Chronic lipoic acid treatment worsens energy imbalances in streptozotocin-induced diabetic rats

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Abstract

**Aim.** – Our objective was to verify the energy balance in streptozotocin-induced diabetic rats chronically treated with lipoic acid (LA).

**Methods.** – Diabetes was induced in rats by streptozotocin and the animals divided into four groups, comprising controls and diabetic rats, with each group receiving either daily intraperitoneal LA (30 mg/kg) or a buffer solution for 30 days. Body weight, food intake and stool and urine collections were recorded daily. On day 30, animals were sacrificed and the carcasses, faeces and urine collected and processed for calorimetric analysis. Blood glucose and insulin were also determined.

**Results.** – All parameters of energy balance were affected by diabetes. LA treatment reduced weight gain, energy gain and gross food efficiency in both control and diabetic animals. However, the LA-treated animals tended to show higher energy expenditure than non-treated animals. Body composition was also affected by diabetes: fat content was impaired by LA treatment in both control and diabetic animals. The latter also showed increased glycaemia and decreased insulinaemia, but LA had no effect on these parameters.

**Conclusion.** – Our results indicate that chronic treatment with LA aggravates energy imbalances in diabetic animals. Moreover, our data suggest the need to reconsider the use of LA as an adjuvant in the prevention and treatment of type 1 diabetes.

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Résumé

L’administration chronique d’acide lipoïque aggrave le déséquilibre énergétique de rats rendus diabétiques par la streptozotocine.

**Objectif.** – Notre objectif était d’évaluer le bilan énergétique de rats rendus diabétiques par la streptozotocine et traités au long cours par l’acide lipoïque (AL).

**Méthodes.** – Le diabète a été induit par la streptozotocine et les animaux ont été répartis en quatre groupes, témoins et diabétiques, qui ont reçu pendant 30 jours par voie intrapéritonéale, soit une dose quotidienne de 30 mg/kg d’AL, soit une solution tampon. Le poids, la prise alimentaire, les selles et les urines ont été mesurés chaque jour. Au 30e jour, les animaux ont été sacrifiés. Les carcasses, les fèces et les urines ont été prélevés et préparés pour l’analyse calorimétrique. La glycémie et l’insulinémie ont été aussi déterminées.

**Résultats.** – Tous les paramètres de l’équilibre énergétique ont été modifiés par le diabète. Le traitement par AL est associé à une réduction de la prise de poids, du gain d’énergie et de la valeur énergétique brute des produits alimentaires chez les animaux témoins et diabétiques. Chez les animaux traités par AL, une tendance à l’augmentation des dépenses énergétiques a été notée. La composition corporelle a été également modifiée par le diabète. Une diminution de la masse grasse a été observée chez les animaux témoins et diabétiques traités par AL. Les animaux diabétiques ont présenté une élévation de la glycémie et une diminution de l’insulinémie. L’AL n’a pas influencé ces paramètres.

**Conclusion.** – Nos résultats montrent que l’administration chronique (30 jours) d’AL aggrave le déséquilibre énergétique d’animaux rendus diabétiques par la streptozotocine. De plus, ces données suggèrent de reconsidérer l’utilisation de l’AL comme adjuvant dans la prévention et le traitement du diabète de type 1.

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Keywords: Lipoic acid; Diabetes mellitus; Animal model of diabetes; Rat; Streptozotocin; Energy balance; Energy expenditure; Energy intake

Mots clés : Acide lipoïque ; Diabète sucré ; Streptozotocine ; Modèle animal de diabète ; Rat ; Équilibre énergétique ; Dépenses énergétiques ; Apports énergétiques

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1. Abbreviations

LA  lipoic acid  
ALA  alpha-lipoic acid  
DM  diabetes mellitus  
STZ  streptozotocin  
EDTA  ethylenediaminetetraacetic acid  
BWG  body weight gain  
MEI  metabolizable energy intake  
EG  energy gain  
EE  energy expenditure  
GFE  gross food efficiency  
AEN  absorbed energy  
DNA  deoxyribonucleic acid  
RIA  radio-immunoassay  
UCP  uncoupling protein

2. Introduction

DM is a chronic metabolic disorder triggered by absolute or relative insulin deficiency. Increased EE is characteristic of type 1 diabetes [1,2] as a consequence of higher rates of gluconeogenesis [3], protein synthesis [4] and increased thermogenic responses to noradrenaline (norepinephrine) [5]. Increased EE usually leads diabetic patients into a state of compensatory hyperphagia that, nevertheless, is unable to counteract the increased EE, thus, resulting in body weight loss as described by Yamada et al. [2].

Free radicals and glycation end products have been suggested to contribute to the development and complications of diabetes [6,7]. Some reports point to the important role of oxidative damage in the onset and progression of DM-associated pathologies. Oxidative damage has previously been shown to damage several cellular components, such as lipids [8], proteins [9] and DNA [10]. ALA, also known as “lipoic acid” (LA), is a powerful natural antioxidant recognized as having unique properties in the therapy and prevention of a broad range of disorders [11]. ALA is a naturally occurring substance commonly found in vegetables and animals. It is readily absorbed in the gut, transported to the tissues and taken up by cells, where a large proportion is converted to dihydrolipoic acid (DHLA) [7]. DM is associated with an increased production of reactive oxygen species and a reduction in antioxidant defences, which is partly responsible for diabetic complications, although some minerals and vitamins or cofactors, such as LA, are also able to exert antioxidant activity. Other researchers have found that dietary supplementation with micronutrients may be a complement to classical therapies for preventing and treating diabetic complications [12]. The beneficial effects of ALA, both in the prevention and treatment of diabetes, have been suggested by different investigators [13–15] and at least one study indicates that ALA has beneficial effects on diabetic neuropathy [16], partly due to its actions as an antioxidant and also by improving the circulation in the small blood vessels that supply nerve tissue [15].

Diabetic patients are predisposed to kidney disease and oxidative stress may play a major role in the progression of this diabetic-related problem. Data from Melhem et al. [17] indicate that LA is effective in the prevention of early diabetic glomerular injury. Laboratory studies also indicate that LA reverses the age-associated decline in the proper functioning of mitochondria [18]. Experiments in vitro have shown that both ALA and DHLA are potent scavengers of reactive oxygen species [19]. ALA is a natural antioxidant and cofactor of several enzymes and causes an increase in glutathione, the most abundant intracellular antioxidant in mammals [11], whereas DHLA is capable of regenerating other antioxidants, such as vitamin E and ascorbate [7].

Kim et al. [20] showed that LA reduces body weight, food intake and fat mass when added to the standard chow of Sprague–Dawley and Otsuka Long–Evans Tokushima fatty rats. LA also increases whole-body EE and uncoupling protein-1 (UCP1) mRNA expression in brown adipose tissue as well as the ectopic expression of UCP1 in white adipose tissue. The authors suggest that the antiobesity effect of LA is not dependent on leptin, but takes place by suppression of hypothalamic AMP-activated protein kinase (AMPK) activity. More recently, Kim et al. [21] have described, in a preliminary investigation, that treatment with LA was able to reduce the antipsychotic drug-induced weight gain in patients with schizophrenia.

Considering that the regulation of energy balance involves the control of food intake and EE, the present study was conducted to evaluate the effect of chronic treatment with LA on the energy balance of STZ-induced diabetic rats, a model of solely insulin-deficient diabetes.

3. Material and methods

Thirty-eight three-month-old female Wistar EPM-1 rats were studied. The animal room was maintained at 21 ± 1 °C on a 12-h to 12-h light-to-dark cycle.

DM was induced by a single intraperitoneal dose of STZ (60 mg/kg; Sigma Chemical Company, St Louis, MO, USA) [22] diluted in citrate solution (0.2 M; pH 4.5) [23]. Non-diabetic animals were injected with an equivalent volume of citrate buffer solution. In the first 48 h after STZ administration, the animals were allowed to choose between two glucose solutions (25 g/L and 50 g/L) to compensate for hypoglycaemia. After this period, all animals received water and food ad libitum. At 72 h after STZ injection, one drop of blood was taken from the tail of active animals to determine glycaemia using a blood-glucose meter (Advantage Roche Diagnostics, São Paulo, Brazil). The animals were considered diabetic only if glycaemia was greater or equal to 250 mg/dL. Ten days after either STZ or citrate buffer administration, the animals were kept in individual metabolic cages for 30 days. For each condition (non-diabetic or diabetic), two groups were studied – LA-treated and non-LA-treated animals – resulting in four experimental groups: control non-treated (C); control treated (CLA); diabetic non-treated (D) and diabetic treated (DLA). Treated animals received daily (for 30 days) intraperitoneal 30 mg/kg injections of DL-α-LA (Sigma, St Louis MO, USA) dissolved in 120 mmol/L of Tris buffer (pH 7.4) [24]. Control animals received isovolumetric injections of buffer.
During the experimental period (30 days), all animals received food (Nuvilab CR-1, Nuvital, Paraná, Brazil) and water ad libitum and their body weight and food intake were recorded daily. At the end of the experimental period (day 30), the animals were sacrificed by decapitation and blood was collected in tubes containing heparin or EDTA.

Following sacrifice and blood collection, the abdominal cavity was opened, the gut removed, emptied and placed back in the carcass. The carcass was then weighed (net weight) and, after softening of hard tissues, homogenized in a blender with an equal volume of water. Two 8-g samples were separated from this material to determine the fat and protein contents of the carcasses. The homogenized material was dried in an oven at 60°C until a constant weight was reached (dry weight) and the resulting powder was dry-homogenized. The percentage of water was obtained by calculating the difference between the carcasses’ dry and net weights. Samples were used to determine the energy content in an adiabatic calorimeter (IKA C-5000, Staufen, Germany). Throughout the entire experimental period, faeces and urine were collected daily for energy-content determination.

Food intake was recorded daily and samples of pellets were frequently analyzed in the calorimeter. Energy intake was calculated by multiplying the amount ingested (in grammes) by the energy content (kilojoules) of the diet, where:

- energy intake (kJ) = amount of chow ingested (g) multiplies by energy content of diet;
- AEN (kJ) = energy intake minus energy in faeces;
- MEI (kJ) = AEN minus energy in urine;
- body EG (kJ) = energy in carcasses minus initial body energy.

Initial-body energy was determined from a baseline group of rats killed on day 1 of the experiment from each treatment group. The regression of body weight over body energy was calculated. The initial-body energy of the experimental animals was calculated from their body weight using the above regression equation. Other calculations were:

- EE (kJ) = MEI minus body EG;
- GFE (%) = EG metabolizable/energy intake multiplies by 100;
- percentage of water in the carcass (%) = wet minus dry weight/wet weight multiplies by 100.

Carcass fat and protein contents were determined in fresh samples of homogenized carcasses using the chloroform-methanol and Lowry [25] methods, respectively. Plasma glucose was measured using an enzymatic colorimetric method (Glucose PAP, Labtest Diagnóstica, Minas Gerais, Brazil) and insulin was determined by RIA (Rat Insulin RIA kit, Linco, Ontario, Canada).

All procedures and methods used on these experimental animals were examined and approved by the ethics committee of the Universidade Federal de São Paulo (UNIFESP-EPM) (#089/03) and the animals were cared for in accordance with the Guide to the Care and Use of Experimental Animals.

Statistical analyses were performed by two-way ANOVA for main condition factors (control, diabetic) and treatment (LA-treated, non-LA-treated). In case of interaction between factors, post-hoc analysis was performed by Tukey’s test. The level of significance to reject the null hypothesis was set at 5%.

4. Results

Fig. 1 shows the body weights and food intakes measured during the 30-day experimental period for both C and D animals, treated or not with LA.

Table 1 shows the parameters of energy balance as well as GFE and AEN determined for C and D animals treated or not with LA. As expected, all of the parameters studied were affected by diabetes (\(P<0.05\)). LA treatment led to a significant reduction of BWG, EG and GFE in both C and D animals (\(P<0.05\)). Although the difference was not significant (\(P>0.05\)), there was a tendency in the LA-treated animals to show higher EE than non-treated animals. D animals showed significant increases (\(P<0.05\)) in MEI and percentage of AEN (Table 1).
Body composition (Table 2) was also affected by diabetes (P < 0.05), with fat content being significantly reduced (P < 0.05) by LA treatment in both C and D animals.

Plasma levels of glucose and insulin (Fig. 2) were not affected by LA treatment in either C or D animals (P > 0.05). As expected, D animals showed increased glycaemia and decreased insulinaemia (P < 0.05).

5. Discussion

The present study confirms some of the data previously reported by our and other laboratories regarding the effects of DM on energy balance in rats. The present results are also in agreement with the literature and showed increased EE in diabetic animals. This increase has been described in both humans and animals and is attributed to higher protein catabolism [26,27] in addition to a greater thermogenic response to adrenaline [5]. This means that, to compensate for the increased EE, D animals enhance their metabolizable energy intake. Yet, despite the hyperphagia, D animals were not able to maintain their body-weight and body-EGs at the same levels as in the C animals, which may be explained by the reduced GFE. The loss of GFE is probably due to the high rates of protein synthesis and gluconeogenesis [3] typically seen in diabetic patients. The percentage of AEN was used as an index of absorption capacity and was increased in both LA-treated and non-LA-treated D animals. Higher intestinal-glucose absorption is documented in animal models of diabetes [28] as well as in human patients [29]. As expected, our D animals showed a significant reduction in carcass fat content. Insulin deficiency is usually accompanied by elevated plasma fatty free acid (FFA) concentrations as a result of an increased outflow from fat depots when the balance of the FFA esterification–triaclylglycerol lipolysis cycle is displaced in favour of lipolysis [30]. This reduction of fat depots is the most likely reason for the marked decrease in circulating leptin in STZ diabetic rats [31]. DM led also to a reduction of protein content in the rat carcass and a negative nitrogen balance and marked substrate-wasting is to be expected in diabetic patients as insulin, when present in normal concentrations, stimulates protein synthesis and amino-acid uptake while inhibiting protein catabolism and the output of amino acid from muscle [30].

Khamaisi et al. [24] have reported that diabetic rats treated for 10 days with LA had a reduced fed-state hyperglycaemia (23%). Consistent with our findings, other authors also found no significant alterations in blood glucose or insulin control [13,32] in type 1 diabetic [7,13,33] and obese rats [34] after treatment with LA. It is interesting to note that both our C and D groups presented with raised glycaemia during the experimental period. This elevation can be attributed to the stress imposed on the animals by being kept in metabolic cages for 30 days. It is well known that different stress stimuli can increase glucose plasma levels [35–37].

In the present study, treatment with LA altered some parameters of energy balance and body composition in both D and C animals. Chronic treatment with LA led to reductions in both body weight and body energy, as well as a reduced GFE and fat content in the carcasses of both D and C animals. Both treated groups (CLA and DLA) showed a tendency to increase EE. These results are in agreement with those previously described by Kim et al., whose findings suggested the antiobesity effect of ALA in control animals [20] and humans [21]. Our study

Table 1
Parameters of energy balance (kJ/30 days), gross food efficiency and absorbed energy in control and diabetic animals treated and non-treated with lipoic acid (LA) (means ± S.E.M.).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control animals</th>
<th>Diabetic animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-treated (C) (n = 10)</td>
<td>Treated (CLA) (n = 10)</td>
</tr>
<tr>
<td>BWG (g)</td>
<td>19.0 ± 2.26b</td>
<td>14.0 ± 2.29b</td>
</tr>
<tr>
<td>MEI (kJ)</td>
<td>5928.9 ± 240.9</td>
<td>5902.3 ± 220.6</td>
</tr>
<tr>
<td>EG (kJ)</td>
<td>246.8 ± 68.5b</td>
<td>72.1 ± 57.6b</td>
</tr>
<tr>
<td>EE (kJ)</td>
<td>5682.0 ± 250.3</td>
<td>5831.0 ± 212.9</td>
</tr>
<tr>
<td>GFE (%)</td>
<td>19.0 ± 2.2</td>
<td>12.9 ± 1.4ab</td>
</tr>
<tr>
<td>AEN (%)</td>
<td>76.7 ± 1.1</td>
<td>76.3 ± 1.5</td>
</tr>
</tbody>
</table>

BWG = body weight gain; MEI = metabolizable energy intake; EG = energy gain; EE = energy expenditure; GFE = gross food efficiency; AEN = absorbed energy.

Table 2
Parameters of body composition in the control and diabetic animals treated and non-treated with lipoic acid (LA) (means ± S.E.M.).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control animals</th>
<th>Diabetic animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-treated (C) (n = 10)</td>
<td>Treated (CLA) (n = 10)</td>
</tr>
<tr>
<td>Protein (mg/g)</td>
<td>242.15 ± 17.01</td>
<td>233.99 ± 14.68</td>
</tr>
<tr>
<td>Fat (mg/g)</td>
<td>151.93 ± 16.63</td>
<td>129.75 ± 5.56b</td>
</tr>
<tr>
<td>Water (%)</td>
<td>62.03 ± 0.71</td>
<td>64.20 ± 0.50</td>
</tr>
</tbody>
</table>

a Versus respective controls.

b Versus respective non-treated animals (P < 0.05).
shows, for the first time, the similar effects in type 1 diabetic animals.

In conclusion, our results indicate that chronic LA treatment aggravates the energy imbalances in diabetic animals. Moreover, our data suggest a need to reconsider the use of LA as an adjuvant in the prevention and treatment of type 1 diabetes and diabetic-related problems.

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