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Evaluation of positive NADPH-diaphorase myenteric neurons in diabetic rats supplemented with Acetyl-L-Carnitine

Avaliação de neurônios mioentéricos NADPH-diaforase positivos do íleo de ratos diabéticos suplementados com Acetil-L-Carnitina

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ABSTRACT
The objective was to evaluate supplementation with acetyl-L-carnitine (ALC) on myenteric neurons of the ileum of rats after induction of diabetes. Diabetic animals supplemented with ALC (DC), diabetic (D), normoglycemic animals supplemented with ALC (CC) and normoglycemic (C) were used. NADPH-d neurons were quantified and measured. There was a reduction in blood glucose and water intake in the DC group. The neuronal density in 12.72mm² of ileum was similar in the four groups (p>0.05): DC (558.8 ± 220.2), D (513.4 ± 72.01), CC (645.2 ± 144.9) and C (934 ± 248.5). The mean cell body area of neurons (µm²) in diabetic animals, DC (303.9 ± 114.2) and D (285.4 ± 111.8), were greater than in the normoglycemic groups, CC (173.6 ± 53.78) and C (158.4 ± 53.73). The ileum area (mm²) was larger in animals of the diabetic groups, CD (190.96) and D (171.62) compared to the normoglycemic groups: CC (138.04) and C (130.04). However, in the DC group, both areas were larger than in D (p<0.05). Thus, a slight increase in neuronal population can be inferred. The data indicated that ALC did not interfere with mechanisms that promote an increase in the production of nitric oxide (NO) by myenteric neurons of the ileum and that the greater dilation of the ileum in the DC group could be the result of a side effect of the dose of carnitine used.

Keywords: Gastrointestinal tract. Myenteric plexus. Nitric oxide.

RESUMO
Objetivou-se avaliar a suplementação com acetil-L-carnitina (ALC) sobre os neurônios mioentéricos do íleo de ratos após a indução de diabetes. Foram usados animais diabéticos suplementados com ALC (DC), diabéticos (D), normoglicêmicos suplementados com ALC (CC) e normoglicêmicos (C). Neurônios NADPH-d foram quantificados e mensurados. Observou-se redução na glicemia e na ingestão de água no grupo DC. A densidade neuronal em 12,72mm² de íleo foi semelhante nos quatro grupos (p>0,05): DC (558,8 ± 220,2), D (513,4 ± 72,01), CC (645,2 ± 144,9) e C (934 ± 248,5). A área média do corpo celular dos neurônios (µm²) nos animais diabéticos, DC (303,9 ± 114,2) e D (285,4 ± 111,8), foram maiores que nos grupos normoglicêmicos, CC (173,6 ± 53,78) e C (158,4 ± 53,73). A área do íleo (mm²) também mostrou-se maior nos animais dos grupos diabéticos, DC (190,96) e D (171,62) quando comparados aos normoglicêmicos: CC (138,04) e C (130,06). Entretanto no grupo DC,

ambas as áreas foram maiores que no D (P<0,05). Assim, pode se inferir discreto incremento na população neuronal. Os dados indicaram que a ALC não interferiu nos mecanismos que promovem aumento na produção de óxido nítrico (NO) pelos neurônios mioentéricos do íleo e que a maior dilatação do íleo no grupo DC poderia ser resultante de efeito colateral da dose de carnitina empregada.

Palavras-chave: Plexo mioentérico. Óxido nítrico. Trato gastrintestinal.

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INTRODUCTION

The relationship between hyperglycemia and diabetic neuropathy has been widely documented in the literature; although its etiology is not fully understood. It is suggested that metabolic disorders, such as: hyperactivity of the polyol pathway, reduced levels of neurotrophic factors, activation of protein kinase C, non-enzymatic glycosylation of Schwann cells[1]; as well as vascular changes[2] and endoneural hypoxia[3]; immunological mechanisms[4]; oxidative stress[5]; and impaired lipid metabolism, including changes in carnitine levels[6], would be the preponderant factors for triggering of neuropathy[7].

Carnitine is important in the oxidation of long-chain fatty acids, transporting them into the mitochondria[8]. Clinical and experimental studies state that the administration of carnitine prevents changes in nerve conduction, contributes to nerve regeneration[9], acting on neuroprotection[10], in addition to exhibiting analgesic effects on the peripheral nervous system[11].

Changes in motility in the gastrointestinal tract are common symptoms of diabetes mellitus (DM) that are manifested by episodes of diarrhea and constipation, as well as gastroparesis[12]. These changes have been associated with poor glycemic control, which in turn is linked to neuropathy.

DM affects differently the subpopulations of enteric neurons, causing a reduction in the number of these neurons[13] in the short or long term, in all segments of the gastrointestinal tract; in addition to causing changes in the levels of different neurotransmitters[14]. Thus, it is feasible that the neuropathy resulting from DM is not selective[15], as it does not affect the myenteric neurons with the same intensity and extension, and it is possible to verify a reduction in some types of neurotransmitters in some neurons, but not in others[16].

Nitric oxide (NO) is the main non-adrenergic and non-cholinergic inhibitory neurotransmitter in the gastrointestinal tract. The role of NO as a link between the neuropathogenic, metabolic and vascular factors triggering diabetic neuropathy has

been proposed; considering that the conversion of glucose to sorbitol by aldose reductase, the synthesis of NO from arginine and the mechanism of glutathione compete for the same cofactor: NADPH[17].

Considering the relevance of neuropathies as one of the chronic degenerative complications of diabetes, as well as the relationship between the reduction of serum and tissue carnitine levels and the pathogenesis of diabetic neuropathy, this study was carried out with the objective of evaluating morphometry and quantification of the population of positive NADPH-diaphorase neurons from the myenteric plexus of the ileum of rats with diabetes induced by streptozootocin and supplemented with acetyl-L-carnitine (ALC); since changes in this neuronal population lead to gastrointestinal dysfunctions such as diarrhea and constipation in diabetic individuals, ALC could serve as an agent of preservation of these neurons contributing to decrease the clinical signs of the pathology.

METHODOLOGY

PROCEDURE WITH ANIMALS

This research was approved by the Animal Experimentation Ethics Committee of the State University of Maringá, according to opinion 161/2003. The techniques used were submitted to the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA). For this purpose, 20 male adult *Wistar* rats (Central Vivarium of the State University of Maringá) were used. At 103 days of age, animals with an average of 350g were transferred to the vivarium of the Department of Morphological Sciences, where they remained in individual cages, kept under controlled ambient temperature conditions (22 ± 2 °C) and light/dark cycle (12/12 hours) and water and food (Nuvilab®) *ad libitum*.

After two days of adaptation to the new environment, rats were weighed and started to be monitored for the experimental period of 105 days. Rats were randomly assigned to four groups containing five (5) animals each, according to the treatments they were subjected to: diabetic supplemented with ALC (group DC); diabetic (group D); normoglycemic supplemented with ALC (group CC); and normoglycemic (group C). Rats in groups C and D received tap water, while those in groups CC and DC received water plus acetyl-L-carnitine-ALC (Spfarma, São Paulo, Brazil), at a daily dose of 200 mg/kg, in an amber bottle. Water intake was monitored daily, and body mass. every 15 days.

For diabetes induction, rats in groups D and DC were fasted for fourteen hours and received an intravenous injection (penile vein) of streptozootocin (Sigma, ST. Louis, MO, USA) at a dose of 35 mg/kg, dissolved in 10 mmol/L citrate buffer (pH 4.5). On the

fourth day after induction, blood glucose was measured (Accu-Chek Active, Roche Diagnostics GmbH, Mannheim, BW, Germany) to confirm the establishment of experimental diabetes. All animals in groups D and DC had glucose levels above 210 mg/dL.

At 210 days of age, rats were anesthetized with Thiopental® (40 mg/kg I.P; Abbott Laboratories, Chicago, IL, USA) and subsequently, cardiac puncture and laparotomy were performed. These last two procedures resulted in the animals' death from hypovolemia. Blood collected by cardiac puncture was used to measure glucose levels, using the glucose oxidase and glycated hemoglobin method.

HISTOCHEMISTRY FOR NADPH-DIAPHORASE

The ileum obtained from each animal was washed and filled with phosphate buffer (0.01M PB, pH 7.4) and fixed in 4% paraformaldehyde (Merck, Darmstadt, Germany) for 30 minutes. Subsequently, it was immersed in 0.3% Triton X-100® (Sigma, St. Louis, USA) dissolved in saline phosphate buffer (PBS, 1 M, pH 7.4) for 10 minutes and washed ten times in PBS (10 minutes each) at room temperature. To demonstrate positive NADPH-diaphorase, myenteric neurons (NADPH-d), according to Scherer-Singler et al.[18], the ileum was incubated for 2h30 under agitation in medium containing for each 200 mL of 0.1 M Tris-HCL buffer (GibcoBRL, NY , USA) pH 7.6: 100 mg of β-NADPH reduced form (Sigma, Steinheim, Germany); 50 mg nitro blue tetrazolium (Sigma Chemical, USA); and 6 mL 0.3% Triton X-100®. After incubation, the ileum was opened at the ends, washed three times in 0.01 M PBS for 5 minutes each and immersed in a 4% paraformaldehyde solution.

After fixation, the ileum was opened along the mesenteric insertion and its circumference was measured. A cross-sectional sample of approximately 1 cm was isolated and microdissected using a stereomicroscope with transillumination to remove the mucous membrane and submucosal tissue, preserving the muscular and serous tissue. Each membrane preparation was dehydrated in an ascending series of ethanol, cleared in xylol and mounted between slide and cover slip with Permount resin (FisherChemical, USA).

QUANTITATIVE AND MORPHOMETRIC ANALYSIS

Quantitative analysis was performed in the antimesenteric (120° to 240°) and intermediate (60° to 120°; 240° to 300°) regions, considering 0 as the mesenteric border[19] in each membrane preparation. Myenteric neurons were then randomly counted, using an Olympus CBA light microscope, with a 40X objective, in 40 microscopic fields of the antimesenteric region and 40 intermediate fields in each

membrane preparation. The area of each field was 12.72 mm^2. Half-neurons were counted in alternate fields.

For morphometry, the computerized image analysis program Image-Pro-Plus 3.0.1 was attached to the Leica DM RX microscope. In the membrane preparation obtained from the ileum of each animal, the cell body area (μm^2) of 100 neurons (50 from the intermediate region and 50 from the antimesenteric region) was measured, making a total of 500 neurons in each group. Neurons were grouped in groups of class intervals of 100 μm^2 and the incidence (%) of each group for each interval was obtained.

STATISTICAL ANALYSIS

Data obtained were tested by One-Way analysis of variance and *Tukey* post-hoc test, all with a significance level of 5%. Data are presented as mean + standard deviation.

RESULTS

The physiological parameters of rats from groups of diabetic animals supplemented with ALC (CD) and diabetics (D) showed typical signs of polydipsia, hyperglycemia and elevated levels of glycated hemoglobin (Table 1).

Table 1. Daily water consumption (DWC), blood glucose (Gl) and glycated hemoglobin (HbG) of animals in the groups: diabetic supplemented with ALC (DC), diabetic (D), normoglycemic supplemented with ALC (CC) and normoglycemic (C). Results are expressed as mean ± standard error of the mean (n = 5 rats per group)

Parameters / Groups	DC	D	C	CC
DWC/mL	156.6 ± 4.9^b	173.4 ± 5.2^a	51.1 ± 1.1^c	62.13 ± 0.9^c
Gl/ $mg.dl^{-1}$	286 ± 21.1^b	362.4 ± 15.1^a	99.8 ± 5.9^c	105.4 ± 11.2^c
HbG/ %	6.7 ± 0.3^a	6.8 ± 0.2^a	3.9 ± 0.2^b	3.9 ± 0.2^b

Means followed by different letters, in the same row, are significantly different ($p<0.05$).

Supplementation with ALC reduced ($p<0.05$) water intake and blood glucose in the DC group. However, there were no differences ($p>0.05$) in the level of glycated hemoglobin between the groups of diabetic rats (Table 1).

The average neuronal density in 12.72 mm^2 of ileum and the average cell body area of 500 positive NADPH-d neurons in the four groups are listed in Table 2. No significant difference ($p>0.05$) was detected in neuronal density between all groups. There was an increase ($p<0.05$) in the area of the neuronal cell body of diabetic rats supplemented or not in relation to the control groups.

Table 2. Ileum area, neuronal density, cell body area of NADPH-d positive myenteric neurons from rats in the groups: diabetic supplemented with acetyl-L-carnitine (DC), diabetic (D), normoglycemic supplemented with acetyl-L-carnitine (CC) and normoglycemic (C)

Group	Ileum area (mm^2)	Neuronal density	Cell body area (μm^2)
DC	190.96[a]	558.8 ± 220.2[a]	303.9 ± 114.2[a]
D	171.62[b]	513.4 ± 72.01[a]	285.4 ± 111.8[b]
CC	138.04[c]	645.2 ± 144.9[a]	173.6 ± 53.78[c]
C	130.06[c]	934 ± 248.5[a]	158.4 ± 53.73[d]

Means followed by different letters, in the same row, are significantly different (p<0.05).

In the DC and D groups, neurons with a cell body area equal to or larger than 200 μm^2 predominated. In groups C and CC, the incidence was higher for neurons with a cell body area smaller than 200 μm^2 (Table 3).

Table 3. Frequency distribution (F) of reactive NADPH-d myenteric neurons in the ileum of rats of the groups: diabetic supplemented with acetyl-l-carnitine (DC), diabetic (D), normoglycemic supplemented with acetyl-L-carnitine (CC) and normoglycemic (C) according to the cell body area by class intervals of 100 μm^2.

Cell body area	DC		D		CC		C	
	F	%	F	%	F	%	F	%
> 100	04	0.8	05	1	30	6	60	12
100 to 200	82	16.4	128	25.6	335	67	348	69.6
200 to 300	195	39.0	174	34.8	120	24	83	16.6
300 to 400	127	25.4	113	22.6	15	3	09	1.8
400 to 500	60	12	58	11.6	-	-	-	
> 500	32	6.4	22	4.4	-	-	-	
TOTAL	500	100	500	100	500	100	500	100

DISCUSSION

Diabetic animals (groups DC and D) exhibited a typical clinical condition of diabetes, throughout the experiment. In our study and in Parvanova et al.[20], there was no difference in glycated hemoglobin in diabetic and diabetic rats supplemented with CLA under varying dosage and time conditions; however, the reduction in fasting glycemia in rats supplemented with CLA observed in our study may be an indication that carnitine may influence the distribution of plasma glucose. This corroborates data by Muoio et al.[21], who reported that carnitine improved glucose metabolism in insulin-resistant humans due to increased metabolic

flexibility; as well as increased the sensitivity of cells to insulin[22].

On the other hand, if we consider that glycated hemoglobin values were similar in the DC and D groups, this reduction in glycemia should not be considered relevant in conditions of diabetes, since the glycated hemoglobin test is a parameter that reflects the average concentration of glycemia to which the erythrocytes were exposed during their stay in the blood[23]. Based on the results of glycated hemoglobin, it can be considered that the degree of diabetes was similar for diabetic rats with and without ALC supplementation. Thus, it is possible to investigate the impact of this supplementation on the population of positive NADPH-diaphorase neurons of the myenteric plexus of the ileum, in the condition of diabetes.

Regarding the population of myenteric neurons NADPH-d of the ileum, in the present experimental condition, ALC did not significantly interfere with neuronal density, either in the comparison between the DC and CC groups, or in the comparison between the DC and D groups. D. It should be noted that animals in group D had neuronal density 45.03% lower than animals in group C, while the total ileum area was 31.95% larger, suggesting neuronal loss by 13.08%. In diabetic animals supplemented with carnitine, neuronal density was 40.17% lower than in group C, while the ileum had a 46.82% larger area, suggesting a neuronal preservation of approximately 6.65%, which is statistically non-significant. In a study by Miranda Neto et al.[24] comparing the colon of diabetic animals with and without ALC supplementation, a reduction in neuronal density associated with significant organ dilation with a concomitant numerical increase in the population of NADPH-d myenteric neurons by 39.9% for the diabetic group and 72% for the diabetic group treated with ALC.

In acute or chronic diabetes, a decrease in the density of the general population of myenteric neurons has been reported[16,25] and in neurons evidenced by the NADH-d histochemical method[26]. Neuronal labeling by NADPH-d histochemistry, however, shows approximately 50% of NADH-d neurons in the small intestine of rats[27], meaning that the decrease in the number of NADH-d neurons does not necessarily imply an equivalent decrease in NADPH neurons-d. The unchanged density of NADPH-d positive neurons suggests that nitrergic neurons are more resistant to pathophysiological conditions resulting from diabetes and cell death[24], thus justifying the neuronal density found for rats in groups DC and D.

On the other hand, the significant increase in the ileum areas of animals in the two diabetic groups, without the corresponding increase in the number of nitrergic neurons, suggests that this increase is related to the reduction in muscle tone due to the greater performance of nitrergic neurons, which are inhibitory. This

hypothesis is reinforced by the fact that the cell area of this neuronal category is significantly increased in diabetics when compared to controls, which generally results from the increase in the neuron synthesis machinery. In the diabetic groups, supplemented or not, neurons with a cell area larger than 200 μm^2 predominated, while in the respective control groups, neurons with a cell area smaller than 200 μm^2 prevailed. Ferreira et al.[28] also found an increase in the area of cell bodies of nitrergic neurons in the small intestine of rats with chronic diabetes.

As with NO, it is also possible that other inhibitory neurotransmitters are increased and/or that there is a decrease in excitatory neurotransmitters interfering with the activity of nitrergic neurons. According to these observations, Defani et al.[29] found an increase in the area of cell bodies of VIP-ergic neurons in the submucosal plexus of the rat jejunum after supplementation with ALC (200 mg/kg), both in diabetic and non-diabetic rats. Miranda Neto et al.[24] observed a decrease in the cellular area of myenteric neurons in the colon of diabetic rats supplemented with ALC.

In addition, Tomlinson et al.[30] observed that the reduction of extrinsic adrenergic innervation of the stomach, related to diabetic neuropathy, could also lead to a compensatory increase in the performance of intrinsic innervation, in an attempt to supply the gastric muscle relaxation induced by the sympathetic pathway. Thus, both the extrinsic denervation and the diabetic state, could promote an increase in the expression of nitric oxide synthase (NOS) generating a large production of NO, with a consequent increase in the cellular area of the myenteric nitrergic neurons of diabetic rats.

As the changes in the small intestine are more evident and extensive than those in the colon, Belai et al.[15] relate the lower sensitivity to the difference in extrinsic innervation and motor activity of the colon in relation to other regions. This fact could justify the differences in results found in the ileum and colon. The increase in cell body area supplemented with ALC described by Defani et al.[29] and what was found in nitrergic neurons in our experiment would not manifest in the colon simultaneously with the ileum.

Supplementation with ALC in the dosage used in our experiment, therefore, does not seem to contribute to a decrease in NO production by myenteric neurons of the ileum, since the area of the cell body of NADPH-d neurons was greater ($p<0.05$) in the supplemented group, when compared with the other groups. The increase in the cell body area was also observed in the CC group in relation to the C group, which may be related to the possible neurotrophic effect of ALC, which in diabetes would not be able to minimize the production of NO. Another fact verified with the dosage of ALC used in this study, is that in the supplemented normoglycemic animals, there was a

weakening of the ileum wall during the microdissection of membrane preparations, suggesting a possible side effect of this substance, since its storage is dose-dependent, that is, the greater the content of carnitine from the diet, the greater its content in the tissues.

CONCLUSION

In conditions of diabetes, ALC supplementation decreased fasting glycemic levels, but was not able to interfere with the density of positive NADPH-d neurons and in the physiological mechanisms that promote an increase in the production of NO by myenteric neurons in the ileum, the increase in the cell body area of neurons in supplemented normoglycemic animals has been suggestive of the neurotrophic action of ALC. Although the use of carnitine has been encouraged for its beneficial effects in several other organic disorders, it is still necessary to carry out further studies to standardize its dosage to avoid possible harmful effects that could result from its administration.

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REFERENCES

1. Naruse K. Schwann Cells as Crucial Players in Diabetic Neuropathy. In: Myelin. Springer; 2019. p. 345–56.

2. Wołoszyn-Durkiewicz A, Myśliwiec M. The prognostic value of inflammatory and vascular endothelial dysfunction biomarkers in microvascular and macrovascular complications in type 1 diabetes. Pediatr Endocrinol Diabetes Metab. 2019;25(1).

3. Fang F, Wang J, Wang YF, Peng YD. Microangiopathy in diabetic polyneuropathy revisited. Eur Rev Med Pharmacol Sci. 2018;22(19):6456–62.

4. Kuhn C, Besancon A, Lemoine S, You S, Marquet C, Candon S, et al. Regulatory mechanisms of immune tolerance in type 1 diabetes and their failures. J Autoimmun. 2016;71:69–77.

5. Malik A, Morya RK, Bhadada SK, Rana S. Type 1 diabetes mellitus: Complex interplay of oxidative stress, cytokines, gastrointestinal motility and small intestinal bacterial overgrowth. Eur J Clin Invest. 2018;48(11):e13021.

6. Ramazani M, Qujeq D, Moazezi Z. Assessing the levels of L-carnitine and total antioxidant capacity in adults with newly diagnosed and long-standing type 2 diabetes. Can J diabetes. 2019;43(1):46–50.

7. Archvadze A, Kistauri A, Gongadze N, Makharadze T, Chirakadze K. MEDICAL BASIS OF DIABETIC NEUROPATHY FORMATION. Georgian Med News. 2018;(283):154–62.

8. Bene J, Hadzsiev K, Melegh B. Role of carnitine and its derivatives in the development and management of type 2 diabetes. Nutr Diabetes. 2018;8(1):1–10.

9. Tomassoni D, Mannelli LDC, Bramanti V, Ghelardini C, Amenta F, Pacini A. Treatment with acetyl-L-carnitine exerts a neuroprotective effect in the sciatic nerve following loose ligation: a functional and microanatomical study. Neural Regen Res. 2018;13(4):692.

10. Ferreira GC, McKenna MC. L-Carnitine and acetyl-L-carnitine roles and neuroprotection in developing brain. Neurochem Res. 2017;42(6):1661–75.

11. Sergi G, Pizzato S, Piovesan F, Trevisan C, Veronese N, Manzato E. Effects of acetyl-L-carnitine in diabetic neuropathy and other geriatric disorders. Aging Clin Exp Res. 2018;30(2):133–8.

12. Kurniawan AH, Suwandi BH, Kholili U. Diabetic Gastroenteropathy: A Complication of Diabetes Mellitus. Acta Med Indones. 2019;51(3):263–71.

13. Pereira RVF, Linden DR, Miranda-Neto MH, Zanoni JN. Differential effects in CGRPergic, nitrergic, and VIPergic myenteric innervation in diabetic rats supplemented with 2% L-glutamine. An Acad Bras Cienc. 2016;88:609–22.

14. Hermes-Uliana C, Panizzon CP do NB, Trevizan AR, Sehaber CC, Ramalho FV, Martins HA, et al. Is L-glutathione more effective than L-glutamine in preventing enteric diabetic neuropathy? Dig Dis Sci. 2014;59(5):937–48.

15. Belai A, Lincoln J, Milner P, Burnstock G. Differential effect of streptozotocin-induced diabetes on the innervation of the ileum and distal colon. Gastroenterology. 1991;100(4):1024–32.

16. Ferreira PEB, Lopes CRP, Alves AMP, Alves ÉPB, Linden DR, Zanoni JN, et al. Diabetic neuropathy: an evaluation of the use of quercetin in the cecum of rats. World J Gastroenterol WJG. 2013;19(38):6416.

17. Stevens MJ. Nitric oxide as a potential bridge between the metabolic and vascular hypotheses of diabetic neuropathy. Diabet Med. 1995;12(4):292–5.

18. Scherer-Singler U, Vincent SR, Kimura H, McGeer EG. Demonstration of a unique population of neurons with NADPH-diaphorase histochemistry. J Neurosci Methods. 1983;9(3):229–34.

19. Miranda-Neto MH, Molinari SL, Natali MRM, Sant'Ana D de MG. Regional differences in the number and type of myenteric neurons of the ileum of rats: a comparison of techniques of the neuronal evidentiation. Arq Neuropsiquiatr. 2001;59(1):54–9.

20. Parvanova A, Trillini M, Podestà MA,

Iliev IP, Aparicio C, Perna A, et al. Blood pressure and metabolic effects of acetyl-L-carnitine in type 2 diabetes: DIABASI randomized controlled trial. J Endocr Soc. 2018;2(5):420–36.

21. Muoio DM, Noland RC, Kovalik J-P, Seiler SE, Davies MN, DeBalsi KL, et al. Muscle-specific deletion of carnitine acetyltransferase compromises glucose tolerance and metabolic flexibility. Cell Metab. 2012;15(5):764–77.

22. Giancaterini A, De Gaetano A, Mingrone G, Gniuli D, Liverani E, Capristo E, et al. Acetyl-L-carnitine infusion increases glucose disposal in type 2 diabetic patients. Metab Exp. 2000;49(6):704–8.

23. Eyth E, Naik R. Hemoglobin A1C. In: StatPearls [Internet]. StatPearls Publishing; 2019.

24. De Miranda Neto MH, Defani MA, Fregonesi C, Natali MRM, Pereira A. Morphometric and quantitative evaluation of the NADH-diaphorase positive myenteric neurons of the jejunum of streptozotocin-diabetic rats supplemented with acetyl-L-carnitine. Anat Histol Embryol. 2005;34(3):154–8.

25. Panizzona CP do NB, de Miranda Netoa MH, Ramalhoa FV, Longhinib R, de Mellob JCP, Zanonia JN. Ethyl Acetate Fraction from Trichilia catigua Confers Partial Neuroprotection in Components of the Enteric Innervation of the Jejunum in Diabetic Rats. Cell Physiol Biochem. 2019;53:76–86.

26. Pereira Alves AM, de Paula AL, Moreira CR, Ferrari F, Coelho Leal JP, Hatoum US, et al. Effects of Quercetin-Supplementation in NADH-Diaphorase Positive Neurons Subpopulations in the Ileum of Rats with Experimental Diabetes Mellitus. Int J Morphol. 2017;35(1):236–41.

27. Santer RM. Survival of the population of NADPH-diaphorase stained myenteric neurons in the small intestine of aged rats. J Auton Nerv Syst. 1994;49(2):115–21.

28. Ferreira PEB, Beraldi EJ, Borges SC, Natali MRM, Buttow NC. Resveratrol promotes neuroprotection and attenuates oxidative and nitrosative stress in the small intestine in diabetic rats. Biomed Pharmacother. 2018;105:724–33.

29. Defani MA, Zanoni JN, Natali MRM, Bazotte RB, Miranda-Neto MH de. Effect of acetyl-L-carnitine on Vip-ergic neurons in jejunum submucous plexus of diabetic rats. Arq Neuropsiquiatr. 2003;61(4):962–7.

30. Tomlinson KC. Functional consequences of streptozotocin-induced diabetes mellitus, with particular reference to the cardiovascular system. Pharmacol Rev. 1992;44:103–50.