Impact of dietary lipoic acid supplementation on liver mitochondrial bioenergetics and oxidative status on normally fed Wistar rats

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ABSTRACT

The aim of this study was to examine the effects of α-lipoic acid (α-LA) on liver mitochondrial bioenergetics and oxidative status for 8 weeks in normal-healthy animals. A pair-fed group was included to differentiate between α-LA direct effects and those changes due to reduced food intake. α-LA decreased body weight gain, liver weight and insulin levels with no differences compared to its pair-fed group. α-LA significantly reduced energy efficiency, the activity of the electron transport chain complexes and induced a lower efficiency of oxidative phosphorylation with reduced ATP production. α-LA supplementation directly decreased plasma triglycerides (TGs), free fatty acids and ketone bodies levels. A significant reduction in hepatic TG content was also observed. A significant up-regulation of Cpt1a, Acadl and Sirt3, all β-oxidation genes, along with a significant deacetylation of the forkhead transcription factor 3a (FOXO3A) was found in α-LA-treated animals. Thus, α-LA along with a standard chow diet has direct actions on lipid metabolism and liver by modulating mitochondrial function in normal-weight rats. These results should be taken into account when α-LA is administered or recommended to a healthy population.

Introduction

α-Lipoic acid (α-LA; 1,2-dithiolane-3-pentanoic acid) is a short chain fatty acid with antioxidant, antimutagenic and anticarcinogenic properties (Goraca et al. 2011; Moura et al. 2015; Pashaj et al. 2015). This fatty acid occurs in different foods such as spinach, broccoli, tomatoes, brussel sprouts, potatoes, garden peas, rice bran and also in animal-derived foods such as red meat, liver, heart and kidney (Matsugo et al. 1997; Moini et al. 2002). α-LA can be also endogenously synthesised by the lipoic acid synthase (LASY), whose impaired formation results in an overall disturbance in the antioxidant defence network, leading to increased inflammation and mitochondrial dysfunction (Matsugo et al. 1997; Moini et al. 2002). In addition, α-LA acts as a cofactor of several mitochondrial bioenergetic enzymes and in several processes of aerobic metabolism (Reed 1998; Solmonson and DeBerardinis 2018). Due to the wide variety of essential functions attributed to α-LA, it has been the focus of numerous research studies and clinical trials as a potential therapy in chronic inflammatory disorders such as diabetes, and obesity (Carbonelli et al. 2010; Koh et al. 2011; Huerta et al. 2015; Huerta et al. 2016) and has been proposed as a potential functional ingredient for disease prevention.

Several investigations have demonstrated that liver is a key target of this antioxidant. Indeed, α-LA has shown to be hepatoprotective, to improve liver circulation and to be useful to treat chronic liver diseases (Bustamante et al. 1998; Khalaf et al. 2017; Sadek et al. 2018; Sena et al. 2018). In this sense, α-LA has shown anti-oxidative and anti-inflammatory properties in endotoxemic livers from rats and in chronic liver
diseases caused by ongoing hepatic damage (Ali et al. 2014; Goraca et al. 2015). α-LA also protects against acute liver injury (Tanaka et al. 2015), against the development of non-alcoholic steatosis (Jung et al. 2012; Valdecantos et al. 2012a, 2012b) and against lipopoapoptosis in hepatocytes (Valdecantos et al. 2015). Some of the aforementioned beneficial effects of α-LA have been attributed, among other mechanisms, to α-LA modulatory actions on liver mitochondria (Valdecantos et al. 2012a). Therefore, α-LA arises as a helpful preventive and/or therapeutic agent to manage diverse liver disturbances. However, most of the beneficial actions described for this fatty acid have been investigated in models where a “challenge” has been carried out (induction of obesity, liver damage, inflammation, oxidative stress, etc.), but α-LA actions on “healthy” models (subjects/animals/cells) have been scarcely analysed. In fact, controversial actions have been described when comparing α-LA effects in “healthy” vs. “non-healthy” models and even some deleterious actions have been recently described with the usage of α-LA under healthy conditions (Kuhla et al. 2016). These controversial results deserve further investigation and should be taken into account when α-LA supplements are administered or recommended as a functional ingredient for health promotion in a general/healthy population. Thus, the aim of this study was to examine the effect of α-LA on mitochondrial bioenergetics and oxidative status in livers from normal rats. Several markers of liver oxidative stress and antioxidant defences, mitochondrial function and SIRT pathway in rats fed with a balanced-standard chow diet supplemented with α-LA were assessed.

Materials and methods

Animals and diets

Six-week-old male Wistar rats (n = 26) were supplied from the Center for Applied Pharmacobiology Research (CIFA, Pamplona, Spain). Animals were housed in cages in a temperature-controlled room (22 ± 2 °C) with a 12-hour light-dark cycle, fed a pelleted chow diet and given deionised water ad libitum for an adaptation period of five days. After this period, rats were assigned into three experimental groups: (i) Control group (n = 10), fed on a standard diet (4.6% w/w of lipids) (Harlan Teklad Global Diets, Madison, WI, USA); (ii) CLIP group (control + α-LA) (n = 10), fed ad libitum with the previously mentioned standard diet supplemented with α-LA in a proportion of 0.25 g α-LA/100 g of diet (Perez-Matute et al. 2009; Prieto-Hontoria et al. 2009) and finally (iii) pair-fed CLIP group (PFC) (n = 6) fed with the same amount of food consumed by the CLIP group, but without adding α-LA. Body weight and food intake were recorded every 2–3 days. At the end of the experimental period (8 weeks), rats were euthanised and blood and tissue samples were immediately collected, frozen in liquid nitrogen and kept at −80 °C for further analyses. All experimental procedures were performed according to National and Institutional Guidelines for Animal Care and Use at the University of Navarra and in accordance with the EU Directive 2010/63/EU for animal experiments.

Blood sampling and analysis

Plasma levels of alanine and aspartate aminotransferases (ALT, AST), triglycerides (TGs), free fatty acids (FFAs), ketone bodies and glucose were measured using a Cobas Mira Autoanalyzer with standardised procedures (Roche Diagnostic, Basel, Switzerland). Insulin levels were determined using a rat/mouse Insulin ELISA kit (EMD Millipore, St. Louis, MO).

Tissue homogenisation procedure and mitochondrial isolation

Livers from experimental animals were immediately excised after the animals were sacrificed and either frozen in liquid nitrogen or placed in ice-cold buffer (250 mM sucrose, 1 mM EDTA and 5 mM NaTES, pH = 7.4). Fresh tissue placed in buffer was used to prepare mitochondrial suspension according to the modified method of Rickwood et al (1987) with slight modifications (Valdecantos et al. 2010).

Electron transport chain (ETC) complexes

Activities of enzymes related to the ETC were assessed in 50 μg of frozen mitochondrial protein using a kinetic method in a Multiskan Spectrum spectrophotometer (Thermo Electron Corporation, Foster City, CA), through standardised reproducible methods as described elsewhere (Aleardi et al. 2005; Benard et al. 2006). All activities were calculated in nmoles per minute and per milligram and expressed as percentage compared to the control. Complex IV and ATP synthase activities were measured in frozen isolated rat liver mitochondria (25 μg and 75 μg of mitochondrial protein, respectively) with a MitoProfile®Rapid Microplate assay kit according to the manufacturer’s instructions (Cat. no. MS447 and MS541, respectively, MitoScience, Eugene, OR). Complex IV and ATP
synthase were immunocaptured within the wells of the microplates, and the enzyme activity was measured by a kinetic colorimetric assay and activities were determined as previously described (Terni et al. 2010; Liu et al. 2015).

**Liver mitochondrial ATP content**

ATP was determined by the firefly luciferin-luciferase assay system according to the method of Lemasters and Hackenbrock (1976). First, the ATP was extracted from mitochondrial matrix following a previously described protocol with minor modifications (Dorta et al. 2005). Bioluminescence was measured in the supernatant with a Protein Kinase sensitive assay kit (Proteinkinase, Biaffin GmbH & Co KG, Kassel, Germany), according to the manufacturer’s instructions with slight modifications by using a Luminoskan Ascent equipment (Thermo Electron Corporation, Foster City, CA).

**Hepatic triglyceride content**

To determine the hepatic TG content, 150 mg of these tissues were sonicated in a Branson Sonifier 250 equipment (duty cycle 40%; output control 4; hold continuous) for 40 seconds in 1.5 mL of buffer (150 mM NaCl, 0.1% Triton and 10 mM Tris pH 8) at 50 °C. After centrifugation at 12,000×g for 10 min, the supernatants were used to measure the TG levels using a COBAS-Mira Autoanalyzer (Roche Diagnostic, Basel, Switzerland).

**Lipid peroxidation**

Malondialdehyde (MDA) levels were quantified following a controlled reaction with thiobarbituric acid (TBAR). Colorimetric changes were measured in liver homogenates with a commercial kit (Cayman Chemical, Ann Arbor, MI) following the manufacturer’s instructions with minor modifications and using a Luminoskan Ascent equipment (Thermo Electron Corporation, Foster City, CA). Results were corrected by the amount of tissue in milligrams, as previously described (Valdecantos et al. 2012b).

**Mitochondrial antioxidant defences**

Manganese superoxide dismutase and glutathione peroxidase activities were measured in isolated rat liver mitochondria using standardised enzymatic colorimetric activity kits (Assay Designs Inc, Ann Arbor, MI) as previously described with slight modifications (Valdecantos et al. 2010). Total reduced (GSH) and oxidised (GSSG) glutathione levels were measured in the same samples using a colorimetric assay (Assay Designs Inc, Ann Arbor, MI). The ratio between GSH and GSSG was also calculated as a marker of antioxidant status in both erythrocytes and the hepatic mitochondrial compartment.

**Mitochondrial oxidative damage and mitochondrial protein mass estimations**

Mitochondrial DNA (mtDNA) oxidative damage was estimated as the ratio of 80-bp fragment (260–339 position), compared to a 162-bp fragment (260–421 position) of the same sample by real-time PCR reactions as previously described (Valdecantos et al. 2012b). The ratio between citrate synthase activity in whole tissue and in isolated mitochondria was used to estimate mitochondrial protein mass, as previously described (Raffaella et al. 2008).

**Real-time quantitative PCR and mitochondrial copy number**

Total RNA was isolated from liver using Trizol® reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. RNA concentrations were determined by Nanodrop 1000 (Nanodrop, Wilmington, DE). To avoid contamination with genomic DNA, DNA digestion and inactivation were assessed using the DNase kit (Ambion Inc, Austin, TX). Four micrograms of RNA were reverse transcribed to cDNA using the M-MLV kit (Invitrogen, Carlsbad, CA) following protocols from the suppliers. Twelve genes were analysed (Supplemental Table 1) using predesigned TaqMan® Assays-on-demand fixed on a low density array microplate (Applied Biosystems, Foster City, CA). The reaction conditions were set up according to the manufacturer’s instructions. Amplification and detection of specific products were performed using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). All the samples were analysed in duplicate. Ct values were generated by the ABI software. Hepatic expression of each gene was normalised by 18s and Actb genes. Finally, the relative expression levels of each gene were calculated with 2−ΔΔCT method (Perez-Matute et al. 2009). The ratio between mitochondrial encoded gen (Mtco2) and nuclear endogenous gene (18s) enabled to estimate the mitochondrial copy number.
**Statistical analyses**

Data are reported as mean ± SEM. Normal distribution was confirmed by two different methods, the Shapiro–Wilk and the Kolmogorov–Smirnov. In order to determine the effects of α-LA treatment, one-way ANOVA followed by the Bonferroni post hoc analysis were carried out. Variables analysed and illustrated in figures were based on a hypothesis driven and did not require a multiple comparison correction. All statistical analyses were performed using the GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA) and SPSS 19.0 (SPSS® Inc., Chicago, IL). Differences were considered as statistically significant at p < .05.

**Results**

**α-LA effects on body weight gain, feed efficiency, liver weight, hepatic triglyceride content, lipid and glucose metabolism in normally fed rats**

α-LA supplementation induced a significant decrease in body weight gain in comparison with the control group (−40%, p < .001). A reduction was also observed in the PF-CLIP, although with a lower intensity (−25%, p < .05). As previously demonstrated, α-LA supplementation decreased food intake (grams and total calories) in comparison with the control group (p < .001), and this was the reason to include a PF-group. In addition, a significant decrease in energy efficiency (p < .001) was found in both the CLIP and PF-CLIP groups compared to the controls, which were more pronounced in the CLIP group (p < .001 CLIP vs. PF-CLIP). α-LA treatment diminished plasma levels of TGs, FFAs and ketone bodies (p < .05 to .01) independently of its ability to reduce food intake, as these decreases were not observed in the PF-group. In contrast, no effects were observed on ALT and AST plasma levels. A significant decrease in liver weight was observed in the CLIP and PF-CLIP groups. A significant reduction in hepatic TG content in the CLIP group was also observed when compared with the controls (p < .001) and also when compared with the pair-fed animals (p < .05). No effects were found on glucose plasma levels, although a significant reduction in insulin levels in both the CLIP and PF-CLIP groups were obtained when compared with the controls (Table 1).

**α-LA actions on mRNA levels of genes involved in lipogenesis and β-oxidation in liver**

α-LA significantly increased the mRNA levels of Srebf1 in liver (p < .001), although no statistical differences were observed when compared with the pair-fed group (Table 2). A significant increase was also recorded on Pparγ expression levels (p < .01), independently of the caloric restriction, as statistical differences were observed among the CLIP and PF-CLIP group (p < .01). No relevant effects were found on Dgat-2 mRNA levels. α-LA induced a significant and direct increase (not secondary to calorie restriction) in the hepatic expression of several genes involved in mitochondrial β-oxidation such as Cpt1a and Acadl (p < .05 and p < .001, respectively) (Table 2).

**Effects of α-LA on the activity of the electron transport chain complexes and the oxidative phosphorylation (OXPHOS) in liver mitochondria**

α-LA strongly inhibited the activity of all the electron-chain complexes (I, II, II + III, IV and V) and a

| Table 1. Body weight gain, liver triglyceride content, plasma levels and energy efficiency. |
|---------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| **CONTROL** (n = 10)                       | **CLIP** (n = 10) | **PF-CLIP** (n = 6) | **ANOVA** p Value |
| Body weight gain (% vs. C)                  | 100.0 ± 4.7      | 61.1 ± 4.4***    | 76.5 ± 5.9**    | <.001           |
| Food intake (g/day)                         | 23.0 ± 0.2       | 20.0 ± 0.1***    | 19.5 ± 0.3**    | <.001           |
| Calorie intake (kcal/day)                   | 71.2 ± 0.7       | 62.1 ± 0.4***    | 60.6 ± 1.1***   | <.001           |
| Feed efficiency (g/kcal × 100)              | 4.2 ± 0.2        | 3.1 ± 0.0***     | 3.4 ± 0.1***    | <.001           |
| Liver weight (g)                            | 9.2 ± 0.4        | 8.5 ± 0.3**      | 7.3 ± 1.5      | <.05            |
| Liver triglycerides (mg/dL)                 | 36.2 ± 4.2       | 21.5 ± 1.3***    | 33.1 ± 1.3     | <.001           |
| **Liver function**                          |                 |                 |                 |                 |
| Plasma ALT levels (U/L)                     | 45.1 ± 2.6       | 50.1 ± 5.4       | 43.1 ± 1.8    | <.001           |
| Plasma AST levels (U/L)                     | 136.5 ± 7.4      | 123.6 ± 3.9      | 130.1 ± 7.9   | <.05            |
| **Lipid metabolism**                        |                 |                 |                 |                 |
| Plasma triglycerides (mg/dL)                | 69.9 ± 7.2       | 47.0 ± 3.9***b   | 71.9 ± 7.7     | <.01            |
| Plasma FFAs (mg/dL)                         | 0.9 ± 0.1        | 0.6 ± 0.0***a    | 0.8 ± 0.0     | <.01            |
| Ketone bodies (mmol/L)                      | 1.8 ± 0.2        | 1.3 ± 0.1***c    | 2.4 ± 0.1     | <.01            |
| **Glucose metabolism**                      |                 |                 |                 |                 |
| Glucose (mg/dL)                             | 105.6 ± 2.9      | 102.0 ± 2.1      | 113.4 ± 6.8    | ns              |
| Insulin (ng/mL)                             | 1.0 ± 0.1        | 0.6 ± 0.1*       | 0.7 ± 0.1*    | <.05            |

Values are mean ± SEM. *p < .05, **p < .01, ***p < .001 vs. control group; †p < .05, ‡p < .01, ††p < .001 vs. PF-CLIP group according to one-way ANOVA followed by post hoc multiple comparisons by the Bonferroni test.
significant reduction was shown in ATP synthesis (Figure 1(a,b)). Noteworthy, these effects were not secondary to calorie restriction. Interestingly, a positive and significant correlation between mitochondrial ATP levels and energy efficiency was identified ($r^2 = 0.6413; p < .001$) (Figure 1(c)).

**α-LA effects on mitochondrial biogenesis and mitochondrial mass**

Current data evidenced that α-LA significantly increased the mitochondrial copy number and mitochondrial mass ($p < .01$ and $p < .05$, respectively), as illustrated in Figure 2(a,b). A significant increase was also found in several genes involved in mitochondrial biogenesis such as *Pparα, Pgc1β, Nrf1* and *Tfam*. The effects of α-LA on mitochondrial biogenesis were independent to the reduction observed in food intake, as pointed out by the statistical differences observed between the CLIP and PF-CLIP groups (Figure 2(c)).

![Image of Figure 1](image-url)

**Role of α-LA on hepatic oxidative damage and mitochondrial antioxidant defences**

α-LA treatment significantly reduced ($p < .001$) hepatic mtDNA oxidative damage (Figure 3(a)) and also lipid peroxidation in the liver ($p < .01$) (Figure 3(b)). α-LA stimulates the activity of the MnSOD enzyme ($p < .001$) (Figure 3(c)). These stimulatory actions were not observed in the PF-group. α-LA did not induce any significant outcome on the activity of the GPX enzyme (Figure 3(d)). Finally, α-LA significantly increased the GSH:GSSG ratio, whereas this increase was not observed on the pair-fed group (Figure 3(e)).

**Effects of α-LA on sirtuin-3 and Foxo3a mRNA and protein acetylation levels**

Our research showed that α-LA supplementation was able to increase the mRNA and protein levels of SIRT-3 ($p < .01$) independently of the caloric intake (Figure 4(a,b)) but no effects were observed on

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**Table 2. Effects of α-LA on mRNA levels of several genes involved in liver lipogenesis and β-oxidation.**

| Gene      | Control | CLIP     | PF-CLIP  | ANOVA |
|-----------|---------|----------|----------|-------|
| Lipogenesis genes                      |
| Srebf1   | 1.0 ± 0.1 | 2.5 ± 0.3*** | 1.7 ± 0.2 | <.001 |
| Dgat2    | 1.0 ± 0.1 | 0.9 ± 0.1 | 1.1 ± 0.1 | n.s.  |
| Ppar-γ   | 1.0 ± 0.1 | 1.6 ± 0.1*** | 0.9 ± 0.1 | <.01  |
| β-Oxidation genes                      |
| Cpt1a    | 1.0 ± 0.0 | 1.3 ± 0.1*** | 0.6 ± 0.1*** | <.001 |
| Acadl    | 1.0 ± 0.0 | 1.4 ± 0.1*** | 1.05 ± 0.1  | <.001 |

Values are means ± SEM. *p <.05, **p <.01, ***p <.001 vs. control group; a $p <.01$ vs. PF-CLIP group according to post hoc multiple comparisons by the Bonferroni test. The p value of one-way ANOVA appears in right column. Gene expression is expressed as fold changes ($2^{-ΔΔCt}$) compared to the control group, which was considered as 1.
SIRT-1 (data not shown). Although a significant increase was observed in Foxo3a mRNA levels ($p < .05$) in those rats treated with $\alpha$-LA (Figure 4(c)), a significant reduction ($p < .001$) in FOXO3A acetylation was observed (Figure 4(d)). This effect seems to be directly attributable to $\alpha$-LA and not secondary to the caloric restriction, as a significant reduction was also observed compared to its pair-fed group ($p < .01$).

**Discussion**

This study demonstrates that $\alpha$-LA supplementation along with a standard chow diet has direct actions on lipid metabolism and on liver by modulating mitochondrial function in controlled normal-weight rats.

The significant reduction in body weight gain and insulin plasma levels after $\alpha$-LA treatment seem to be more related to the well-known anorexigenic actions of $\alpha$-LA (Kim et al. 2004; Shen et al. 2005; Song et al. 2005; Prieto-Hontoria et al. 2009), rather than to a direct effect of the $\alpha$-LA, as no differences were observed among the CLIP and PF-CLIP groups. However and interestingly, $\alpha$-LA was able to influence the amount of energy absorbed from food, similarly to what was observed when it was supplemented along with a high fat diet (HFD) (Prieto-Hontoria et al. 2009). $\alpha$-LA also reduced the activity of the ETC complexes, which, in turn, leads to a lower efficiency of OXPHOS and lower amounts of ATP. Thus, rats fed with $\alpha$-LA produced less ATP and, therefore, they are less energy efficient. Similar energy unbalances after $\alpha$-LA treatment were observed in control and diabetic rats (Luz et al. 2009) and in HFD-obese rats (Valdecantos et al. 2012a). While these effects could be of interest in the context of obesity and associated disorders as it prevented the increase in body weight gain induced by a HFD, the impact in normal-weight animal’s needs to be fully understood.

Our study also evidenced that $\alpha$-LA supplementation decrease the plasma levels of TGs, FFAs and ketone bodies with no effects on hepatic transaminases (ALT and AST). Although the hypolipidaemic actions have been previously documented in several studies (Butler et al. 2009; El Midaoui et al. 2011; Valdecantos et al. 2012a), this is the first assay where these actions have been reported in normal-weight and not-diseased animals with no “challenges” apart from the supplementation with the fatty acid. A significant reduction in hepatic TG content was also observed. The stimulation of mitochondrial $\beta$-oxidation in liver could explain, at least in part, this decrease. In fact, a significant increase in the mRNA levels of Acadl and Cpt1a, two key enzymes with a
key role in mitochondrial β-oxidation, has been observed, in contrast with the data presented by Butler et al. (2009). An explanation for these discrepancies could be that in the study of Butler, obese male Zucker rats were used instead of Wistar normal-weight rats. In addition, a shorter treatment with α-LA was also carried out (5 weeks vs. 8 weeks). In this context, Acadl is a specific target of Pparα, a key transcriptional factor involved in fatty acid metabolism. In fact, Pparα is able to decrease fat accumulation by increasing fatty acid degradation (El Midaoui et al. 2011). PPARγ and its agonists have been demonstrated to improve non-alcoholic steatohepatitis by enhancing insulin action and also by increasing FFA oxidation (Ip et al. 2004). Thus, the increase observed in the mRNA levels of Pparα and Pparγ could also contribute to the lower hepatic TG content observed after α-LA supplementation. In addition, Pgc-1β is known to regulate the transcriptional activity of Pparγ (Meirhaeghe et al. 2003). Thus, and similarly to what we previously observed in HFD-fed rats (Valdecantos et al. 2012b), the increased mRNA levels of this transcription factor could suggest a potential relationship among Pgc-1β, Pparγ and Acadl in liver after α-LA supplementation. This association seems to be independent of the nutritional status of the rats, since similar results were observed when α-LA was supplemented in a HFD (Valdecantos et al. 2012a). Other mechanism that could also contribute to the decrease in the hepatic lipid content is a reduced lipogenesis, as previously demonstrated (Park et al. 2008). However, we failed to feature such down-regulation as no actions of α-LA supplementation on Dgat2 gene expression, (pivotal gene responsible for the majority

Figure 3. Effects of α-LA on different markers of oxidative stress and damage and on mitochondrial antioxidant defences. (a) mtDNA oxidative damage and (b) liver MDA levels. (c) Manganese superoxide dismutase (SOD2) activity; (d) glutathione peroxidase (GPx) activity and (e) mitochondrial GSH:GSSG ratio. Values are mean ± SEM of 6-10 animals. *p < .05, **p < .01, ***p < .001 vs. control group; *p < .05, **p < .01, ***p < .001 vs. PF-CLIP according to post hoc multiple comparisons by the Bonferroni test. The p value of one-way ANOVA appears in up corner when appropriate.
of TG synthesis), were found, in contrast with the results observed in HFD-fed rats (Valdecantos et al. 2012a).

Finally, our data revealed that $\alpha$-LA supplementation increased mitochondrial biogenesis and improved mitochondrial antioxidant status in liver of normal-weight rats, even above of the values observed in control rats. It is plausible that the increase observed in the activity of the main mitochondrial antioxidant defences could be due to the stimulation of mitochondrial biogenesis as demonstrated in HepG2 cells (Zhang et al. 2016) but also due to an increased expression of SIRT3 (Chen et al. 2011). In this context, sirtuins (SIRTs) can be activated by $\alpha$-LA (Chen et al. 2012; Valdecantos et al. 2012b; Liu et al. 2015). Specifically, SIRT3, which is expressed in mitochondria, plays a role in regulating in vivo energy homeostasis (Ahn et al. 2008) and is stimulated by caloric restriction in a wide variety of tissues (Shi et al. 2005; Hallows et al. 2011). In addition, SIRT3 stimulates mitochondrial antioxidant defences by the activation of SOD2 through acetylation (Chen et al. 2011). Forkhead transcription factor 3a (Foxo3a), a substrate of SIRT3, is also able to stimulate several antioxidant defences (Sundaresan et al. 2009). Thus, deacetylation of FOXO3A and the increased expression of SIRT3 could mediate the stimulation of the hepatic antioxidant defences observed in our study, similarly to what was previously demonstrated in HFD-fed rats (Valdecantos et al. 2012b). It is important to mention that the induction of SIRT3 by $\alpha$-LA seems to be a direct effect of this fatty acid and independent of the nutritional status (caloric restriction or HFD ingestion) of the animals. Thus, the physiological relevance of these findings needs to be further investigated in non-diseased animals where transcriptomic or epigenetic phenomena may be also involved (Huerta et al. 2017).

Figure 4. $\alpha$-LA on SIRT3 and on forkhead transcription factor 3a (Foxo3a). (a) SIRT3 mRNA and (b) protein levels. (c) mRNA levels of Foxo3a and (d) lysine acetylation of Foxo3a expressed in fold changes vs. control group. Values are mean ± SEM of 6–10 animals. *$p < .05$, **$p < .001$ vs. control group; *$p < .05$, **$p < .01$ vs. PF-CLIP group according to post hoc multiple comparisons by the Bonferroni test. The $p$ value of one-way ANOVA appears in up corner when appropriate.
Conclusions

Our results clearly demonstrate that α-LA supplementation along with a standard/healthy dietary pattern has direct actions on lipid metabolism and on liver by modulating mitochondrial function in normal-weight rats (Figure 5). These effects were very similar than those described for α-LA supplementation in HFD-fed rats (Valdecantos et al. 2012a, 2012b). In fact, the effects previously observed in obese rats were very positive as the supplementation with α-LA prevented the development of non-alcoholic steatosis induced by a HFD. Thus, our current data could be considered as a first step to promote the potential usage of α-LA as a functional ingredient not only for disease prevention but also to promote health, although the physiological relevance of our present findings deserves further investigation before making nutritional recommendations in a general/healthy population.

Acknowledgements

The authors thank Ana Lorente and Verónica Ciaurriz for their technical assistance.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work has been supported by Línea especial “Nutrición, Obesidad y Salud” (University of Navarra LE/97) and Ministry of Science and Innovation (AGL2006-04716/ALI and AGL2009-10873/ALI). CIBER and RETICS networks are also gratefully credited.

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