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BIOMEDICAL SCIENCES

β-Cyclodextrins alter the energy metabolism-related enzyme activities in rats

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Abstract: Although widely used in medicine, separation technology, and other fields, the effects of cyclodextrins on the activities of phosphoryl transfer enzymes have not been previously evaluated. *In vivo* studies evaluated the function of cyclodextrins as active compounds. Despite the use of cyclodextrins as active compounds, the effects of cyclodextrins on hepatic and renal tissues remain to be fully elucidated. The primary objective of this study was to evaluate the effects of β- cyclodextrins, methyl-β-cyclodextrin (M-β- cyclodextrins), and (2-hydroxypropyl)-β-cyclodextrin (HP-β-cyclodextrins) on enzyme activities regulating the maintenance of energy homeostasis in the kidney and liver tissues in relation to toxicity. Serum levels of liver and kidney markers were measured, and oxidative stress parameters were assessed. After 60-day treatments, we observed that the administration of β-cyclodextrins and M-β-cyclodextrins inhibited the hepatic activity of pyruvate kinase, an irreversible enzyme within the glycolytic pathway. Additionally, administration of HP-β-cyclodextrins inhibited creatine kinase activity and increased the total sulfhydryl content in kidneys. Here, we demonstrated for the first time that β-cyclodextrins, M-β-cyclodextrins, and HP-β-cyclodextrins cause bioenergetic dysfunction in renal and hepatic tissues. These findings suggest that understanding the balance between cyclodextrins' efficacy and adverse effects is essential for better accepting their use in medicine.

Key words: creatine kinase, adenylate kinase, pyruvate kinase, oxidative stress, biochemical, blood serum.

INTRODUCTION

The α-, β-, and γ-cyclodextrins (CDs) are cyclic oligosaccharide molecules. These molecules possess nanometric cavities and are composed of 6 – 8 D-glucose units (Souza et al. 2016). These cavities are hydrophobic and interact with drugs that are poorly soluble in water. This reaction forms inclusion complexes that improve the biocompatibility of drugs and their use within biological environments. This property promotes the sustained release of drugs (Yang et al. 2013) and allows for the lodging of organic compounds. Hydroxyls in CDs cause the external environment to become hydrophilic (Souza et al. 2016).

For these attributes, CDs have been widely used for applications in the pharmaceutical field, the pesticide, foodstuffs, toilet articles, and textile processing industries, and for supramolecular host-guest chemistry and molecular encapsulation (Gao et al. 2006, Mura 2020, Tang et al. 2020). CDs can interact

with cholesterol and phospholipids in the biomembranes of the gastrointestinal tract. They can also pass through the stomach and small intestine but–are degraded in the intestinal colon. This characteristic is associated with the potential adaptability of these drug carriers (Uekama et al. 2006).

βCDs possessing either methyl (M-βCD) or hydroxypropyl (HP-βCD) groups have been used in pharmaceutical preparations, as toxicity studies have shown that these CDs are practically non-toxic after oral administration (Mura 2020). Nishihira et al. (2017) demonstrated that resveratrol complexed with HP-β-CD did not cause any significant differences in hematological and biochemical parameters. Therefore, CDs can carry hydrophobic molecules in an aqueous environment, act as non-toxic and biocompatible carriers, and are widely used throughout the pharmaceutical field (Monteil et al. 2017, Souza et al. 2018).

However, the effects of CDs on the activities of phosphoryl transfer enzymes, pyruvate kinase (PK), creatine kinase (CK), and adenylate kinase (AK) have yet to be previously evaluated. The coupling between the processes of consumption and production of cellular ATP must be tightly balanced, and this coupling is fundamental for various metabolic processes within living organisms and cell signaling (Rech et al. 2018). The enzymes PK, CK, and AK allow this connection between cell compartments by transferring the γ-phosphoryl group of ATP from mitochondria to the cytosol (Dzeja & Terzic 2003). Based on this, these enzymes are crucial for maintaining cellular energy homeostasis in most mammalian tissues. Imbalances between the flow and distribution of cellular energy have been associated with cardiovascular and neurodegenerative diseases, and these imbalances can influence the uncontrolled growth and metastatic potential of tumor cells (Dzeja & Terzic 2003, 2009). A decrease in the functions of these enzymes may be associated with a crucial role in the physiopathology of neurodegeneration and the subsequent loss of neurons (Bortoluzzi et al. 2014). Consequently, the study of the activities of these enzymes is critical to corroborate reports that CDs are not toxic to living organisms.

In addition to the phosphotransfer network system, oxidative stress is associated with several diseases, including neurodegenerative processes. Oxidative stress is an imbalance in relation to the pro-oxidant side of pro-oxidant/antioxidant homeostasis that occurs in several human diseases, including diabetes, chronic renal failure, and respiratory distress syndrome (Dalle-Donne et al. 2003). Yalcin et al. (2016) demonstrated that using HP-βCD microspheres decreased lipid peroxidation levels and the production of reactive oxygen species in a model of Aβ (1-42)-induced neurotoxicity. Considering the importance of CDs as drug carriers or as active compounds, we evaluate the effect of βCD administration on energy parameters and oxidative stress in hepatic and renal tissues to better understand their mechanisms of action.

MATERIALS AND METHODS

Animals and Reagents

Thirty-two male Wistar rats (sixty-day-old) were obtained from the Central Animal House of the Federal University of Santa Maria and used in the experiments. The animals were maintained on a 12:12 h light/dark cycle in a room acclimatized at constant temperature (22 ± 1 °C). The rats were provided free access to water and commercial chow (Guabi, RS, Brazil). The Ethics Committee approved the experimental protocol for Animal Research of the Franciscan University, Santa Maria, Brazil, under protocol number 010/2011 and followed the 'Principles

of Laboratory Animal Care' (NIH publication 85-23, revised 1996). All chemicals, including βCD, M-βCD, and HP-βCD, were purchased from Sigma (St. Louis, MO, USA).

Treatment of Rats

The animals were randomly divided into four groups of eight rats, each that included groups I (Control), II (βCD), III (M-βCD), and IV (HP-βCD). The animals received intraperitoneal treatments of saline solution (0.9% NaCl), 66 mg βCD/kg (body weight), M-βCD/kg (body weight), and HP-βCD/kg (body weight), respectively, for 60 days. These doses were chosen based on previous Bellringer et al. (1995) data. The intraperitoneal route was chosen according to Trautwein et al. (1999), as some CDs interfered with the absorption of certain nutrients.

Preparation of Hepatic and Renal Tissues

After sixty days of treatment, the animals were euthanized by decapitation. The liver and kidneys were quickly removed, dissected, and frozen at -80 °C for no more than a week until the determination of energy metabolism and oxidative stress parameters.

Kidneys and liver were homogenized to evaluate the parameters of the phosphoryl transfer network (1:10, w/v with a Potter–Elvehjem glass homogenizer) in SET buffer (0.32 M sucrose, 1 mM EGTA, 10 mM Tris-HCl, pH 7.4). The homogenate was centrifuged at 800 × *g* for 10 min at 4 °C to discard nuclei and cell debris, and the pellet was discarded. The supernatant that contained a suspension of mixed and preserved organelles, including mitochondria, was then separated and fractionated. An aliquot of the supernatant was used for AK activity determination. The pellet was discarded, and the remaining supernatant was centrifuged at 10,000 × *g* for 15 min at 4 °C.

The supernatant of this second centrifugation corresponded to the cytosolic fraction. It was collected to determine PK and cytosolic CK activities (CKcyt). The pellet, containing mitochondria, myelin, synaptosomes, and membrane fragments, was washed twice with the same Tris–sucrose isotonic buffer and then resuspended in 100 mM $MgSO_4$–Trizma buffer (pH 7.5) for determination of mitochondrial CK activity (CKmit). The samples were stored for no more than a week at -80 °C. The other half of the liver and the other kidneys were weighed and homogenized (1:10, w/v) in 20 mM sodium phosphate buffer (pH 7.4) containing 140 mM KCl. Homogenates were centrifuged at 800 × *g* for 10 min at 4 °C, and the pellet was discarded. The supernatant containing a suspension of mixed and preserved organelles, including mitochondria, was separated and used for the various oxidative stress analyses.

Determination of energy parameters

The activities of CK, PK, and AK enzymes were determined in liver and kidney homogenates according to the method of Hughes (1962), Leong et al. (1981), and Dzeja et al. (1999), respectively. The results were expressed as βmol of creatine formed per min per mg of protein for CK, as βmol of pyruvate formed per min per mg of protein for PK, and as βmol of ATP formed per min per mg of protein for AK.

2'7' Dihydrodichlorofluorescein oxidation assay (H2DCF-DA)

The levels of reactive species were evaluated according to LeBel et al. (1992) using reduced 2', 7'-dihydrodichlorofluorescein diacetate (H_2DCF-DA). H_2DCF-DA is enzymatically hydrolyzed to H_2DCF by intracellular esterases. H_2DCF is oxidized to DCF by reactive oxygen species (ROS) or reactive nitrogen species (RNS) present in the samples. The intensity of the DCF fluorescence

corresponds to the proportion of reactive species present within the sample. Fluorescence was measured using excitation and emission wavelengths of 480 and 535 nm, respectively. The results are expressed as nmol of DCF. mg protein^{-1}.

Thiobarbituric acid-reactive substances (TBARS)

Thiobarbituric acid-reactive substances were determined according to the method described by Ohkawa et al. (1979), and this method essentially evaluates malondialdehyde (MDA), a lipid peroxidation product. This attack on the lipids primarily occurs due to hydroxyl radicals ($^{\bullet}OH$) derived from H_2O_2 by the iron-catalyzed Fenton reaction or the Haber–Weiss reaction (Kehrer 2000). Each sample was assessed at 532 nm using a spectrophotometer. The results are expressed as nmol of MDA. mg protein^{-1}.

Determination of carbonyls

Carbonyl protein content was determined using the method described by Reznick & Packer (1994). The oxidation of amino acids forms carbonyls in proteins or adducts formed along with lipoperoxidation products such as 4-hydroxy-2-nonenal (HNE). Each sample was measured at 370 nm, and total carbonylation was calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1} as described by Levine et al. (1990). The results are expressed as nmol of carbonyls. mg protein^{-1}.

Total sulfhydryl content

The sulfhydryl assays are based on the method of Aksenov & Markesbery (2001), where the reduction of 5, 50-dithio-bis (2-nitrobenzoic acid) (DTNB) by thiols generates a yellow derivative (TNB) with absorption that can be measured spectrophotometrically at 412 nm. The results are expressed as nmol of TNB per mg of protein.

Protein determination

The protein content of the homogenates was determined according to the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Serum Biochemical Parameters

Blood samples were carefully collected and centrifuged at 900 × *g* for 15 min. The serum was removed and stored at -80 °C until analysis. Glucose, triglycerides (TG), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and total protein were measured by an automated analyzer (Vitros 250, Ortho-Clinical Diagnostics, Rochester, NY) using Johnson and Johnson kits that incorporated a chemical drying method. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, uric acid, lipase, albumin, gamma-glutamyl transferase, phosphatase alkaline, iron, phosphate, sodium, potassium, calcium, and magnesium were also measured.

Statistical analysis

Data were analyzed by repeated ANOVA tests followed by the Tukey test when the F values were significant. Differences between groups were rated as significant at $p < 0.05$. All analyses were performed using SPSS software.

RESULTS

After treatment for 60 days, the animals were initially evaluated to assess the effects of the intraperitoneal administrations of the CDs on the activities of the enzymes AK, PK, and CK, all of which are important enzymes involved in the phosphotransfer network. Subsequently, we

studied the effects of the treatments on serum biochemical parameters.

Creatine kinase activity

First, we observed that CKcyt activity in kidney tissue was not significantly different among the groups ($p > 0.05$) (Table I). CKmit activity in the kidney tissue of rats [$F (3, 22) = 5.07$; $p < 0.05$] exhibited a significant decrease in the HP-βCD group compared to that of the control group (Table I). In contrast, CKmit activity was not altered by βCD or by M-βCD ($p > 0.05$) (Table I).

Pyruvate kinase activity

PK activity in the kidney tissue of rats was not significantly different among the groups ($p > 0.05$) (Table I). However, the activity of this enzyme in the liver was significantly reduced [$F (3.23) = 7.11$; $p <0.05$] in the βCD and M-βCD groups compared to that in the co-control group (Table I).

Adenylate kinase activity

AK activity in the kidney and liver tissues of rats was not significantly different among the groups ($p > 0.05$) (Table I).

2',7'-Dihydrodichlorofluorescein (DCFH) oxidation levels

The generation of reactive species as evaluated by 2',7'-dichlorofluorescein production was not significantly different among the groups ($p > 0.05$) in the kidney tissue of rats (Table II).

DCFH oxidation levels in the liver were significantly increased in response to M-βCD and HP-βCD administration [$F (3, 25) = 4.822$; $p < 0.05$] in rats; however, these levels were not increased in response to βCD (Table II).

Lipid peroxidation

Next, we evaluated the effects of CDs on MDA levels. We observed that this metabolite did not exhibit any significant differences among the groups ($p > 0.05$) in the kidney and liver tissues of rats (Table II).

Carbonyl formation

Carbonyl formation in the kidney and liver tissues of the rats was not significantly different among the groups ($p > 0.05$) (Table II).

Total sulfhydryl content

Total sulfhydryl content in the liver was not altered by CD administration ($p > 0.05$) compared to levels in the control group (Table

Table I. Effect of intraperitoneal administration of βCD, M-βCD, and HP-βCD on cytosolic creatine kinase (CKcyt), mitochondrial creatine kinase (CKmit) activity in kidney tissue and adenylate kinase (AK) pyruvate kinase (PK) activity in kidney and liver tissues from rats.

Tissues	Enzime activity	Control	βCD	M-βCD	HP-βCD
Kidney	CKcyt	0.53 ± 0.08	0.5 ± 0.08	0.48 ± 0.1	0.46 ± 0.07
	CKmit	0.38 ± 0.03	0.33 ± 0.03	0.35 ± 0.08	0.28 ± 0.02*
	AK	0.13 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.16 ± 0.01
	PK	6.50 ± 0.88	7.78 ± 0.84	7.55 ± 0.85	6.77 ± 0.35
Liver	AK	0.33 ± 0.02	0.33 ± 0.05	0.34 ± 0.03	0.34 ± 0.07
	PK	15.7 ± 3.2	9.8 ± 3.3*	10.3 ± 2.1*	13.7 ± 3.6

Values are mean ± standard deviation for six to eight independent experiments (animals) performed in triplicate and are expressed as βmol creatine per min per mg of protein for CK; performed in duplicate and are expressed as βmol of ATP per min per mg of protein for AK; performed in duplicate and are expressed as βmol of pyruvate per min per mg of protein for PK. *p < 0.05, compared to the Control group (ANOVA followed by Tukey test). CD: cyclodextrins; M-βCD: methyl; HP-βCD: hydroxypropyl.

II). In contrast, total sulfhydryl content was significantly increased in the kidney following HP-βCD administration [F (3, 23) = 87.488, p < 0.001] in rats; however, this content was not increased in response to βCD or M-βCD (Table II).

Serum Biochemical Parameters

The results for serum biochemical parameters are shown in Table III. AST levels were significantly increased by M-βCD and HP-βCD administration in the serum [F (3, 26) = 7.950; p < 0.05] of rats, and these levels were not increased in response to βCD. ALT, lipase, gamma-glutamyl transferase, and glucose levels were not significantly different among groups.

BCD or HP-βCD administration did not alter the serum's albumin, total protein, and phosphatase alkaline. However, the M-βCD administration significantly increased albumin [F (3, 27) = 6.259; p < 0.05], total protein [F (3, 28) = 4.606; p < 0.05], and phosphatase alkaline [F (3, 22) = 7.467; p < 0.05] levels in the serum of rats.

The analysis of the serum electrolyte concentrations shows (Tables III and IV) a significant reduction in the concentration of iron in response to βCD, M-βCD, and HP-βCD administration [F (3,23) = 19.682; p < 0.05] in the serum of rats. However, only M-βCD administration resulted in a significant increase in sodium [F (3, 28) = 5.759; p < 0.05], calcium [F (3, 28) = 5.505; p < 0.05], magnesium [F (3, 28) = 4.810; *p* < 0.01] and phosphate [F (3,28) = 2.755; p > 0,05] in the serum of rats, but not by βCD or HP-βCD.

Renal function (Table V) was evaluated according to urea, creatine, and uric acid levels. We observed a significant increase in urea [F (3, 28) = 3.161; p < 0.05] and creatinine [F (3, 28) = 3.400; p < 0.05] concentrations following βCD administration in the serum of rats, and this was not observed in response to M-βCD or HP-βCD administration.

We then analyzed the lipid profiles (Table VI). We found that M-βCD administration increased LDL levels [F (3, 24) = 7.929; p < 0.05], while βCD or HP-βCD administration did not alter this parameter. Moreover, other parameters, including total cholesterol, HDL, VLDL, and TG, were not significantly different among the groups in the serum of rats.

Table II. ***In vivo*** **effect of βCD, M-βCD and HP-βCD on the oxidative stress parameters from kidney and liver of rats.**

Tissues	Tests	Control	βCD	M-βCD	HP-βCD
Kidney	Sulfhydryl content	18.4 ± 4.9	22.2 ± 1,7	24.4 ± 7.5	60.9 ± 5.4*
	DCF	9.4 ± 0.7	9.7 ± 1.1	9.8 ± 0.6	9.3 ± 1.0
	TBARS	1887 ± 354	1918 ± 252	2064 ± 203	1958 ± 262
	Carbonylation protein	6.4 ± 1.6	5.7 ± 0.4	7.8 ± 1.8	6.2 ± 2.3
Liver	Sulfhydryl content	28.6 ± 3.8	33.7 ± 5,4	27 ± 4.1	35.1 ± 8.6
	DCF	4.3 ± 0.2	4.6 ± 0.2	4.7 ± 0.3*	4.7 ± 0.2*
	TBARS	1134 ± 88	1252 ± 72	1239 ± 86	1192 ± 105
	Carbonylation protein	5.0 ± 16	4.8 ± 0.8	5.5 ± 1.7	5.7 ± 0.5

Values are means ± standard deviation for six to eight independent experiments (animals) per group, performed in triplicate. *p < 0.05, compared to Control group (ANOVA followed by Tukey test). CD: cyclodextrins; M-βCD: methyl; HP-βCD: hydroxypropyl; DCF: dichlorofluorescein; TBARS: thiobarbituric acid-reactive substances.

Table III. ***In vivo*** **effect of βCD, M-βCD and HP-βCD on the serum biochemical parameters from rats.**

Tests	Control	βCD	M-βCD	HP-βCD
AST (U/L)	247 ± 58	267 ± 29	518 ± 248*	550± 121*
ALT (U/L)	69.3 ± 9.8	70.8 ± 11	68.7 ± 7.6	70.6 ± 4.7
Albumin (g/dL)	2.7 ± 0.1	3.0 ± 0,2	3.2 ± 0.3*	3.0 ± 0.2
Gamma glutamyl transferase (U/L)	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.3 ± 0.7
Glucose (mg/mL)	101 ± 5	104 ± 8	102 ± 12	100 ± 11
Total protein (g/dL)	6.1 ± 0.3	6.2 ± 0.3	6.7 ± 0.3*	6.3 ± 0.4
Lipase (U/L)	88 ± 19	67 ± 21	99 ± 26	97 ± 26
Phosphatase alkaline (U/L)	137 ± 25	124 ± 17	197 ± 47*	165 ± 19

Values are means ± standard deviation for six to eight independent experiments (animals) performed in triplicate. *p < 0.05, compared to Control group (ANOVA followed by Tukey test). CD: cyclodextrins; M-βCD: methyl; HP-βCD: hydroxypropyl; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

Table IV. ***In vivo*** **effect of βCD, M-βCD and HP-βCD on the serum electrolyte concentrations from rats.**

Tests	Control	βCD	M-βCD	HP-βCD
Sodium (mg/dL)	141 ± 5	144 ± 4	155 ± 12*	144 ± 3
Potassium (mmol/L)	6.7 ± 0.7	7.3 ± 0.4	7.4 ± 0.7	7.1 ± 0.3
Iron (mg/dL)	226 ± 31	143 ± 16*	192 ± 16*	149 ± 26*
Calcium (mg/dL)	9.6 ± 0.3	9.6 ± 0.3	10.5 ± 0.8*	9.8 ± 0.3
Magnesium (mg/dL)	2.1 ± 0.2	2.2 ± 0.2	2.4± 0.2*	2.1 ± 0.2
Phosphate (mg/dL)	8.3 ± 1.0	8.7 ± 0.9	9.3 ± 0.7*	8.6 ± 0.6

Values are means ± standard deviation for six to eight independent experiments (animals) performed in triplicate. *p < 0.05, compared to Control group (ANOVA followed by Tukey test). CD: cyclodextrins; M-βCD: methyl; HP-βCD: hydroxypropyl.

DISCUSSION

The effects of βCD and M-βCD on bioenergetic dysfunction in rats' renal and hepatic tissues have been characterized here for the first time. Our data revealed that βCD and M-βCD administration inhibited hepatic PK activity. Administration of HP-βCD inhibited CK activity and increased the total sulfhydryl content in the kidney. In general, these findings may suggest that understanding the balance between the efficacy and adverse effects of using CDs is fundamental for better accepting their use in medicine.

The literature has strong evidence that CDs are virtually non-toxic to the biological environment (Loftsson & Duchene 2007, Nishihira et al. 2017). However, when associating drugs with CDs, it is expected that there is no toxicity on the part of the carrier and that possible toxicity of the associated drug is minimized through this association. Thus, there is an immense need for further studies related to the possible toxicity of CDs. Many mechanisms from the action of cyclodextrins in the biological environment will still be reported in future research, enriching our understanding of them.

It is also important to evaluate the bioenergetic function in tissues. Cell viability is highly dependent on ATP required for the function of ATPase enzymes responsible for many cellular processes, including intracellular

Table V. ***In vivo*** **effect of βCD, M-βCD and HP-βCD on the measurements of renal function in rats.**

Tests	Control	βCD	M-βCD	HP-βCD
Urea (mg/dL)	36.7 ± 5	45.5 ± 6.6*	41.3 ± 6.3	41.5 ± 4.3
Uric acid (mg/dL)	1.3 ± 0.3	1.5 ± 0.2	1.5 ± 0.3	1.4 ± 0.3
Creatinine (mg/dL)	0.5 ± 0.1	0.6 ± 0.1*	0.56 ± 0.1	0.56 ± 0.1

Values are means ± standard deviation for six to eight independent experiments (animals) performed in triplicate. *p < 0.05, compared to Control group (ANOVA followed by Tukey test). CD: cyclodextrins; M-βCD: methyl; HP-βCD: hydroxypropyl.

and extracellular electrolyte and biomolecule homeostasis (Wallimann et al. 1992, Rech et al. 2018). The creatine kinase/phosphocreatine system (CK/PCr system) is responsible for the rapid replacement of ATP (Wallimann et al. 1992, Rech et al. 2018) in tissues or cells that require high levels of energy (Machado et al. 2016).

From the analysis of the CK system, it was observed that there are distinct CK isoenzymes that are compartmentalized specifically in areas that produce (mitochondria) or use (cytosol) ATP (Wallimann et al. 1992). In this study, HP-βCD inhibited CKmit activity in the kidneys of rats. Inhibition of CKmit enzyme activity can cause energy loss, ADP accumulation, and excess intracellular calcium, ultimately leading to oxidative stress (Machado et al. 2016). Some oxidative damage biomarkers include protein oxidation (indexed by protein carbonyls) and lipid peroxidation products (Dalle-Donne et al. 2003). In the interim, it is important to determine if the activities of thiol-containing enzymes such as PK, CK, and AK can change in response to oxidative stress (Rech et al. 2008, De Franceschi et al. 2013). In this study, it was observed that the administration of HP-βCD caused an increase in total sulfhydryl levels but did not affect the levels of TBARS, DCFH oxidation, and protein carbonylation in the kidney, indicating that HP-β-CD did not induce oxidative stress in the rat kidney. Based on this, it can be concluded that there is a possibility that a relationship exists between the increase in total sulfhydryl levels and the reduction of CKmit activity. We also observed that βCD and M-βCD administration did not alter CK activity in the kidney.

In liver tissue, the administration of HP-βCD increased the oxidation of DCFH but did not alter the activity of the enzymes PK and AK. In contrast, the administration of M-βCD, in addition to increasing the oxidation of DCFH due to an increase in oxidative species, also reduced the activity of PK. Here, it must be emphasized that PK activity is the primary route for producing pyruvate in the liver, as demonstrated by Burgess et al. (2008). This reference highlights the importance of analyzing this route, and the results of this study shed light on the possible effects of the use of CDs on bioenergetic dysfunction. Moreover, PK activity in the liver was also reduced by βCD administration; however, this treatment did not affect any of the oxidative stress parameters evaluated. The observed inhibited activity of PK implies a reduction in the amount of cellular pyruvate, an important antioxidant agent (Figueiredo et al. 2009), and a reduction in the ATP cell pool (Rech et al. 2018) that can induce cell death (Gabardo et al. 2015).

According to the proposed experimental design, it was observed that there was no induction of oxidative stress in response to the administration of βCD, M-βCD, or HP-βCD in animals compared to that observed in the control group. This result highlights the non-toxic characteristics of CDs concerning the evaluated parameters. Our findings regarding the non-toxicity of CDs in biological environments are corroborated by the study performed by

Table VI. ***In vivo*** **effect of βCD, M-βCD and HP-βCD on the lipids measurements in rats.**

Tests	Control	βCD	M-βCD	HP-βCD
Cholesterol (mg/mL)	83 ± 13	86 ± 13	99 ± 14	85 ± 11
VLDL (mg/mL)	9.9 ± 1.2	9.1 ± 1.9	9.1 ± 1.2	8.9 ± 1.2
LDL (mg/mL)	26.3 ± 5.3	23.4 ± 4.7	36.4 ± 7.2*	24.2 ± 4.9
HDL (mg/mL)	48.8 ± 5.5	51.3 ± 3.9	55.8 ± 6.6	52 ± 6.0
TG (mg/mL)	52 ± 8	46 ± 9	46 ± 6	45 ± 6

Values are means ± standard deviation for six to eight independent experiments (animals) performed in triplicate. *p < 0.05, compared to Control group (ANOVA followed by Tukey test). CD: cyclodextrins; M-βCD: methyl; HP-βCD: hydroxypropyl; VLDL: very low-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TG: triglycerides.

Nishihira et al. (2017). Although the DCs affected the activities of some enzymes involved in energy metabolism, no correlation between these effects and the evaluated parameters of oxidative stress was observed.

From the analysis of bioenergetic functions, it can be determined that there is a significant relationship between CK and AK enzymes. Both CK and AK are closely associated in such a manner that when the activity of one enzyme is reduced, the activity of the other enzyme is enhanced (Dzeja & Terzic 2009, Figueiredo et al. 2009). The AK enzyme catalyzes the reversible reaction of nucleotide phosphoryl exchange among ATP, ADP, and AMP. Thus, this enzyme transfers the phosphoryl group from ATP, produced in mitochondria, to the cytosol, where it is consumed. However, no interaction between CK and AK activities was found in this study.

Furthermore, it was observed that the administration of M-βCD and HP-βCD significantly increased the serum levels of AST. According to Mello et al. (2012), an increase in serum AST levels indicates the induction of chronic hepatocellular damage. Despite this, it is generally observed that hepatic changes cause variations in AST levels accompanied by increased ALT levels (Spanish Society of Clinical Biochemistry and Molecular Pathology 2012).

Additionally, in this study, it was observed that the administration of M-βCD significantly increased the serum levels of albumin, total proteins, and alkaline phosphatase. It is widely established that serum albumin is present in much greater amounts in the blood than other proteins (Chandrasekaran et al. 2016). It should be considered that the increase in the serum albumin level might be related to the increase in the total protein. It is well established that in liver disorders, serum albumin levels decrease; however, it is also established that these levels increase in response to dehydration. These results, together with increased sodium, calcium, magnesium, and phosphate levels, are characteristic of dehydration rather than liver damage caused by M-βCD. In agreement with Moe (2008), it has been observed that most patients with renal disease in conjunction with hyperphosphatemia and hypermagnesemia exhibit increased gastrointestinal absorption. Furthermore, magnesium, phosphate, and calcium are essential for many biological and cellular functions. Thus, it must be noted that maintaining the homeostasis of these ions is a central role performed by the kidneys (Blaine et al. 2015).

Among the findings of the present study, it was observed that treatment with βCD significantly increased urea and creatinine levels in the rats' serum. These parameters are associated with liver damage. Mosher & Thompson (2002) observed liver damage when

performing experiments that included the oral administration of βCD. However, it was also observed in the present study that βCD did not alter AST and ALT levels. Thus, it is advisable to perform further studies to elucidate possible tissue alterations that the administration of βCD may cause.

A significant reduction in iron levels was observed based on the analysis of serum electrolyte concentrations. In response to βCD and HP-βCD treatments, the reduction was approximately 40% and around 15% for M-βCD. It is common knowledge that iron is a facilitator of reversible redox reactions, and this element is, therefore, essential for life (O'Brien 2011). From our study, we observed that the administration of M-βCD increased the iron levels in rats. It is known that excess iron can lead to the production of reactive oxygen species (ROS) that can damage cell structures. To prevent this harmful process, homeostasis is necessary to avoid tissue damage (Grotto 2008). In addition to the analysis of iron, another study highlighted the effects of CDs on blood components such as LDL. LDL comprises an important group of plasma lipoproteins that transport fats (mainly cholesterol) to cells. In this study, the administration of M-βCD increased the serum levels of LDL without affecting the levels of total cholesterol, VLDL, HDL, and TG. However, an *in vitro* study found that CDs could induce lipid depletion of native LDL (Ao et al. 2016).

Oxidative stress occurs because of the overproduction of ROS and a failure in the function of antioxidant molecules (Dalle-Donne et al. 2003). Thiol compounds are reducing agents that contain a sulfhydryl or thiol group (-SH), and they are found in free form or linked to proteins in the biological environment. In this study, the total thiol content was increased 3-fold in the kidneys of animals treated with HP-βCD. This significant increase was not reflected in DCFH, TBARS, and carbonylation protein measurements. However, βCD and M-βCD did not alter the parameters tested in this tissue. Based on the findings from renal tissue, the increase in serum levels of creatinine and urea in animals treated with βCD does not correlate with the findings of energy metabolism and oxidative stress.

The hepatic tissue exhibited an increase in the oxidation of DCFH in response to the administration of M-βCD and HP-βCD, indicating an increase in free radicals. The other parameters related to oxidative stress were not affected in this tissue. Inhibition of hepatic PK may be associated with increased reactive oxygen species and DCFH oxidation in response to M-βCD. However, this analysis cannot inhibit the enzyme influenced by βCD. Similar to the results reported by Bellringer et al. (1995), these results indicate a possible kidney injury caused by βCD. However, these results do not rule out a possible liver injury even without altering ALT, gamma-glutamyl transferase, or alkaline phosphatase (indicators of liver injury). This possibility can be considered in this tissue based on the observation that the enzyme PK is inhibited, and this typically occurs in the presence of gluconeogenesis. We also assessed pancreatic function by measuring the lipase enzymes and glycemia, which were not affected by the treatments.

These results demonstrate that the βCDs used in this study did not affect most of the evaluated oxidative stress parameters. It is possible that CDs can be modulated by mechanisms other than oxidative stress. CDs have been widely used as drug carriers. Regardless, understanding the balance between the efficacy and adverse effects of using CDs is fundamental for a better acceptance of the clinical use of these compounds.

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V. C. Rech, A. L. de Oliveira and F. R. Ianiski, and I. Z. da Silva designed the research. A. L. de Oliveira, N. J. Mezzomo, G. M. do Carmo, C. R. Cremonese, M. D. Baldissera, J. P. Zanon, J. Kolling, J. D. Friederich, were involved in rat care and treatment. I. D. de Franceschi, A. L. de Oliveira, L. R. Feksa, and J. L. Giongo performed the measurements of enzymes of phosphoryl transfer network. N. J. Mezzomo, G. M. do Carmo, and R. A. Vaucher performed serum biochemical analyzes. I. D. de Franceschi, A. L. de Oliveira executed the measurements of oxidative stress. V. C. Rech and F. R. Ianiski performed the statistics analysis. V. C. Rech, L. R. Feksa, and C. D. Wannmacher collected and analyzed the data. V. C. Rech and F. R. Ianiski wrote the paper. V. C. Rech, F. R. Ianiski, R. A. Vaucher, I. Z. da Silva, L. R. Feksa, J. L. Giongo, and C. M. D. Wannmacher revised it critically the paper.