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ORIGINAL CONTRIBUTION

Effects of lipoic acid on AMPK and adiponectin in adipose tissue of low- and high-fat-fed rats

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Abstract
Background Lipoic acid (LA) is an antioxidant with antiobesity and antidiabetic properties. Adiponectin is an adipokine with potent anti-inflammatory and insulin-sensitizing properties. AMP-activated protein kinase (AMPK) is a key enzyme involved in cellular energy homeostasis. Activation of AMPK has been considered as a target to reverse the metabolic abnormalities associated with obesity and type 2 diabetes.
Aim of the study The aim of this study was to determine the effects of LA on AMPK phosphorylation and adiponectin production in adipose tissue of low-fat (control diet) and high-fat diet-fed rats.
Results Dietary supplementation with LA reduced body weight and adiposity in control and high-fat-fed rats. LA also reduced basal hyperinsulinemia as well as the homeostasis model assessment (HOMA) levels, an index of insulin resistance, in high-fat-fed rats, which was in part independent of their food intake lowering actions. Furthermore, AMPK phosphorylation was increased in white adipose tissue (WAT) from LA-treated rats as compared with pair-fed animals. Dietary supplementation with LA also upregulated adiponectin gene expression in WAT, while a negative correlation between adiposity-corrected adiponectin levels and HOMA index was found. Our present data suggest that the ability of LA supplementation to prevent insulin resistance in high-fat diet-fed rats might be related in part to the stimulation of AMPK and adiponectin in WAT.

Keywords Lipoic acid · Obesity · Adiponectin · AMPK

Abbreviations

AMPK	AMP-activated protein kinase
HOMA	Homeostasis model assessment
LA	Lipoic acid
LASY	Lipoic acid synthase
TZDs	Thiazolidinediones
WAT	White adipose tissue
BAT	Brown adipose tissue

Introduction

Obesity is a complex disease affected by genetic and environmental factors [1]. Obesity is currently reaching epidemic levels worldwide and is a major predisposing factor for a variety of life-threatening diseases including type 2 diabetes and hypertension, which are major components of the metabolic syndrome [2–4]. Obesity involves a state of chronic low-grade inflammation and oxidative stress, which predisposes to some obesity-related comorbidities [5–7].

LA is a naturally occurring short chain fatty acid, present in plants and animals, and synthesized by lipoic acid synthase (LASY) within the mitochondria [8, 9]. It has been shown that downregulation of LASY reduced endogenous levels of LA as well as critical components of the antioxidant defense network. It also increased oxidative

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stress, which, in turn, leads to increased insulin resistance, mitochondrial dysfunction, and inflammation. Moreover, LASY gene expression is downregulated in animal models of diabetes and obesity both in skeletal muscle and adipose tissue [10]. Thus, LA has been proposed as a novel therapeutic approach for chronic inflammatory diseases such as diabetes and obesity. Several studies have demonstrated the potent anti-obesity properties of LA in rodents through the inhibition of hypothalamic adenosine monophosphate-activated protein kinase (AMPK) activity in hypothalamus, which leads to decreased food intake, and stimulated energy expenditure [11–13]. More importantly, two recent trials in humans have also supported that LA is a promising antioxidant candidate for the therapy of obesity-related diseases [14, 15].

White adipose tissue (WAT) is an important endocrine organ that secretes several immunomodulators and bioactive peptides termed adipokines [16, 17]. Dysregulated adipokine secretion from the expanded WAT of obese individuals contributes to the development of systemic insulin resistance and metabolic disease [2]. Adiponectin is one of the adipocyte-derived adipokines with potent lipid-lowering, anti-inflammatory, and insulin-sensitizing properties. Circulating levels of adiponectin are decreased in obesity and increased after weight loss [18]. Thus, hypoadiponectinemia then mediates the metabolic effects of obesity on the other peripheral tissues, such as liver and skeletal muscle [19]. Circulating adiponectin levels are increased by many commonly used insulin-sensitizing molecules such as thiazolidinediones (TZDs) and n-3 polyunsaturated fatty acids [20, 21]. Therefore, stimulation of adiponectin could be a potential mechanism contributing to the anti-obesity and anti-diabetic properties of LA. However, this possibility remains unclear since there are few trials that have determined changes in adiponectin after LA supplementation and data obtained up to now are controversial reporting increase, no change, or even decrease in adiponectin levels [22–24]. Therefore, further studies are needed to get a better knowledge about the effects of LA actions on adiponectin in animal models of obesity and type 2 diabetes.

AMPK is a key enzyme involved in cellular energy homeostasis. AMPK stimulates pathways that increase energy production (glucose transport and fatty acid oxidation) and switches off pathways that consume energy (lipogenesis and gluconeogenesis) [25]. Changes in AMPK activity have been reported in obesity, type 2 diabetes, the metabolic syndrome, and cardiovascular disease [26, 27]. AMPK has also a broader role in metabolism through the control of appetite. Regulation of AMPK activity at the whole-body level is coordinated by a growing number of hormones including adiponectin and leptin. Furthermore, activation of AMPK by bioactive food components or drugs has been considered as target to reverse the metabolic abnormalities associated with obesity and type 2 diabetes [28].

Several investigations have analyzed the effects of LA on AMPK in different tissues including the hypothalamus, liver, muscle, and pancreas [13, 29–31]. The role of AMPK in mediating the metabolic actions of LA is complex and seems to be tissue-dependent. However, there is no information regarding the ability of LA to modulate AMPK in adipose tissue under an obesogenic environment (high-fat diet).

The objective of this study was to determine the effects of dietary supplementation with LA on insulin-sensitivity markers and on adiponectin production, as well as on AMPK in WAT, brown adipose tissue (BAT), and muscle in control and high-fat-fed rats.

Materials and methods

Animal and diets

Six-week-old male Wistar rats ($n = 54$) were obtained from the Centre of Applied Pharmacology (CIFA, Pamplona, Spain). Animals were housed in polycarbonate cages (3–4 rats per cage) in a temperature-controlled room (22 ± 2 °C) with a 12 h light–dark cycle, fed a pelleted chow diet, and given deionized water *ad libitum* for an adaptation period of 5 days.

Afterward, rats were assigned to two dietary groups: 1) Animals receiving normal chow standard diet (Harlam Tekland Global Diets) containing 20 % of energy as proteins, 67 % as carbohydrates, and 13 % as lipids per dry weight; 2) Animals receiving a high-fat diet (OpenSource diets Research Diets Inc) containing 60 % of energy as lipids, 20 % as carbohydrates, and 20 % as proteins per dry weight, which has been widely used to induce obesity in rodents [32]. The two dietary groups were assigned into different experimental subgroups: (a) Control group which was fed *ad libitum* with the standard chow diet, (b) the CLIP group fed *ad libitum* with the standard chow diet supplemented with racemic α-Lipoic acid (Sigma, St Louis, MO) in a proportion of 0.25 g LA/100 g of diet, (c) the Obese group fed *ad libitum* with the high-fat diet, and (d) the OLIP group fed *ad libitum* with the high-fat diet supplemented with LA (0.25 g LA/100 g). LA was thoroughly and homogeneously mixed with both diets (chow and high-fat) using a blender. Two pair-fed groups (PF-CLIP and PF-OLIP) receiving the same amount of food ingested by the groups CLIP or OLIP, respectively, but without LA supplementation were also included in the experimental design. These two groups are necessary to distinguish what proportion of the LA actions is independent of LA effects on food intake. Body weight and food intake were recorded every 2–3 days before the onset of

the dark period. At the end of the experimental period (56 days), rats were killed by decapitation and blood and tissue samples including WAT depots (epididymal, retroperitoneal, mesenteric, and subcutaneous), BAT depot, and gastrocnemius muscle were collected as previously described [32]. All organs were weighed and kept at −80 °C for subsequent analysis. All experimental procedures were performed according to National and Institutional Guidelines for Animal Care and Use with the approval of the Ethical Committee for Animal Care and Use at the University of Navarra.

Western blotting

In order to determine AMPK levels (total and phosphorylated), epididymal fat, BAT, and skeletal muscle (gastrocnemius) from LA-treated and untreated control and high-fat-fed rats were lysed in cold-lysis buffer 50 mM Tris HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 2 mM NaF, 1 % protease inhibitor cocktail 1 (Sigma), 2 mM Sodium orthovanadate, 1 % Triton x-100, 0.25 % sodium deoxycholate, and 6 mM octil-β-glucopiranoside. Western blot assays were performed [33] using antibodies specific for phospho-AMPKα (Thr172) and AMPKα (Cell Signaling, MA). The results were densitometrically analyzed using a GS-800 calibrated densitometer (Bio-Rad Laboratories).

Adiponectin assay

Adiponectin levels in rat serum were measured using an ELISA kit from Millipore (Linco Research, Missouri, USA) according to the manufacturer's protocol. The lowest level of adiponectin that can be detected by this assay is 0.155 ng/ml.

Real-time PCR

Total RNA was extracted from epididymal adipose tissue from LA-treated and untreated control and high-fat-fed rats using TRIzol® reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. RNA concentrations and quality were measured by Nanodrop Spectrophotometer 1000 (Thermo Scientific, DE, SA). RNA was then incubated with an RNAse-free kit DNase (Ambion, Austin, TX) for 30 min at 37 °C. Then, RNA (2 μg) was reverse-transcribed to cDNA using MMLV (Moloney murine leukemia virus) reverse transcriptase (Invitrogen). Adiponectin mRNA levels were determined using predesigned TaqMan® Assays-on-Demand (Rn00595250_m1, Applied Biosystems, CA, USA). Taqman Universal Master Mix was also provided by Applied Biosystems. The reaction conditions were followed as described by manufacturer's instructions. Amplification and detection of specific products were performed using the ABI PRISM 7900HT (Applied Biosystems). Adiponectin mRNA levels were normalized using Cyclophilin (Rn00690933_m1, Applied Biosytems) as housekeeping gene. All samples were analyzed in duplicate. The relative expression level of each gene was calculated as $2^{-\Delta\Delta Ct}$ [32].

Data analysis

Data are expressed as means with standard errors (SE). Differences were set up as statistically significant at $P < 0.05$. Differences between the values for different variables were analyzed by two-way ANOVA, followed by Bonferroni post hoc test. Furthermore, Pearson correlation analysis was performed to screen potential association between two variables. GraphPad Prism 4.0 (Graph-Pad Software Inc., CA, USA) was used for the statistical analyses.

Results

Effects of LA on body weight gain, white adipose tissue weights, and serum glucose and insulin levels

Table 1 shows that consuming a high-fat diet for 56 days induced a significant increase ($P < 0.001$) in body weight gain, which was prevented ($P < 0.001$) by LA supplementation. Furthermore, LA also reduced ($P < 0.001$) the body weight gain in control-fed rats. The body weight-lowering actions of LA were accompanied by a significant reduction ($P < 0.001$) of WAT weight in both control (CLIP group) and high-fat-fed rats (OLIP group). Moreover, LA supplementation was able to counteract hyperinsulinemia ($P < 0.001$) and the increase observed in the HOMA index ($P < 0.001$) induced by the high-fat diet.

Because the body weight-lowering actions of LA are due in part to reduced food intake (Control: 71.16 ± 0.69, CLIP: 62.08 ± 0.40, Obese: 101.60 ± 1.40, OLIP: 84.84 ± 1.01 kcal/day; $P < 0.001$ for untreated vs. LA-treated groups), we compared the effects of LA supplementation with pair-fed (PF) animals. Our data showed that the body weight gain (Fig. 1a), the WAT weights (Fig. 1b), serum insulin levels (Fig. 1c), and the HOMA index (Fig. 1d) were significantly lower in LA-supplemented groups than in their corresponding PF groups.

Effects of dietary supplementation with LA on adiponectin

The levels of the insulin-sensitizing adipokine adiponectin were significantly ($P < 0.001$) elevated in the CLIP group (fed with control diet supplemented with LA). Surprisingly,

Table 1 Effects of LA supplementation (0.25 g/100 g) on body weight gain, white adipose tissue weights, and serum markers of glucose metabolism and insulin sensitivity in control (low-fat) and high-fat diet-fed rats

	Control ($n = 10$)	CLIP ($n = 10$)	Obese ($n = 10$)	OLIP ($n = 12$)	ANOVA 2×2		
					D	LA	DXLA
Initial body weigh	215.1 ± 6.4	212.3 ± 5.8	218.1 ± 5.6	214.7 ± 6.0	n.s.	n.s.	n.s.
Final body weigh	391.8 ± 12.2	320.3 ± 10.9	482.0 ± 13.7	378.4 ± 13.1	***	***	n.s.
Body weight gain (g)	176.70 ± 8.31	108.00 ± 7.76	263.90 ± 11.67	163.70 ± 10.52	***	***	n.s.
WAT (g)	28.51 ± 2.18	13.86 ± 1.20 ***	64.65 ± 4.22***	28.82 ± 2.42###	–	–	***
Glucose (mg/dl)	99.74 ± 1.87	100.70 ± 2.13	122.70 ± 5.89	119.40 ± 3.87	***	n.s.	n.s.
Insulin (pM)	154.20 ± 22.55	93.86 ± 8.66 *	305.20 ± 24.39***	167.80 ± 14.28###	–	–	*
HOMA-IR[a]	5.26 ± 0.97	3.32 ± 0.39	13.54 ± 1.47	6.31 ± 0.80	***	***	n.s.
Adiponectin (μg/ml)	22.47 ± 1.76	41.17 ± 2.13 ***	34.11 ± 2.52***	35.56 ± 2.39	–	–	***
Adiponectin/WAT	0.83 ± 0.09	3.14 ± 0.25 ***	0.54 ± 0.04*	1.33 ± 0.13###	–	–	***

Data are expressed as mean ± SE. Data were analyzed by two-way ANOVA: *** $P < 0.001$; * $P < 0.05$. When an interaction was found, comparison between groups were analyzed by a Student's t test

D diet; *LA* lipoic acid treatment; *DxLA* interaction between diet and LA treatment

*** $P < 0.001$; * $P < 0.05$ when compared with control group; ### $P < 0.001$ when compared with obese group

[a] HOMA-IR = fasting insulin (μUI/ml) × fasting glucose (mmol/l)/22.5

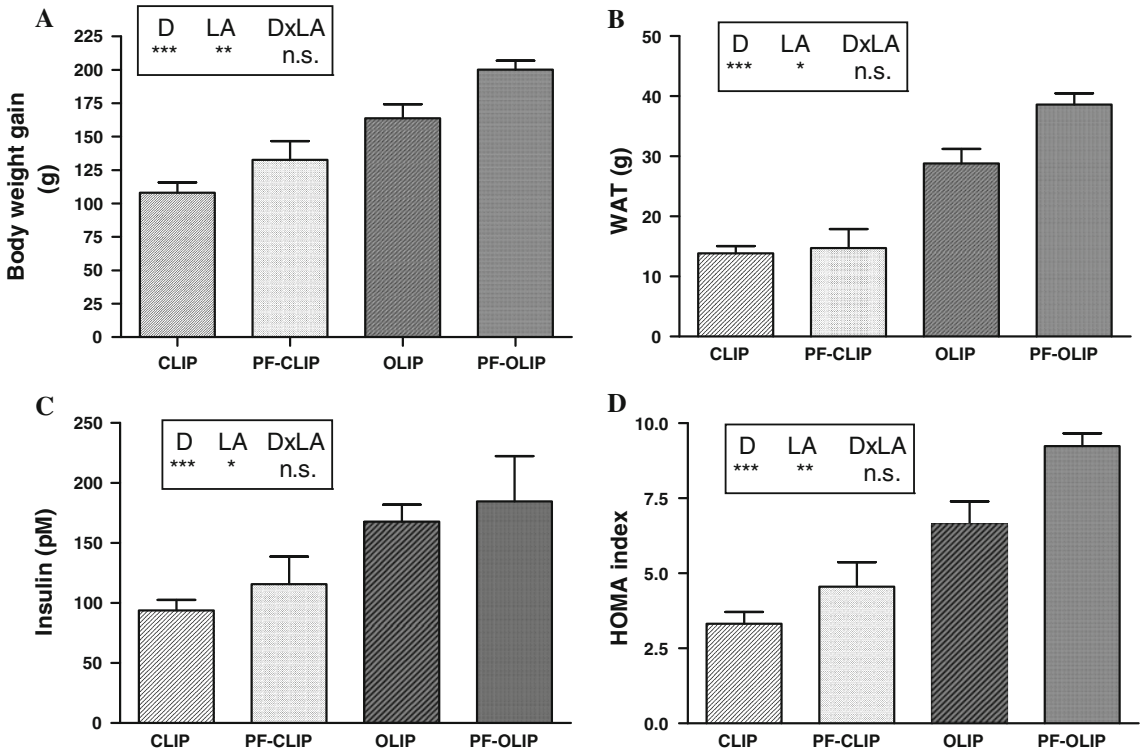

Fig. 1 Effects of LA (0.25 g/100 g diet during 56 days) on **a** body weight gain, **b** total white adipose tissue, **c** serum insulin levels, and **d** HOMA index. Data are expressed as mean ± SE. ($n = 6$–12). Data were analyzed by two-way ANOVA: ***$P < 0.01$; **$P < 0.01$; *$P < 0.05$. *D* diet; *LA* lipoic acid treatment; *DxLA* interaction between diet and LA treatment

serum adiponectin levels were also higher in high-fat-fed animals and no differences were induced by the LA treatment (Table 1).

However, when adiponectin concentrations were corrected for adiposity, a marked reduction ($P < 0.05$) in the amount of adiponectin per g of WAT was observed in the high-fat-fed obese animals, which was reversed ($P < 0.001$) by dietary supplementation with LA in both control (Table 1). A similar pattern was observed for adiponectin gene expression showing that LA supplementation was able

to stimulate adiponectin in control-fed rats and it was also able to override the inhibition in adiponectin gene expression induced by the high-fat diet (Control: 1.00 ± 0.09, CLIP: 1.95 ± 0.94, Obese: 0.65 ± 0.16; OLIP: 1.31 ± 0.25, $P < 0.01$ for untreated vs. LA-treated groups). Interestingly, the ability of LA to stimulate adiponectin was not only secondary to its body-lowering actions since statistically significant differences were observed between LA-treated groups (CLIP and OLIP) and their corresponding PF groups (PF-CLIP and PF-OLIP) in both the amount of adiponectin/g WAT ($P < 0.01$) and in adiponectin gene expression levels ($P < 0.05$) in adipose tissue (Fig. 2b, c). Furthermore, our data showed an inverse relationship between the HOMA index, a marker of insulin resistance and adiponectin mRNA levels ($r = -0.422$; $P < 0.05$) as well as with serum adiponectin levels corrected for adiposity (Fig. 2d).

Effects of dietary supplementation with LA on AMPK in WAT, BAT, and skeletal muscle

Figure 3a shows representative Western blots reflecting the effects of LA supplementation on total and phosphorylated (at Thr^{172}) AMPK. Interestingly, dietary supplementation with LA significantly increased ($P < 0.05$) phospho-AMPK/total AMPK ratio in comparison with both *ad libitum*-fed control and obese groups (Fig. 3b) as well as with the corresponding PF groups (Fig. 3c). In contrast to what was observed in WAT, in BAT LA treatment significantly decreased ($P < 0.05$ and $P < 0.01$) the phosphoAMPK/total AMPK ratio when comparing with both *ad libitum*-fed control and obese groups (Fig. 3b) and corresponding PF groups (Fig. 3c). Levels of AMPK phosphorylation remained unchanged in gastrocnemius muscle of LA-treated animals.

Discussion

Our current data clearly show that dietary supplementation with LA prevents the hyperinsulinemia and the elevation of the HOMA index when a high-fat saturated diet is consumed, supporting the ability of LA to counteract the development of insulin resistance even under this obesogenic condition. These results are in agreement with those of Timmers et al. [34], who found that fasting plasma insulin levels were lower if receiving LA in rats fed

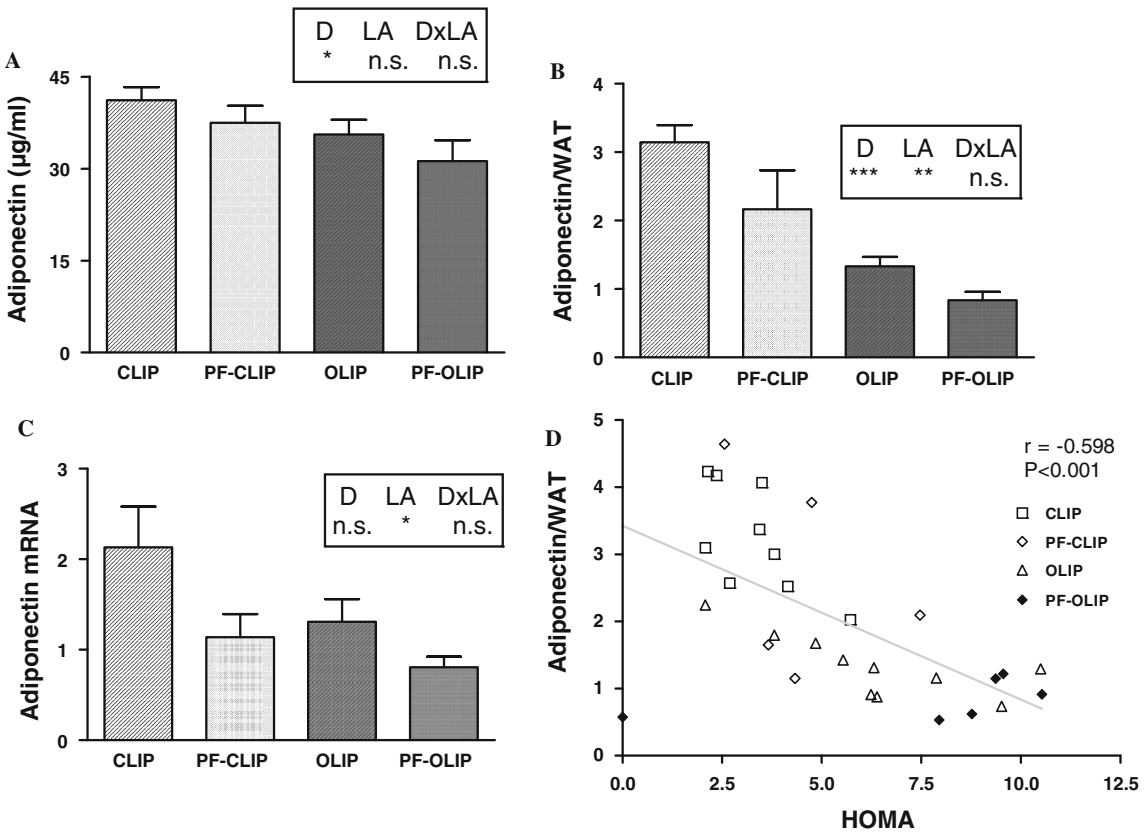

Fig. 2 Effects of LA (0.25 g/100 g diet during 56 days) on **a** adiponectin circulating levels, **b** adiponectin concentrations expressed per g of white adipose tissue (WAT), **c** adiponectin gene expression in epididymal fat, and **d** Pearson's correlation between Adiponectin/WAT and HOMA index. Data are expressed as mean ± SE. (n = 6–12). Data were analyzed by two-way ANOVA: $***P < 0.001$; $**P < 0.01$; $*P < 0.05$. *D* diet; *LA* lipoic acid treatment; *DxLA* interaction between diet and LA treatment

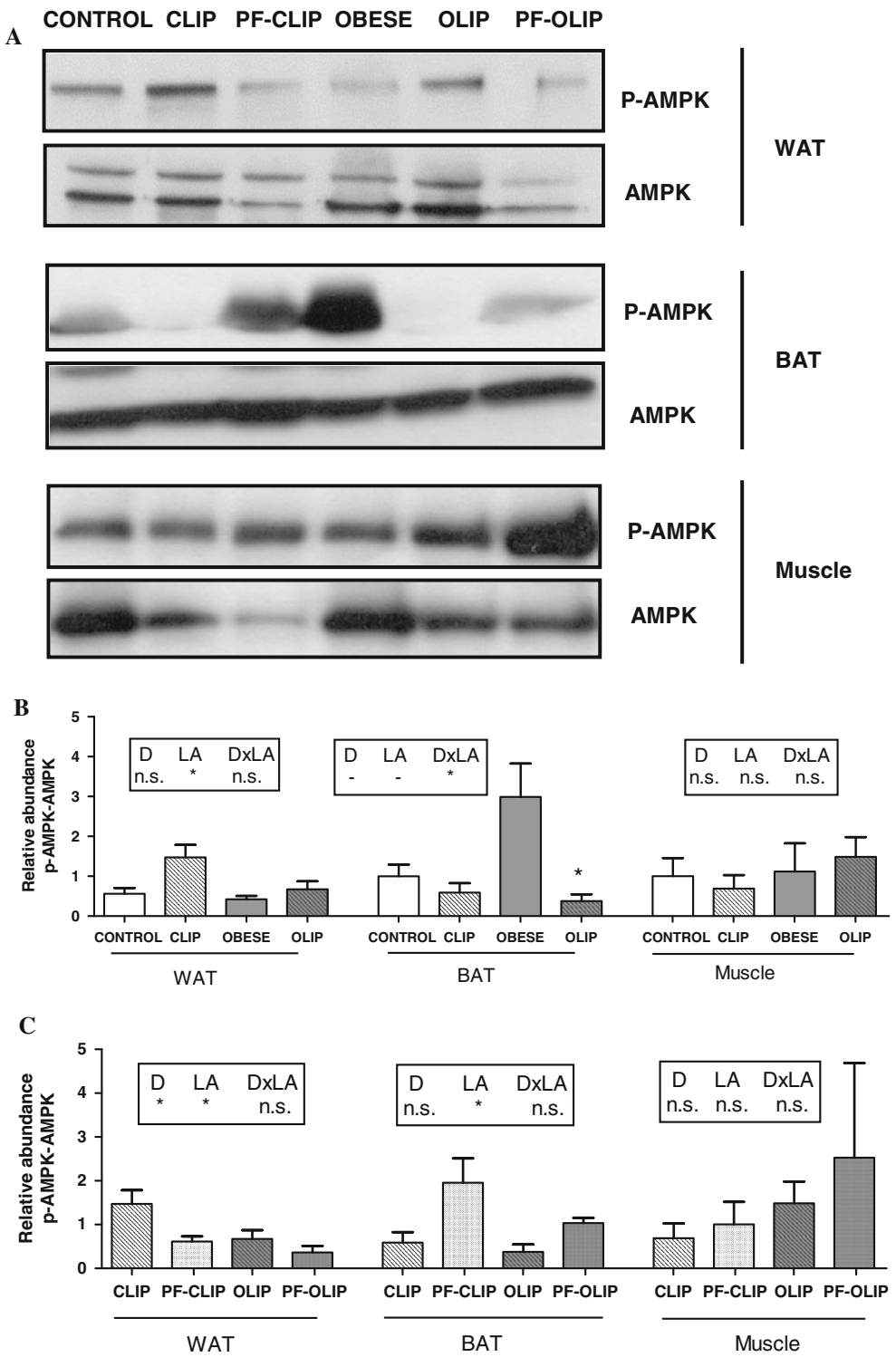

Fig. 3 Effects of LA (0.25 g/100 g diet during 56 days) on AMPK phosphorylation in epididymal fat, brown adipose tissue, and gastrocnemius muscle from control and high-fat-fed rats. Whole cell lysates were subjected to gel electrophoresis and immunoblotted using specific antibodies for p-AMPKα (Thr-172) and AMPKα as described in "Materials and methods". **a** Representative blots and **b–c** densitometric analysis of 4–5 independent experiments showing the effects of different treatments on phosphoAMPK/ total AMPK ratio. Data, expressed as mean ± SE, were analyzed by two-way ANOVA: $^{*}P < 0.05$. *D* diet; *LA* lipoic acid treatment; *DxLA* interaction between diet and LA treatment

low- and high-fat diet. Interestingly, our data demonstrated that the ability of LA to ameliorate insulin resistance was not only secondary to its anorexigenic and body weight-lowering actions since statistically significant differences were observed between LA-treated groups and their corresponding PF groups. This outcome is in agreement with a recent study showing that LA (at doses that do not affect energy intake or body weight) ameliorates the onset of type 2 diabetes induced by fructose in UCD-T2DM rats [23]. In our study, we have only tested the effects of LA supplementation on basal glycemia and insulinemia, but several other studies have described the glucose-lowering and insulin-sensitizing action of LA in an insulin-stimulated status such as after a glucose tolerance test or even in a euglycemic–hyperinsulinemic clamp in both in rodents [23, 34, 35] and in humans [36], respectively.

Several mechanisms have been proposed to mediate the insulin-sensitizing action of LA, including the improvement of glucose homeostasis by the preservation of beta-cell function [23]. In fact, similar to metformin, LA inhibits insulin secretion in vitro from MIN-6 cells and isolated rat islets [30]. It has been also suggested that LA prevents the development of diabetes in diabetes-prone obese rats by reducing triglyceride accumulation in non-adipose tissues such as muscle, pancreatic beta-cells, and liver [29]. Some of these positive effects on insulin sensitivity have been proposed to be mediated by the modulation of AMPK. Thus, it has been demonstrated that LA increases insulin sensitivity by activating AMPK in skeletal muscle [31] and beta-cells [30]. Moreover, Park et al. [29] described that LA decreases lipogenesis in liver through AMPK-dependent and AMPK-independent pathways. In the present study, we did not find any significant changes in AMPK phosphorylation in skeletal muscle of LA-treated rats. Similarly, the study of Timmers et al. [34] observed that the prevention of high-fat diet-induced muscular lipid accumulation in rats by alpha-LA is not mediated by AMPK activation.

Regarding the role of AMPK activation in WAT, it has been suggested to be beneficial in insulin-resistant states, particularly as AMPK activation also reduces cytokine secretion in adipocytes [25]. In the present trial, we have evaluated the role of LA in AMPK phosphorylation in epididymal fat depot of Wistar rats. Our data revealed the ability of LA to activate AMPK phosphorylation in WAT. A recent study also reported an increased phosphorylation of AMPK in WAT from ovariectomized rats treated with LA in parallel with the inhibition of food intake and adipose tissue size. However, the authors did not address if this stimulation of AMPK is merely the consequence of weight loss and reduced adiposity as pair-fed groups were not included in the study. The novelty of our study relies on the fact that we observed a significant stimulation of AMPK phosphorylation in LA-treated groups as compared with pair-fed animals, suggesting a direct ability of LA to activate AMPK. This finding is further supported by our observation that LA increase AMPK phosphorylation in cultured adipocytes (data not shown). Previous studies have suggested that the activation of AMPK in rodent adipocytes leads to a decreased lipogenic flux and triglyceride synthesis as well as increased fatty acid oxidation [37], which could contribute to the adipose-lowering effect of LA. Moreover, LA-induced activation of AMPK in adipose tissue may also contribute to its insulin-sensitizing properties since high levels of FFA have been suggested to cause insulin resistance in all major insulin target organs [25, 38].

In contrast to what was observed in WAT, LA treatment caused a significant reduction in AMPK phosphorylation in BAT, supporting that the regulation of AMPK by the same bioactive compound is tissue-specific [39]. In this context, it has been shown that the omega-3 fatty acid DHA stimulates AMPK activation mainly in WAT and in a less extend in muscle, but not in liver [40].

Adiponectin is an adipokine with key insulin-sensitizing properties [41]. Stimulation of adiponectin production has been described for several insulin-sensitizing molecules including PPARγ activator rosiglitazone, metformin, and omega-3 fatty acids [42–44]. However, few studies have addressed the effects of LA supplementation on adiponectin and controversial data have been obtained. Thus, the recent study of Cheng et al. [22] suggests that LA treatment (200 mg/kg by gavage during 7 weeks) suppresses the elevation of adiponectin levels induced by ovariectomy. On the other hand, Cummings et al. [23] did not observe any significant change in fasting plasma adiponectin levels in UCD-T2DM rats fed with fructose in the absence or presence of LA (80 mg/kg b.w.). In contrast, Houng and Ide [24] described an elevation in adiponectin circulating levels after dietary supplementation with LA (1–5 g/kg of diet) for 21 days. Our data indicate that dietary supplementation with LA is able to upregulate adiponectin gene expression in WAT and suggest an increase in the amount of adiponectin produced per gram of adipose tissue. Interestingly, this ability of LA to stimulate adiponectin was not only secondary to its body weight-lowering actions since statistically significant differences were observed between LA-treated groups and their corresponding PF groups. These data suggest that the increase in adiponectin production by adipocytes could be involved in the insulin-sensitizing properties of LA. In fact, we found a negative correlation between adiposity-corrected adiponectin plasma levels and the HOMA index, a recognized marker of insulin resistance. The different outcomes obtained in those trials could be related to differences in the animal models, the dose, and the duration of treatments with LA.

A question that remains to be answered is the mechanisms leading to the LA-induced upregulation of

adiponectin and activation of AMPK in WAT. The regulation of adiponectin gene expression, secretion into, and clearance from the circulation is highly complex [45]. It is well known that the activation of the transcription factor PPARγ increases adiponectin expression and production in adipocytes. However, we did not find any significant change in the expression level of this transcription factor in WAT of LA-treated rats (data not shown). Several studies have related AMPK activation with the production of adiponectin. Thus, some in vitro trials described that the AMPK activator AICAR upregulates adiponectin [45, 46]. These findings lead us to suggest that the activation of AMPK by LA could contribute to the upregulation of adiponectin in WAT. On the other hand, evidence is accumulating that adiponectin, at least in part, acts by activating AMPK [47, 48]. Therefore, it is possible that the upregulation of adiponectin by LA also could also contribute to activate AMPK, revealing a complex regulation and cross-talk between adiponectin and AMPK activation in adipocytes.

A myriad of studies have highlighted the importance of oxidative stress in the development of insulin resistance [49]. Increased oxidative stress has been demonstrated to dramatically affect the secretion of adipokines by adipose tissue, including adiponectin, and the regulation of redox state in adipose tissue has been proposed as a potentially useful therapeutic target for obesity-associated metabolic syndrome [50]. In this context, it has been described that LA protects against oxidative stress-induced impairment in insulin function in adipocytes [51]. Moreover, a recent study of our group have demonstrate that the LA supplementation is able to restore the oxidative balance by increasing antioxidant defenses [52] through the deacetylation of Foxo3a and PGC1β by SIRT1 and SIRT3 in fatty liver of high-fat-fed rats [53]. Although we have not tested the direct effects of LA supplementation on redox balance in WAT, all the previously mentioned facts seem to suggest that the beneficial effects of LA supplementation on adiponectin and insulin resistance observed in the present study might be also related to its ability to restore the oxidative balance in these animals.

In conclusion, our present data demonstrated that dietary supplementation with LA upregulates adiponectin and activates AMPK in WAT, suggesting that both facts might somehow contribute in part to LA ability to prevent hyperinsulinemia and insulin resistance in high-fat-fed rats.

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Conflict of interest The authors state no conflict of interest.

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