increase ( $P < 0.05$ ) in serum insulin and HOMA-IR levels, as well as in MMP-2 and MMP-9, in rats fed a HF diet. L-arg supplementation (alone or combined with vitamin C) protected against increases in MMP-2 and MMP-9, and partially protected against increases in the concentration of insulin and HOMA-IR levels.

### Discussion

A decreased accumulation of visceral fat was observed after L-arg supplementation, a new finding discovered during our study, which could be related to diminished MMP-2 and MMP-9 serum concentrations. Furthermore, our study confirms the beneficial influence of L-arg on insulin resistance.

Our study demonstrates that the relative mass of the visceral fat (as a percentage of body mass) was significantly higher in rats fed a high-fat diet than in the rats from the control group. Both L-arg as well as the combination of L-arg with vitamin C diminished the relative mass of visceral fat comparably. Dietary supplementation with L-arg resulted in white-fat mass reduction in Zucker diabetic rats and in diet-induced obese rats; this fact has been confirmed by several studies [14, 15]. In Zucker diabetic rats, L-arg supplementation reduced the epididymal and abdominal fat deposits by 28% and 46%, respectively. The weight of other organs remained unchanged. Supplementation with citrulline (a precursor of L-arg) also decreased the amount of fat [19]. The beneficial influence of L-arg supplementation on fat accumulation may also be associated with the role of nitric oxide (NO) in the regulation of fatty acid metabolism, glucose, and amino acids in all mammals, as well as in rats. Physiological concentrations of NO increase fatty acid

**Table III.** Effects of dietary L-arginine with and without vitamin C supplementation on serum insulin concentration, resistance index (HOMA-IR), and MMP-2 and MMP-9 serum concentration in high-fat-diet rats**Tabela III.** Wpływ suplementacji L-argininą i witaminą C na wskaźnik insulinooporności HOMA-IR, stężenia insuliny, MMP-2 i MMP-9 u szczurów, żywionych dietą bogatołuszczową

Groups		Parameters			
		Insulin [ $\mu\text{g/L}$ ]	HOMA-IR	MMP-2 [ng/mL]	MMP-9 [ng/mL]
Group 1	Control	0.31 $\pm$ 0.05 <sup>a</sup>	0.08 $\pm$ 0.01 <sup>a</sup>	1.64 $\pm$ 0.72 <sup>a</sup>	4.55 $\pm$ 1.87 <sup>a</sup>
Group 2	High fat diet	0.62 $\pm$ 0.07 <sup>c</sup>	0.16 $\pm$ 0.03 <sup>c</sup>	2.42 $\pm$ 0.90 <sup>b</sup>	7.00 $\pm$ 2.15 <sup>b</sup>
Group 3	L-arginine	0.46 $\pm$ 0.08 <sup>b</sup>	0.11 $\pm$ 0.02 <sup>b</sup>	1.55 $\pm$ 1.00 <sup>a</sup>	4.85 $\pm$ 2.33 <sup>a</sup>
Group 4	L-arginine and vitamin C	0.41 $\pm$ 0.05 <sup>b</sup>	0.10 $\pm$ 0.01 <sup>b</sup>	1.53 $\pm$ 0.82 <sup>a</sup>	5.13 $\pm$ 1.69 <sup>a</sup>

Values are means  $\pm$  SEM for twelve rats, HOMA-IR—insulin resistance index, MMP-2 — metalloproteinase 2; MMP-9 — metalloproteinase 9; <sup>a,b,c</sup>significant differences between all groups (Anova test,  $p < 0.005$ )

oxidation in skeletal muscle, liver, and adipose tissue, while decreasing the synthesis of lipids in liver and adipose tissue. A possible explanation of the molecular relationship between NO and the amount of fat is the variable regulation of gene expression, affecting oxidation, fat accumulation, and adipocyte differentiation [20]. Therefore, dietary L-arg supplementation reduces the size, although not the number, of adipocytes in obese rats [14, 15].

An important finding of our study is that the beneficial effect of L-arg on visceral fat could be explained by a decrease of MMPs activity. In the group of rats fed a HF diet, a significant increase in visceral fat content was associated with elevated levels of MMP-2 and MMP-9, while supplementation of L-arg protected the rats fed a HF diet from increases in visceral fat and MMP concentration. It is possible that inhibition of MMPs activity, induced by L-arg, protected the HF-diet-fed rats from an increase in visceral fat. This potential beneficial effect of MMPs on adipose tissue was shown in earlier studies. In mice, after 15 weeks of a high-fat diet, the body weight gain in Ro 28-2653 (a synthetic MMP-inhibitor treated group) was found to be lower, and the amounts of the isolated subcutaneous or gonadal fat deposits were diminished by 10–15%. Moreover, the number of adipocytes in adipose tissue was higher (at 10–17%), but their diameter was smaller (by about 10%) [21]. Another MMP inhibitor (tolylsam), administrated to obese wild-type mice fed a HF diet, reduced total, subcutaneous, and gonadal adipose tissue [22]. *De novo* development of fat in mice injected with preadipocytes and kept on a HF diet was significantly impaired upon treatment with tolylsam; which was also responsible for the preservation of collagen and the reduction of blood-vessel size in adipose tissue [23]. A possible explanation for this is that MMPs degrade the basement membrane of adipocytes, and are responsible for hypertrophic development of adipocytes and the formation of adipocyte clusters.

Another explanation of the beneficial influence of L-arg on the visceral fat content in rats is its role in the regulation of carbohydrate metabolism and insulin sensitivity. We demonstrated a significant increase in serum insulin and HOMA-IR levels in rats fed a HF diet. L-arg alone and in combination with vitamin C partially decreased the insulin concentration and the HOMA-IR level when compared to the rats fed a HF diet. The results of a study by Alam et al. showed that L-arg supplementation in Wistar rats improved glucose tolerance while decreasing blood pressure, abdominal fat deposits, and oxidative stress [24]. On the other hand, NO synthesis inhibitors, such as NG-nitro-L-arginine methyl ester (L-NAME), decrease insulin sensitivity and lipid metabolism, while the addition of L-arg reverses this effect. Moreover, a significant decrease in insulin sensitivity and plasma levels of postprandial triglyceride was detected by chronic treatment of L-NAME in diabetic rats [15]. It has been shown that short-term L-arginine supplementation in rats with ischemia-reperfusion syndrome causes a decrease in the insulin binding capacity of insulin receptors in skeletal muscle, and an increase in insulin level [25]. Newer studies show a pathophysiological role of NO in obesity-related insulin resistance; however, the results have been controversial. After 12 weeks of either a standard diet (SD) or a HF diet, alone or combined with L-NAME (100 mg/kg/d), it was found that L-NAME treatment significantly attenuated body-weight gain in both groups of mice without affecting calorie intake. Chronic NOS blockade by L-NAME in mice ameliorated HF diet induced adiposity and glucose intolerance; it also reduced adipose inflammation and improved insulin signalling in skeletal muscle [26].

The favourable effect of L-arg on insulin resistance could be explained partly by its positive influence on MMPs. MMP concentrations are elevated in hyperinsulinaemia and insulin resistance. Boden et al. observed the influence of elevated levels of insulin and FFA on

MMPs and TIMP in the aortic tissue of male rats during euglycaemic-hyperinsulinaemic clamping. Hyperinsulinaemia led to increases in MMP-2 (approximately six-fold), MMP-9 (approximately 13-fold), but did not affect tissue inhibitors of MMPs [27]. Monti et al. demonstrated that sucrose-induced insulin resistance increased the expression of cardiac MMP-2 by a factor of 2.4, and of MMP-9 by a factor of 10.5, compared to Sprague-Dawley rats fed a standard diet. The explanation that was discussed focused on insulin or insulin growth factor-1 (IGF-1) signalling PI3 kinase activation and the subsequent stimulation of MMPs [28].

Surprisingly, when L-arg supplementation was combined with vitamin C, we did not observe any effect on the levels of MMPs, the amount of visceral fat, insulin concentration, or HOMA when compared to L-arg supplementation alone. The effect of vitamin C has been consistently demonstrated in several studies; dietary supplementation with ascorbic acid reduces body weight and the amounts of retroperitoneal and subcutaneous fat deposits in cafeteria diet-induced obese rats, but our study did not confirm this finding [29]. The synergic action of dietary supplementation with phytosterol and ascorbic acid has been shown to reduce body mass accumulation and alter food transit time in a diet-induced-obesity mouse model [30]. Other studies also confirmed the advantageous effect of vitamin C on MMP. In pigs fed a high-cholesterol diet [31] the supplementation of vitamins C and E markedly prevents lipid peroxidation, increases neointimal collagen content, and reduces hypercholesterolaemia-induced changes in vascular MMP-1. The fibroblasts of aged human skin cultured in fragments, manifest raised intracellular oxidant levels; conversely, treatment with antioxidant MitoQ (10) significantly reduces MMP-1 expression [32]. The underlying mechanisms that have been discussed include: upregulation of the expression of genes involved in cell proliferation, regulation of transcription, as well as downregulation of genes involved in lipid metabolism, cell adhesion, and differentiation. A possible explanation for the lack of an effect of vitamin C combined with L-arg on MMP levels and insulin concentration, found in our study, is that the accumulation of vitamin C oxidation products may predispose tissues toward destabilisation or damage. This occurs partly through increased advanced glycation, especially under disadvantageous conditions such as diabetes, end-stage renal disease, or overalimentation [33].

## Clinical relevance

In this study, we showed that L-arg supplementation, whether alone or in combination with vitamin C, protects rats fed a HF diet against increases in MMP-2,

MMP-9, and visceral fat accumulation. Additionally, we confirmed the favourable effect of L-arg on insulin resistance. Although neither the decrease in serum concentrations of MMPs nor the augmentation of insulin sensitivity completely explains the beneficial effect of L-arg and vitamin C on visceral fat accumulation. Identification of contributing factors may help in the prevention of obesity and its consequences, such as atherosclerosis or inflammation.

## Limitations

The activation of MMPs in different organs and tissues is regulated by NO through many potential mechanisms [34–39]. This relationship is described as being either organ-specific or tissue-specific [40–42]. We did not evaluate the levels of MMPs in adipose tissue; therefore, we could not explain how a change in fat mass, with a subsequent alteration in local environment, influences circulating blood levels of these proteins. Another limitation of this study was the absence of a group fed with a HF diet supplemented with vitamin C alone. Higher doses of L-arg and vitamin C, as well as a longer treatment period, could also influence the results more significantly. As our study is experimental, future studies that aim to verify its results should be performed in humans.

## Conclusions

1. L-arginine protects rats fed a high-fat diet from an increase of visceral fat, possibly through a change in MMP-2 and MMP-9 activity and amelioration of insulin sensitivity.
2. The addition of vitamin C did not improve the beneficial effects of L-arginine supplementation.

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