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Preliminary report: Leucine supplementation enhances glutamate dehydrogenase expression and restores glucose-induced insulin secretion in protein-malnourished rats

Priscilla Muniz Ribeiro da Silva[a], Claudio Cesar Zoppi[a], Eliane Filiputti[a], Leonardo Reis Silveira[b], Ivan Quesada[c], Antonio Carlos Boschero[a], Everardo Magalhães Carneiro[a,*]

[a]*Department of Anatomy, Cellular Biology and Physiology and Biophysics, Institute of Biology, State University of Campinas (UNICAMP), Caixa Postal 6109, Campinas, SP CEP, 13083-970, Brazil*
[b]*School of Physical Education and Sports, University of Sao Paulo (USP), Ribeirao Preto, SP14040-900, Brazil*
[c]*CIBERDEM and Institute of Bioengineering, University of Miguel Hernández, Elche 03202, Spain*

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Abstract

Low-protein diet impairs insulin secretion in response to nutrients and may induce several metabolic disorders including diabetes, obesity, and cardiovascular disease. In the present study, the influence of leucine supplementation on glutamate dehydrogenase (GDH) expression and glucose-induced insulin secretion (GIIS) was investigated in malnourished rats. Four groups were fed with different diets for 12 weeks: a normal-protein diet (17%) without or with leucine supplementation or a low (6%)-protein diet without (LP) or with leucine supplementation (LPL). Leucine (1.5%) was supplied in the drinking water. Western blotting analysis revealed reduced GDH expression in LP, whereas LPL displayed improved GDH expression, similar to control. The GIIS and leucine-induced insulin release were also enhanced in LPL compared with LP and similar to those observed in rats fed a normal-protein diet without leucine supplementation. In addition, GDH allosteric activators produced an increased insulin secretion in LPL. These findings indicate that leucine supplementation was able to increase GDH expression leading to GIIS restoration, probably by improved leucine metabolic pathways.

1. Introduction

Protein malnutrition impairs glucose-induced insulin secretion (GIIS) by reducing the transcription of several genes related to β-cell signaling [1]. Actually, it has been reported that the insulin signaling pathway is impaired in protein-undernourished rats because of reduced PI3K and p70S6k expression in pancreatic islets [2]. In this process, leucine was demonstrated to improve insulin release [3], an effect that was associated with elevated mitochondrial energy production [4]. Although the mechanism remains unclear, leucine metabolism might supply the tricarboxylic acid cycle (TCA) with different anaplerotic substrates including α-ketoisocaproate (KIC), which can be further metabolized to acetyl–coenzyme A and acetoacetate [5]. Leucine may also allosterically activate glutamate dehydrogenase (GDH), the enzyme that converts glutamate to α-ketoglutarate, an important anaplerotic substrate for the second span of TCA. The observation that β-cells exhibit a high level of anaplerotic mitochondrial enzymes suggests that this process is particularly important for the insulin secretion mechanism [5]. In addition, leucine might activate mammalian target of rapamycin (mTOR) downstream events, affecting elongation and translation of intracellular signaling proteins [6]. In the β-cell line RINm5F, the leucine-induced GDH activation was shown to increase the p70S6k phosphorylation and mitochondrial activity [4], suggesting that leucine might be directly associated with

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The experiments with animals are in adherence with the institutional State University of Campinas Committee for Ethics in Animal Experimentation.

* Corresponding author: Tel.: +55 19 3521 6203; fax: +55 19 3521 6185.
E-mail address: emc@unicamp.br (E.M. Carneiro).

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improvement of insulin release. In addition, preliminary data from our group demonstrated that, after leucine supplementation, undernourished rats exhibit an increased PI3K/mTOR pathway activation as well as enhanced GIIS despite reduced glucose oxidation [7]. However, although leucine plays an important function as an allosteric activator of GDH, whether this enzyme has a role in the amelioration of islet function in malnourished rats has not been examined.

Therefore, the purpose of this work was to test the hypothesis that, in undernourished rats, leucine supplementation restores GIIS capacity via enhanced GDH expression.

2. Material and methods

2.1. Animals and treatment

The experiments were approved by the institutional Committee for Ethics in Animal Experimentation–UNICAMP. Male Wistar rats (21 days old) from the breeding colony at UNICAMP were housed at 24°C on a 12-hour light/dark cycle. Rats were separated into 4 groups of isocaloric diets and treated for 12 weeks with the following: normoprotein diet (NP) (17% protein), normoprotein diet plus leucine (NPL) (17% protein plus leucine supplementation during the last 4 weeks), low-protein diet (LP) (6% protein), and low-protein diet plus leucine (LPL) (6% protein plus leucine supplementation during the last 4 weeks). The 2 isocaloric diets compositions were previously described elsewhere [2]. Leucine (1.5%) was supplied in the drinking water.

2.2. Insulin secretion

Islets were isolated by collagenase digestion of the pancreas [8]. For static incubation, 5 islets from each group were incubated with Krebs-Ringer-bicarbonate containing glucose 5.6 mmol/L for 45 minutes at 37°C as previously described [2]. This medium was then replaced with Krebs-Ringer-bicarbonate with the following secretagogues: glucose (22.2 mmol/L), leucine (20 mmol/L), 2-amino-2-norbornane-carboxylic acid (BCH) (20 mmol/L), and KIC (20 mmol/L) for 1 hour. Samples from independent experiments were collected on different days and stored at −80°C. Insulin concentration was determined by radioimmunoassay method.

2.3. Western blotting assay

Isolated islets were pelleted and resuspended in buffer-containing protease inhibitors, as previously described [9]. Total protein content was determined by the Bradford method. Samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Glutamate dehydrogenase was detected in the membrane after 12 hours of incubation at −4° temperature with a rabbit polyclonal antibody against GDH (Rockland Immunochemicals, Gilbertsville, PA). Antibody was diluted in TRIS-Tween buffer saline (TTBS) containing 30 g/L dry skimmed milk. Detection was performed by chemiluminescence (SuperSignal West Pico; Pierce, Rockford, IL) after incubation with a horseradish peroxidase–conjugated secondary antibody. Band intensities were quantified by optical densitometry (Scion Image, Frederick, MD).

2.4. Statistical analysis

Results are expressed as means ± SEM of the indicated number (n) of experiments. Analysis of variance for unpaired groups, followed by Newman-Keuls post hoc test, was used for multiple comparisons of parametric data. The significance level adopted was $P < .05$.

3. Results

The LP group showed reduced GDH content ($P < .05$), whereas leucine supplementation (LPL) restored its expression to the same levels observed for NP and NPL groups (Fig. 1).

Interestingly, LPL showed improved GIIS to the same level observed in NP. Fig. 2 presents insulin secretion stimulated by several secretagogues and gives clues about the possible metabolic pathways by which leucine supplementation could enhance GIIS. Although similar levels of GDH expression were observed in NP, NPL, and LPL groups, our findings showed higher insulin release in response to glucose, leucine, and KIC in NPL ($P < .05$). In addition, insulin release presented similar profiles among groups. The secretory response to allosteric GDH activators such as leucine and BCH as well as KIC in LP was, as expected, significantly lower ($P < .05$) as compared with control. Remarkably, LPL islets showed improved insulin secretion in response to all other tested secretagogues compared with LP islets. Actually, the secretory response of LPL in the majority of cases was similar to that observed in control NP. Thus, these results indicate that leucine

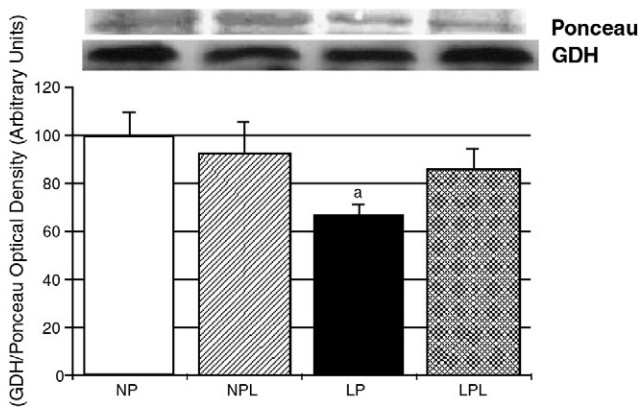

Fig. 1. Increased levels of GDH protein in islets lysates from LPL rats. Western blotting analysis of GDH expression in NP, NPL, LP, and LPL. Values are means ± SEM indicated by vertical bars. Different letters indicate significant differences ($P < .05$) among all groups (n = 7-10).

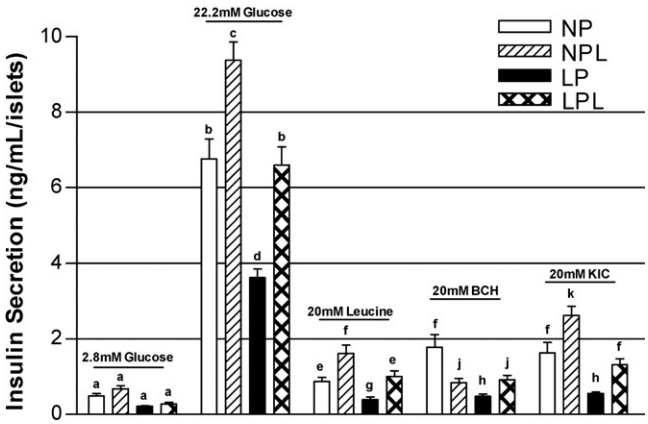

Fig. 2. The LPL rats exhibited increased insulin secretion. Static insulin secretion in NP, NPL, LP, and LPL in response to glucose 2.8 and 22.2 mmol/L, leucine 20 mmol/L, BCH 20 mmol/L, and KIC 20 mmol/L. Values are means ± SEM indicated by vertical bars. Different letters indicate significant differences ($P < .05$) among NP, NPL, LP, and LPL in response to the same secretagogue condition (n = 10).

supplementation enhances GDH activity and leucine oxidation pathway in LPL, which may lead to the increased GIIS during protein undernourishment status.

4. Discussion

Direct evidence linking GDH and GIIS was previously reported by using adenovirus-mediated GDH overexpression and deletion, which increased and reduced GIIS, respectively [10]. Likewise, we showed here in protein malnutrition rats that the reduced β-cell GDH expression and GIIS were restored by leucine supplementation. Xu et al [4] demonstrated that the leucine-induced effect on GDH activity is coupled with higher levels of GIIS and is associated with mTOR pathway. Previous results from our group are in agreement with the aforementioned report suggesting that the leucine-induced increase in PI3K/mTOR activity [7] might be directly related to upper GDH expression also in protein-undernourished rats supplemented with leucine.

Given that the LPL group exhibited both an increased GDH expression and an augmented secretory response to leucine, BCH, and KIC compared with islets from the LP rats, the improved GIIS with leucine supplementation may imply an enhanced allosteric activation of GDH as well as improved leucine oxidation pathway. Indeed, a previous study showed evidence for a possible interaction between GDH and leucine oxidation pathways to enhance leucine-induced insulin secretion in pancreatic islets of BTBR mice [11]. Thus, leucine metabolism would provide higher anaplerotic replenishment, supporting glucose metabolism to increase metabolic coupling factors including adenosine triphosphate, malonyl–coenzyme A, and nicotinamide adenine dinucleotide phosphate (NADPH) [5]. This statement is reinforced by previous results from our group that demonstrated that leucine supplementation did not enhance glucose oxidation in protein-malnourished rats [7]. Taken together, these data suggest that leucine might be the major anaplerotic substrate supplying oxidative substrates to TCA under protein undernourishment.

In conclusion, our results showed that leucine supplementation could restore GIIS in low-protein–fed rats by enhanced GDH expression, activating amino acid–dependent anaplerotic routes. However, further studies are needed to also investigate leucine oxidation pathway alterations to better understand the mechanisms by which leucine supplementation enhances GIIS.

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