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MARIANE CARNEIRO

INIBIDOR DO METABOLISMO LIPIDICO, CLODINAFOP-PROPARGYL ATENUA A SÍNDROME METABÓLICA EM RATOS OBESOS

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas (área de concentração - Biologia Celular e Molecular), da Universidade Estadual de Maringá para a obtenção do grau de Mestre em Ciências Biológicas.

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Coorientador: Prof. Dr. Paulo Cezar de Freitas Mathias

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Aprovado em: 26 de agosto de 2022
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BIOGRAFIA

Paranaense, nascida na cidade de Loanda, interior do estado, no dia 27 de janeiro de 1992, filha do pedreiro Aparecido Ferreira da Silva, e da pedagoga Ercilia Carneiro dos Santos, a também missionária voluntária morou em Paranavaí cidade do extremo Noroeste do Estado, onde se formou em Ciências Biológicas (licenciatura) em 2019 pela Universidade Estadual do Paraná- UNESPAR. Ainda em 2019, ingressou no Programa de Pós-Graduação em Bioquímica – PBQ nesta universidade, após 5 meses, já no ano de 2020 ingressou no Programa de Pós-Graduação em Ciências Biológicas – PBC, com área de concentração em Biologia Celular e Molecular, na Universidade Estadual de Maringá - UEM, Paraná.

Para esta instituição agora apresenta esta dissertação, para obtenção do grau de Mestre em Ciências Biológicas.

DEDICATÓRIA

Dedico esta dissertação primeiramente a Deus, a ele toda honra e toda glória, bem como a Nossa Senhora das Graças por estarem comigo em todos os momentos deste trabalho que é consagrado a eles. Como disse Aristóteles: “Nenhum obstáculo é grande demais quando confiamos em Deus.

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Esta dissertação é composta por um artigo científico, em consonância com a Resolução 056/2019 do Programa de Pós-graduação em Ciências Biológicas, PBC, sendo redigido de acordo com a revista *Frontiers in Pharmacology*, ISSN 1663-9812. Qualis A1. IF 5,98

Title: Lipid metabolism inhibitor, clodinafop-propargyl attenuates the metabolic syndrome in obese rats

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RESUMO

A obesidade é considerada uma das principais causas de morte no mundo. Diante desse fato têm-se realizados estudos em busca de tratamentos para esta que é considerada uma pandemia. Do ponto de vista bioquímico, o metabolismo hepático exerce um papel fundamental no metabolismo de carboidrato e síntese de triglicerídeos. O excesso de carboidrato aumenta a secreção de insulina promovendo aumento no estoque de triglicerídeos. Uma das enzimas envolvidas nesse processo, a acetil-CoA carboxilase (ACC), que se tornou alvo para intervenções terapêuticas no tratamento da obesidade. Neste estudo, consideramos a origem homóloga evolutiva de conservação das estruturas e utilizamos o composto clodinafop-propargyl (CP), da família dos inibidores seletivos da ACC utilizado na cultura do trigo para combater as plantas daninhas, com o intuito de inibir a ACC e com isso atenuar os efeitos da obesidade e suas consequências metabólicas. O estudo utilizou ratos Wistar machos adultos, tratados por 30 dias com 0,8 mg/kg/dia de CP, divididos em 4 grupos: veículo controle (NLV), controle tratado (NL 0,8), veículo obeso (SLV) e obeso tratado (SL 0,8). Aos 120 dias de idade, os animais foram eutanasiados e avaliados os parâmetros *in vivo* e *ex vivo*. Os testes realizados foram o teste de tolerância à insulina intraperitoneal (ipITT), teste oral de tolerância a glicose (Ogtt), perfusão hepática e analise bioquímica no sangue.

O estudo observou que os ratos obesos tratados com clodinafop-propargyl apresentaram uma interação benéfica entre o metabolismo hepático e da glicose, evidenciando uma melhora na sensibilidade a insulina e o déficit na captação de oxigênio. Os animais tratados não mostraram uma redução no peso corporal se comparados aos animais não tratados. Observou-se melhora no perfil lipídico dos animais tratados, bem como na sensibilidade periférica a insulina e intolerância a glicose. Não houve melhora na redução dos depósitos de gordura, o que pode sugerir que a exposição a inibidor tende a ser tempo-dependente. Diante dos resultados encontrados, sugerimos que a expressão de algumas enzimas e vias metabólicas devem ser investigadas para elucidar as razões por trás do mecanismo subjacente a essas respostas. Mesmo que a administração do clodinafop-propargyl não reduziu depósitos de gordura, ocorreu melhora no metabolismo da glicose e nos marcadores bioquímicos de disfunção hepática. O estudo, portanto, sugere que o inibidor foi capaz de inibir o metabolismo lipídico e possui potencial para novas abordagens terapêuticas, visando o tratamento da obesidade e síndrome metabólica.

Palavras-chave: Acetil-CoA carboxilase, clodinafop-propargyl, obesidade, ninhada reduzida, síndrome metabólica

ABSTRACT

Obesity is considered one of the leading causes of death in the world. Given this fact, studies have been carried out in search of treatments for what is considered a pandemic. From a biochemical point of view, hepatic metabolism plays a key role in carbohydrate metabolism and triglyceride synthesis. Excess carbohydrate increases insulin secretion promoting an increase in triglyceride storage. One of the enzymes involved in this process, acetyl-CoA carboxylase (ACC), has become a target for therapeutic interventions in the treatment of obesity. In this study, we considered the evolutionary homologous origin of conservation of structures and used the compound clodinafop-propargyl (CP), from the family of selective ACC inhibitors used in wheat crops to combat weeds, in order to inhibit ACC and with this mitigate the effects of obesity and its metabolic consequences. The study used adult male Wistar rats, treated for 30 days with 0.8 mg/kg/day of CP, divided into 4 groups: control vehicle (NLV), treated control (NL 0.8), obese vehicle (SLV), and treated obese (SL 0.8). At 120 days of age, the animals were euthanized and parameters were evaluated in vivo and ex vivo. The tests performed were the intraperitoneal insulin tolerance test (ipITT), oral glucose tolerance test (Ogtt), liver perfusion, and biochemical analysis in the blood.

The study observed that obese rats treated with clodinafop-propargyl showed a beneficial interaction between hepatic and glucose metabolism, evidencing an improvement in insulin sensitivity and a deficit in oxygen uptake. Treated animals did not show a reduction in body weight compared to untreated animals. There was an improvement in the lipid profile of the treated animals, as well as in peripheral insulin sensitivity and glucose intolerance. There was no improvement in the reduction of fat deposits, which may suggest that inhibitor exposure tends to be time-dependent. In view of the results found, we suggest that the expression of some enzymes and metabolic pathways should be investigated to elucidate the reasons behind the mechanism underlying these responses. Even though the administration of clodinafop-propargyl did not reduce fat deposits, there was an improvement in glucose metabolism and in biochemical markers of liver dysfunction. The study, therefore, suggests that the inhibitor was able to inhibit lipid metabolism and has potential for new therapeutic approaches, aiming at the treatment of obesity and metabolic syndrome.

Keywords: Acetyl-CoA carboxylase, clodinafop-propargyl, obesity, small litter, metabolic syndrome

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65 # Lipid metabolism inhibitor, clodinafop-propargyl attenuates metabolic
66 syndrome in obese rats

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80 **Keywords: Acetyl-CoA carboxylase, clodinafop-propargyl, obesity, small litter, metabolic**
81 **syndrome**

82 **Abstract**

83 Obesity is considered a pandemic and one of the leading causes of death in the world. Given
84 this fact, studies have been carried out in search of treatments. From a biochemical point of
85 view, hepatic metabolism plays a key role in carbohydrate metabolism and triacylglyceride
86 synthesis. One of the enzymes involved in this process, acetyl-CoA carboxylase (ACC), has
87 become the target of therapeutic interventions in the treatment of obesity. In this study, we
88 considered the homologous evolutionary origin of the conservation of structures, and we
89 hypothesized that the compound clodinafop-propargyl (CP), from the selective ACC inhibitor
90 family used in agriculture, could attenuate the effects of obesity by inhibiting lipid biosynthesis.
91 The study used adult male Wistar rats, treated for 30 days with 0.8 mg/kg/day of CP, divided
92 into 4 groups: vehicle control (NLV), treated control (NL 0.8), obese vehicle (SLV), and treated
93 obese (SL 0.8). At 120 days of age, the animals were euthanized, and *in* an *ex vivo* parameters
94 were evaluated. The study observed that obese rats treated with clodinafop-propargyl showed
95 a beneficial interaction between hepatic and glucose metabolism, completely reversing the
96 glucose intolerance characteristic of litter reduction animals. There was a significant
97 improvement of 20% in peripheral insulin sensitivity and a complete reversal of the deficit in
98 oxygen uptake. There was also a significant improvement in the lipid profile, with 41%
99 triglycerides, 21% total cholesterol, 30% VLDL cholesterol, and 74% LDL. Given the results

100 found, we can conclude that the administration of clodinafop-propargyl was able to attenuate
101 the effects on glucose metabolism and biochemical markers of liver dysfunction.

102 1 Introduction

103 It is estimated that obesity affects 13% of the adult population worldwide, totaling more
104 than 650 million individuals (WHO, 2021). Globally, obesity is a major public health problem,
105 with annual costs estimated at US$2 trillion, equivalent to 2.8% of the world's gross domestic
106 product (GDP) and equal to the costs of smoking (Swinburn et al., 2019). The exponential
107 increase in obesity in the world can be explained by social changes such as a sedentary lifestyle
108 and increased consumption of energy-rich foods (Christakis and Fowler, 2007). To contain the
109 obesity pandemic and its consequences, efforts have been made in the search for treatments,
110 whether preventive or chronic, from multidisciplinary interventions considering dietary
111 approaches, physical activity, behavioral changes, and the development of new drugs.

112 Obesity is complex and multifactorial, both in its etiology and pathophysiology, but the
113 expansion of adipocytes leads to an underlying inflammatory state, which together with
114 lipotoxicity, glucotoxicity, insulin resistance, oxidative stress, and appetite dysregulation, can
115 cause irreversible damage to fabrics (Crewe et al., 2017). In this context, liver regulation plays
116 a key role in the energy balance of the body, where the liver is the main site of carbohydrate
117 metabolism and triglyceride synthesis. Lipogenesis is regulated by key enzyme activities
118 controlled by insulin and the presence of glucose (Rui, 2014). Thus, excess carbohydrate
119 increases the availability of glucose that stimulates insulin secretion, and both activate lipogenic
120 and glycolytic enzymes promoting a greater stock of triacylglycerols (Westin, 2007).

121 The first step in the formation of triacylglycerols is the conversion of acetyl-CoA to
122 malonyl-CoA through the addition of a molecule of CO2. This is a critical step in the regulation
123 of lipid biosynthesis, catalyzed by a key enzyme, acetyl-CoA carboxylase (ACC), making this
124 enzyme a potential target for therapeutic interventions for the treatment of obesity (Tong 2005;
125 Jr. and James Harwood 2005).

126 ACC inhibitors are commonly added as an active ingredient in selective herbicides for
127 ACC, so the weed loses its nutritional capacity, interrupting its development which makes
128 planting safe. One of these inhibitors is clodinafop-propargyl (CP), from the
129 aryloxyphenoxypropionate chemical family used in wheat (*Triticum*) cultivation. Considering
130 the evolutionary concept of homology and preservation of structures and that ACC has shared

131 domains between plants and animals, we hypothesize that inhibition of lipid biosynthesis
132 through the compound clodinafop-propargyl will attenuate the effects of obesity and metabolic
133 syndrome in obese rats.

134 **Material and methods**

135 The experimental protocol was approved by the Ethics Committee in Research for
136 Animal Use and Experimentation at the State University of Maringa (protocol number
137 5134280920).

138 **2.1 Animal model**

139 Female and male Wistar rats aged 70 days were obtained from the Central Animal
140 Facility of the State University of Maringa and kept in the Sectorial Animal Facility of the
141 Department of Biotechnology, Genetics and Cell Biology, in polypropylene cages (45 × 30 ×
142 15 cm) at room temperature controlled (23 ± 2 °C) and light-dark cycle (07:00–19:00). After
143 one week of adaptation, the animals were mated in the proportion of two females (n = 24) for
144 each male (n = 12).

145 Pregnant Wistar rats were housed in individual polypropylene cages with free access to
146 food and water during the entire gestation and lactation period. At delivery, mothers were
147 randomly divided into two groups, normal litter (NL – n = 12) and small litter (SL – n = 12).
148 On the third postnatal day (PND), NL litters were adjusted to 9 pups and SL litters were adjusted
149 to 3 pups (Junior et al. 2019). In PND 21, pups from both litters were weaned (n = 36 male rats
150 per group). After weaning, the pups were housed in collective polypropylene cages (3 animals
151 per cage). Both groups were provided *ad libitum* access to water and commercial standard chow
152 (Nuvilab® CR-1) from PND 21 to PND 120. During the lactation period, pups were weighed at
153 PND 3, 7, 14, and 21. After weaning, body weight was measured weekly.

154 In PND 90 the animals were separated into 4 new groups by blind test: (NLV) vehicle
155 control, (NL 0.8) control treated with a dose of 0.8 mg/kg/day of CP, (SLV) vehicle, and (SL
156 0.8) obese treated with a dose of 0.8 mg/kg/day, ordered so that there is always one control
157 animal and one treated animal per box, to maintain variability and minimize research bias due
158 to kinship of animal per litter. Thus, the treatment was terminated at PND 120, and these
159 animals were directed to *in vivo* experiments (ipITT and OGTT) and *ex vivo*, euthanasia by
160 decapitation for tissue and plasma collection. Emphasizing that the results presented here are
161 different in their origin according to each technique, therefore, different animals were directed
162 to the *in* and *ex vivo* experiments.

163 **2.2 Intraperitoneal insulin tolerance test (ipITT)**

164

165 On PND 120, after a 6-hour fast, 6 animals per group received an intraperitoneal
166 injection of recombinant human insulin (1U/kg BW, Eli Lilly®, São Paulo, Brazil). Blood
167 samples were collected via a small tail cut at 0 (before insulin injection) and 15, 30, 45,
168 and 60 minutes after injection, and glucose was measured at each corresponding time point
169 using a glucometer (ACCU-CHEK® Advantage, Roche Diagnostics, Manheim, Germany).
170 Subsequently, the constant rate of glucose decay (K_{itt}) was calculated using the formula
171 0.693/ $t_{1/2}$, where $t_{1/2}$ is the plasma glucose half-life calculated including the curve obtained
172 during the linear decay phase of plasma glucose detected within 0 to 60 minutes after
173 insulin application (Bonora *et al.*, 2000; Miranda *et al.*, 2018). Insulin sensitivity was
174 greater the faster and more intense the drop in glucose during the test (Geloneze &
175 Tambascia, 2006).

176

177 **2.3 Oral glucose tolerance test (oGTT)**

178

179 After two days of ipITT recovery, the animals (n= 06/group) fasted for 12 hours.
180 Weighed and a blood sample was collected from the tail (time 0) through a heparinized
181 capillary tube (75mm long, 1.5mm outside diameter). Rats received 1 U/kg BW of glucose
182 in 50% aqueous solution via oral gavage. Blood samples were obtained at 15, 30, 45, 60,
183 and 120 minutes, and glucose was measured at each corresponding time point using a
184 glucometer (ACCU-CHEK® Advantage, Roche Diagnostics, Mannheim, Germany) (Islam
185 *et al.*, 2009). Blood samples were centrifuged at 10,000 rpm for 5 min for plasma collection
186 and stored at -20°C for subsequent quantification.

187

188 **2.4 Euthanasia and samples collection**

189

190 Adult animals from each experimental group (PND 120; n = 06 - 08 /group) fasted
191 for 12 hours. The animals were euthanized by decapitation. Subcutaneous (SUB),
192 periepididymal (EPI), retroperitoneal (RP), and mesenteric (MES) adipose tissue were
193 removed and weighed.

194

195 **2.5 Biochemical assays**

196

197 Blood samples were collected in the same 12-hour fast of the euthanized animals
198 for biometric evaluation and stored at -20 ºC for later quantification. Blood glucose was
199 quantified using the glucose oxidase method by spectrophotometry using a 96-well ELISA
200 plate (SpectraMax® Plus 384 Microplate Reader from Molecular), using a commercial kit
201 (Gold Análisa®, Belo Horizonte/MG, Brazil) (Trinder, 1969 adapted).
202 Total cholesterol was measured by the colorimetric method of total cholesterol (TC)
203 oxidase and triglycerides (TG) were measured by the colorimetric method of glycerol-3-
204 phosphate oxidase using commercial kits (Gold Análisa®, Belo Horizonte/MG, Brazil),
205 both readings were performed in spectrophotometry equipment using a 96-well ELISA
206 plate (SpectraMax® Plus 384 Microplate Reader by Molecular). HDL-cholesterol (HDL-
207 c) was determined after precipitation of chylomicrons and low-density lipoproteins were
208 determined with a commercial kit (Gold Analisa®, Belo Horizonte/MG, Brazil). After
209 chylomicron precipitation, HDL-c was determined using the method described above for
210 the measurement of TC. Results were expressed in mg/dL. We used the calculation
211 methodology to determine the VLDL and LDL values, according to the aforementioned
212 commercial kit protocol.

213

214 **2.6 Liver perfusion**

215

216 Perfusion of the isolated liver was performed in a non-recirculating system (Comar et
217 al., 2003). The animals were anesthetized with a combination of xylazine (9 mg/kg) and
218 ketamine (90 mg/kg) i.p. and underwent laparotomy. Briefly, after cannulation of the cava and
219 portal veins, the hepatic circulation was isolated and the liver perfused in situ under a surgical
220 table with Krebs/Henseleit bicarbonate buffer (pH 7.4) containing 25 mg% bovine serum
221 albumin (Scholz et al.., 1965). The perfusion fluid flow through the liver was kept constant by
222 a peristaltic pump (Minipuls 3, Gilson, France) and adjusted to approximately 35 mL per
223 minute. The perfusion fluid was saturated with a mixture of oxygen and carbon dioxide (95:5)
224 using a membrane oxygenator and simultaneous adjustment of the temperature (37°C). A
225 platinum electrode was connected to the acrylic chamber at the perfusate outlet to monitor
226 oxygen consumption. Samples of effluent perfusate will be collected at regular intervals and
227 analyzed for metabolite content.

228 **2.7 Ketogenesis**

229

230 To measure the transformation of fatty acids into ketone bodies, the liver was perfused
231 with Krebs buffer and 0.3 mM palmitic acid complexed with fatty acid-free bovine serum
232 albumin (0.15 mM) for 30 minutes as previously described (Wendt et al., 2019; Sá-Nakanishi
233 et al., 2020). The levels of β-hydroxybutyrate and acetoacetate were measured in the effluent
234 perfusate as described previously (Bergmeyer, 1974).

235

236 **2.8 Statistical analysis**

237 The variables evaluated were body weight, tissue, and biochemical parameters, ipITT,
238 and oGTT. Data were submitted to Student's t-test and Two-way ANOVA analysis, with a
239 significance level of 5%. When significant differences were observed between the groups, the
240 data were submitted to the Tukey test ($p \leq 0.05$). The number of repetitions used was 12. Prism
241 software version 8.01 (GraphPad, San Diego, CA, USA) was used for data analysis and graph
242 construction. Data are expressed as the percentage value of the p-value difference.

243

244 **3. Results**

245 **3.1 Biometric parameters of obesity induction**

246 At PND 21 it is possible to confirm obesity induction and metabolic dysfunction through
247 neonatal supplementation, with the SL animal ($p < 0.0001$) being 35% heavier than the NL
248 control animal ($p < 0.05$). This difference remains until adulthood with 9.5% (PND 90 p
249 <0.0001) as evidenced by the curve and area under the curve (Fig. 1a) compared to the control.
250 At the end of the experimental protocol, the animals in the SL group still had a higher body
251 weight than the control ($p < 0.01$). Evolution of the body weight on days of treatment (Fig. 1b).

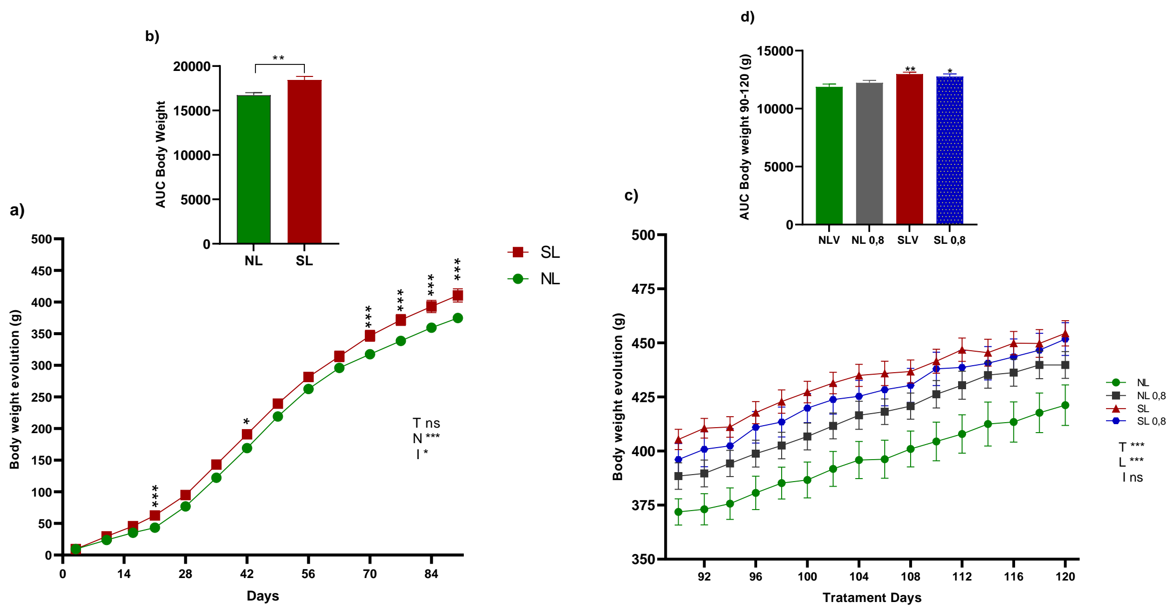
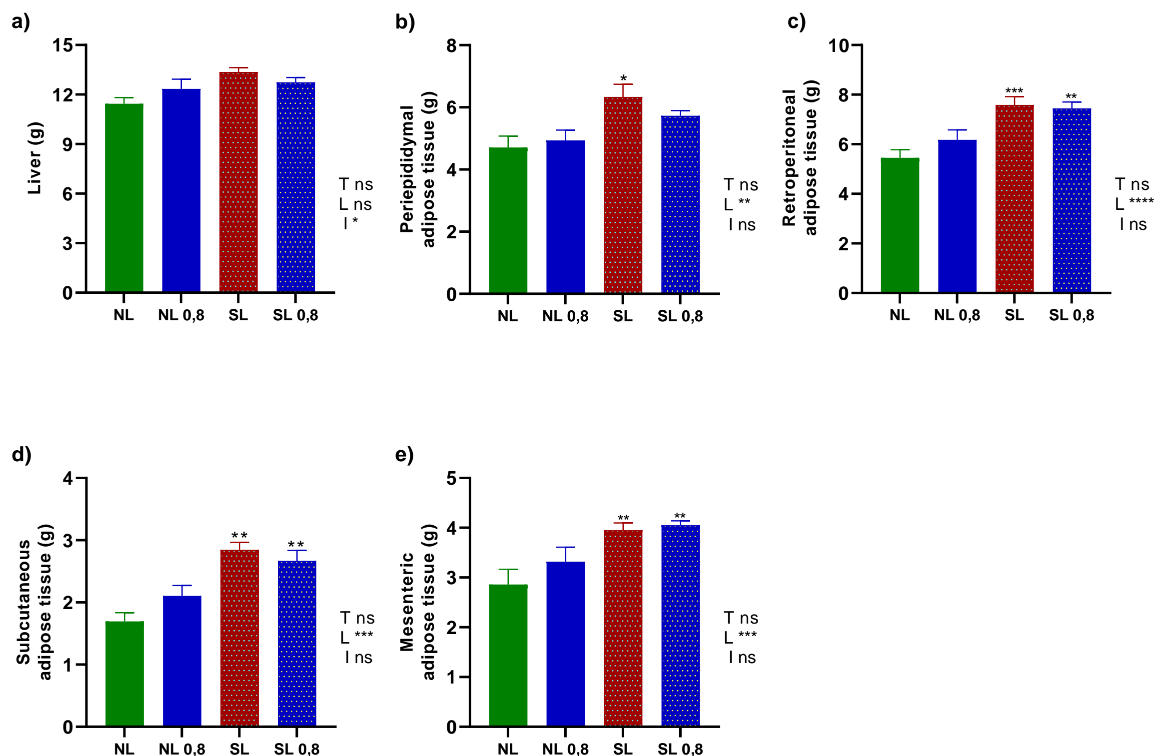


Fig. 1: Effects of litter reduction on obesity induction. The curve of the evolution of body weight in the 21 days of lactation in grams (a), AUC during the period (b), the curve of the evolution of body weight from weaning to adulthood in grams (c) area un the der curve (AUC) during the period (d). N = 12-16 animals per group NL, normal litter; NL 0.8, normal litter treated with a dose of 0.8 mg/kg of the inhibitor, SL, small litter, SL 0.8, small litter treated with a dose of 0.8 mg/kg of the inhibitor. the I, the interaction between treatment and litter, L, litter factor, and T, treatment factor. Data are presented as the mean ± SEM. Results based on Student's t-test, Two-way Anova and Tukey post-test, *p < 0.05, **p < 0.01 and *** p <0.001.

In addition, when the animals of the SL group were compared to the animals of NL group, they presented an increase of in periepididymal adipose tissue (NL: 4.71 ± 0.37 *vs* SL: 5.73 ± 0.17; p < 0.01, Fig 2b) retroperitoneal (NL: 5.451 ± 0.3254 *vs* SL: 7.588 ± 0.3312 p < 0.01, Fig 2c), subcutaneous (NL: 1.695 ± 0,1411 *vs* SL: 2.844 ± 0.1214 p < 0.01, Fig 2d) and mesenteric (NL: 2.860 ± 0.3056 *vs* SL: 3.951 ± 0.1489 p < 0.01, Fig 2e).



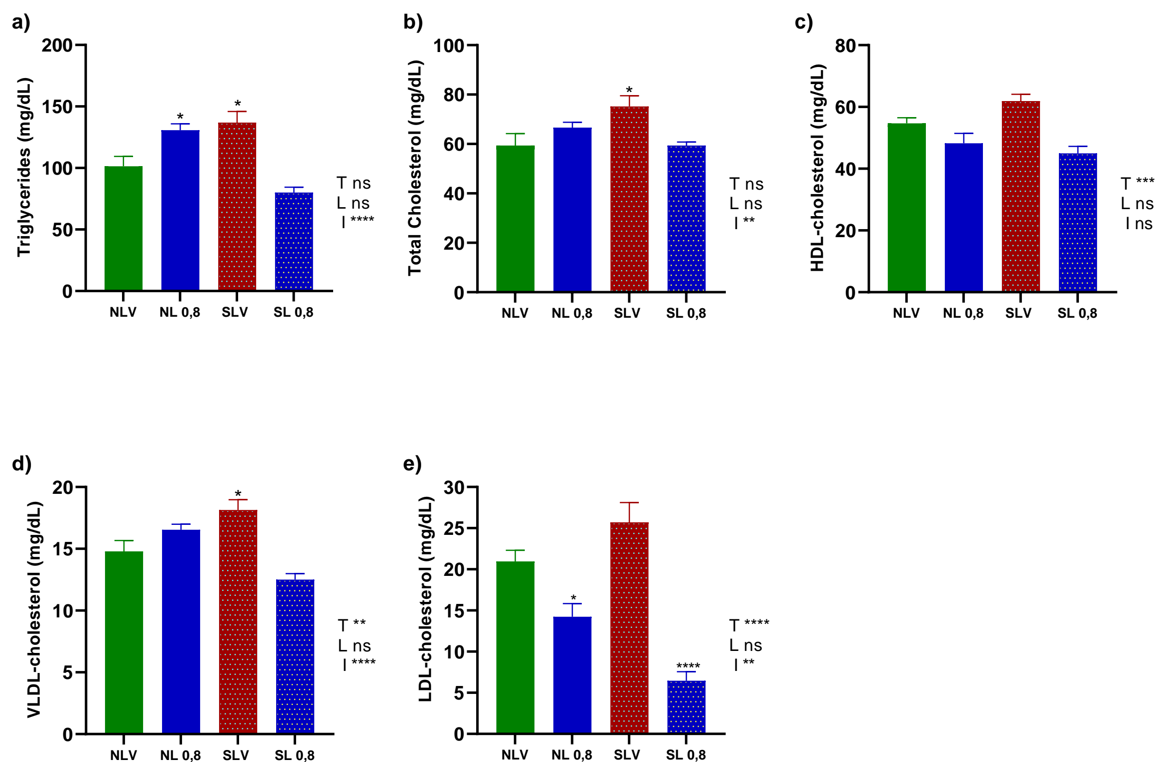
267

268 **Fig. 2: Effects of ACC inhibitor treatment on tissue parameters.** Liver (a) Periepididymal
269 (b), retroperitoneal (c), subcutaneous (d), and mesenteric adipose tissue (e), N= 6–8 animals
270 per group NL, normal litter; NL 0.8, normal litter treated with a dose of 0.8 mg/kg of the
271 inhibitor, SL, small litter, SL 0.8, small litter treated with a dose of 0.8 mg/kg of the inhibitor.
272 I, the interaction between treatment and litter, L, litter factor and T, treatment factor. Data are
273 presented as the mean ± SEM. Two-way ANOVA and Tukey post-test, *p< 0.05, **p < 0.01
274 and *** p <0.001.

275

276 **3.2 Biochemical parameters of lipid profile**

277 The SL animals showed an increase in triglycerides (NL: 101.4 ± 7.94 *vs* SL: 136.9 ±
278 9.00, p= 0.026, Fig 4a), total cholesterol (NL: 59.38 ± 4.850 *vs* SL: 75.21 ± 4.368, p= 0.049,
279 Fig 4b). VLDL and LDL, (NL: 14.79 ± 0.881 *vs* SL: 18.14 ± 0.840, p= 0.013, Fig 4d, NL: 20.96
280 ± 1.350 *vs* SL: 25.71 ± 2.398, Fig 4e). Despite the increased values of the obese control SL
281 group about the normal control NL, we observed a significant improvement of all these
282 parameters in the obese treated group SL 0.8, being 41% in triglycerides, 21% total cholesterol,
283 30% VLDL and 74 % LDL. In contrast, we also obtained a decrease in HDL cholesterol (NL:
284 54.67 ± 1.854 *vs* SL: 61.92 ± 2.215, with -27% SL 0.8: 44.98 ± 2.238, p= 0.002) which can be
285 justified due to the low VLDL and LDL levels, since it has the same route of origin.



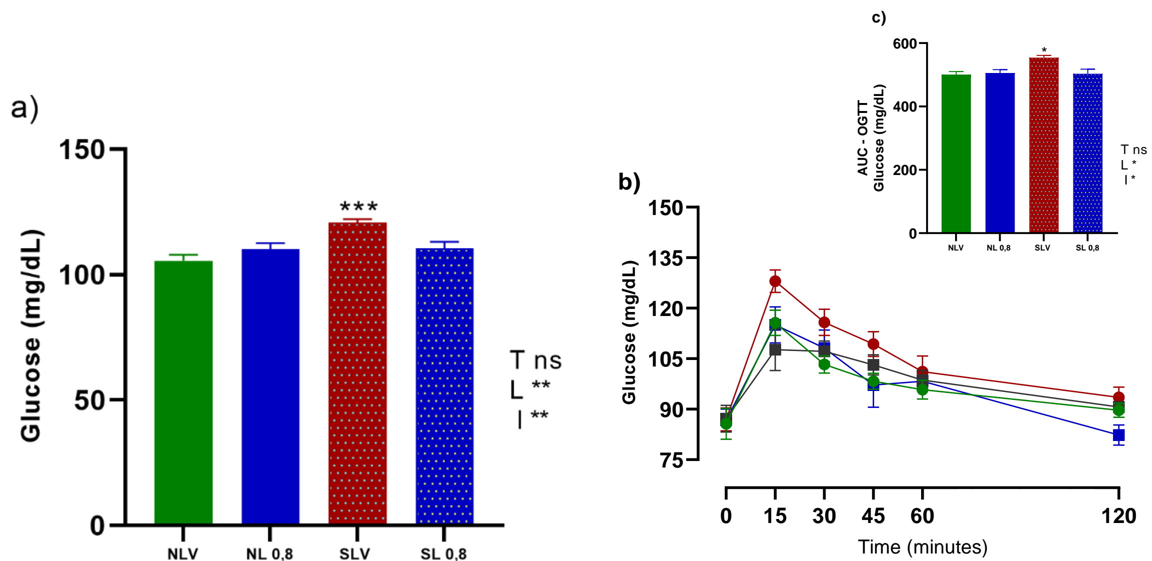
286

287 **Fig. 4: Effect of ACC inhibitor on lipid profile.** Triglycerides (a), Total cholesterol (b), HDL
288 cholesterol (c), VLDL cholesterol (d) and LDL cholesterol (e). N= 6–8 animals per group NL,
289 normal litter; NL 0,8, normal litter treated with a dose of 0.8 mg/kg of the inhibitor, SL, small
290 litter, SL 0,8, small litter treated with a dose of 0.8 mg/kg of the inhibitor. I, the interaction
291 between treatment and litter, L, litter factor and T, treatment factor. Data are presented as the
292 mean ± SEM. Two-way ANOVA and Tukey post-test, *p< 0.05, **p < 0.01 and *** p <0.001.

293

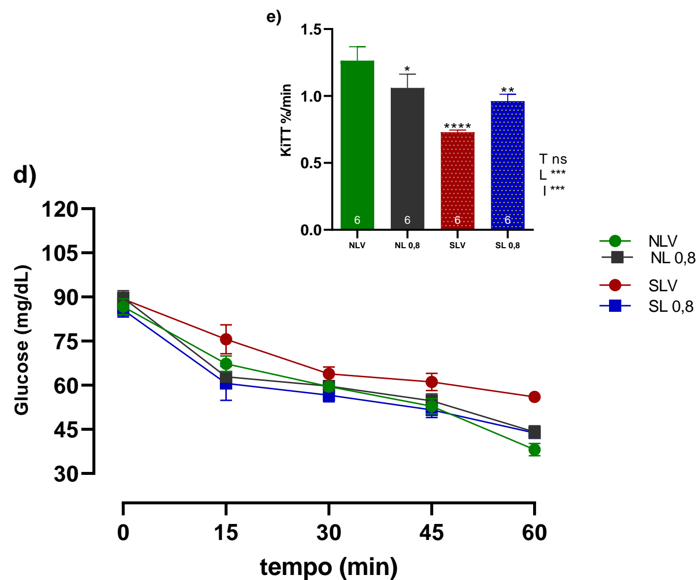
294 ## 3.2 Glucose homeostasis

295 Fasting plasma glucose was measured on the day of euthanasia, expressing the same
296 behavior of glucose intolerance as the SL group (NL: 105.6 ± 2.335 *vs* SL: 120.7 ± 1.353,
297 p=0.0005; Fig. 3a). During oGTT, a blood glucose spike was observed 15 minutes after glucose
298 injection and euglycemia was restored at approximately 120 minutes. At times 15 (+10%), 30
299 (+12%), and 45 (+11%) min of the curve, SL rats exhibited higher blood glucose concentration
300 compared to NL animals, which translated into a greater area under the blood glucose curve
301 (+10%, p=0.011 Fig. 3b). Contrasting this parameter, the treated obese group SL 0.8, obtained
302 improvement in both times, being statistically not different from the control group NL in the
303 area under the curve (Fig 3c).



304

305 As shown in Fig. 3d, the glucose level during ipITT remained higher in the SL group
306 (p=0.0003; Fig. 3d), a parameter confirmed by Kitt, which was significantly low in these
307 animals -30% (NL: 1.264 ± 0.32 *vs* SL: 0.729 ± 0.152, p<0.0001; Fig. 3e).



308

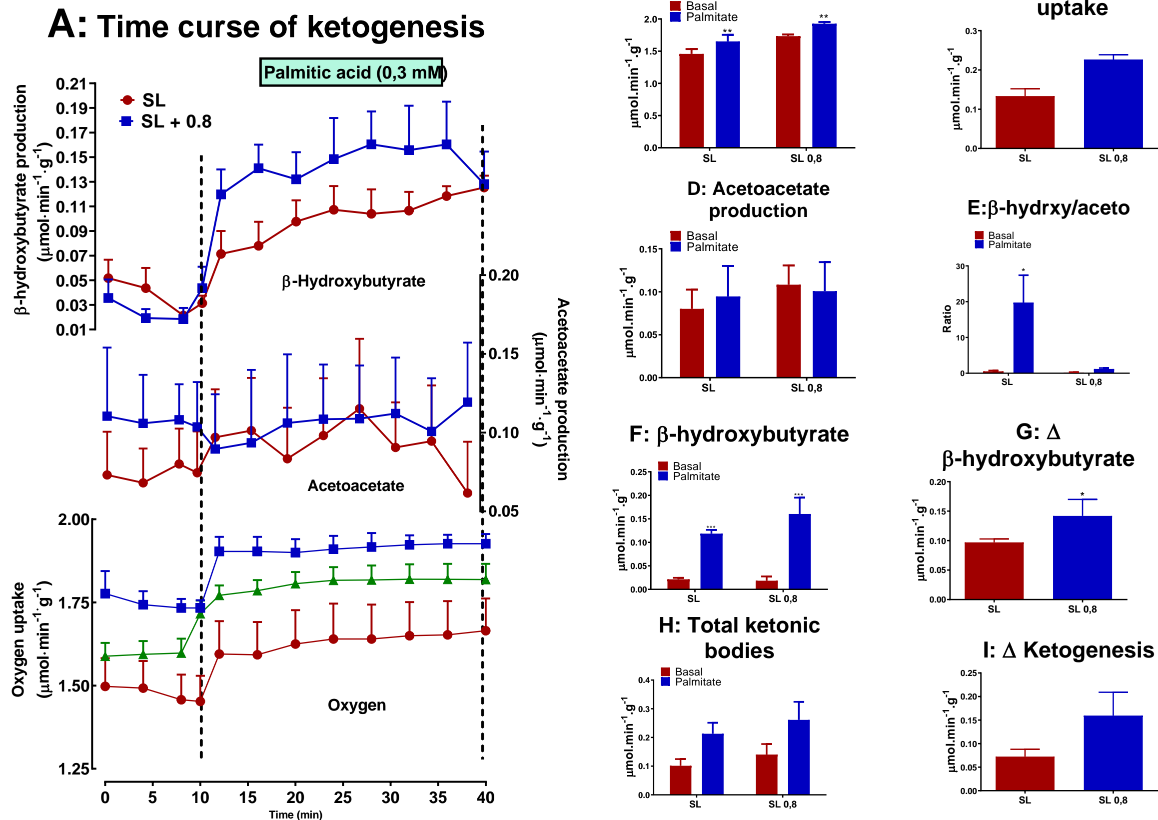
309 **Fig.3: Effect of ACC inhibitor treatment on glucose homeostasis.** And fasting plasma
310 glucose (a) blood glucose curve (b) and area under the curve (AUC) of blood glucose curve
311 durithe ng oGTT (c) Blood glucose curve (d) and blood glucose decay (Kitt) during ipITT (e).
312 N = 6-8 animals per group NL, normal litter; NL 0,8, normal litter treated with a dose of 0.8
313 mg/kg of the inhibitor, SL, small litter, SL 0,8, small litter treated with a dose of 0.8 mg/kg of
314 the inhibitor. I, the interaction between treatment and litter, L, litter factor and T, treatment
315 factor. Data are presented as the mean ± SEM. Two-way ANOVA and Tukey post-test, *p <
316 0.05, **p < 0.01 and *** p <0.001.

317

318 **3.3 Liver perfusion and evaluation of Ketogenesis**

319 Rat liver perfusion experiments were performed to evaluate whether treatment with the
320 inhibitor can interfere with hepatic metabolism specifically with carbohydrate metabolism.
321 Ketogenesis was chosen because it is a compartmentalized pathway and is particularly sensitive
322 to changes in cellular integrity. Rats fasted for 12 hours to deplete endogenous glycogen. In
323 these animals, the glucose concentration of the perfused effluent mainly reflects the rate of
324 ketogenesis. The first ten minutes of perfusion (0-10 min) correspond to the baseline period.
325 The one with palmitic acid (10-40min) promoted a progressive increase in the production of β-
326 hydroxybutyrate ($p=0.0128$) and acetoacetate ($p=0.0084$) of and in the oxygen consumption of
327 the animals ($p<0.0001$).

328 We can observe the difference in oxygen consumption ($p= 0.0001$) between the NL and
329 SL groups, which corroborates the other findings confirming the induction of the metabolic
330 syndrome. In the SL 0.8 group, the opposite occurs and this deficit is attenuated, reaching the
331 same level as the NL animal, and even with greater uptake (Fig.5A). In the evaluation of the
332 ketone bodies produced during the perfusion, it can be observed that the SL 0.8 animals present
333 higher production ($p=0.0277$) when compared to the basal state (Fig.5H). Such parameters
334 indicate an increased oxidative status in the treatment group.



335

336 **Fig. 5. Effects of ACC inhibitor on ketogenesis in the liver of obese rats perfused with**
337 **palmitic acid.** A: Time course of ketone body production and oxygen consumption in the
338 perfused liver caused by infusion with palmitic acid. The livers of 16-hour fasted rats were
339 perfused with Krebs/Henseleit buffer (basal). 0.3 mM palmitic acid was introduced as indicated
340 by the horizontal bars. The outgoing perfusate was sampled at regular intervals and analyzed
341 for its acetoacetate and β-hydroxybutyrate contents. Oxygen consumption was monitored by
342 polarography. The values in Panels B, D, F, and H are the rates of production of liver
343 metabolites at baseline steady state (black bars: time 8 minutes in Figure 6A) and steady state
344 after the introduction of palmitic acid (white bars: time 36 minutes in Figure 5A). Total ketone
345 bodies correspond to acetoacetate + β-hydroxybutyrate. The values in Panels C, G and I are the
346 increments in the production of metabolites caused by the palmitic acid infusion and were
347 calculated as (values at the end of the palmitic acid infusion period; 32 min) – (values before
348 palmitic acid infusion; 8 min) in Figure 5A. Data are the mean ± SEM of 3 - 5 animals for each
349 condition. * $p<0.05$; ** $p < 0.01$; *** $p < 0.0001$: difference between baseline and steady state
350 after addition of palmitic acid; # $p < 0.05$: difference between control and treatment (ANOVA
351 one way and Newman-Keuls post-test). The statistical difference is shown by horizontal bars
352 in panels B, D, F, and H: paired Student's t-test.

353

354

355

356 **4. Discussion**

357 The major finding of the present study is that obese rats treated with clodynafop-propargyl
358 present a beneficial interaction between hepatic lipid and glucose metabolism. To our
359 knowledge, the first published study showing the therapeutic use of this inhibitor can reverse
360 glucose intolerance and improve insulin sensitivity and the oxygen uptake deficit.

361 Previous studies have shown that the model of litter size reduction induces increased
362 breast milk consumption, energy intake, and, consequently, weight gain, hypertriglyceridemia,
363 glucose intolerance, and peripheral insulin resistance in adult offspring (Malta et al. 2016;
364 Previate et al. 2020 and Pavanello et al. 2022). In this research, adult animals SL treated for
365 thirty days with 0.8 mg/kg of CP showed a 2% reduction in body weight during the treatment
366 period when compared to untreated SL (Fig.1c). On the other hand, we did not observe a
367 reduction in fat pad stores in animals treated, suggesting that exposure to inhibitor can be time-
368 dependent.

369 The present study also showed that the lipid profile of SL 0.8 animals was attenuated
370 about SLV animals, and this was observed in the triglycerides (35%), total cholesterol (17.5%),
371 VLDL (30%), LDL (70%) and HDL (27%) levels. These findings are similar to those of Kim
372 et al. 2017, which obtained a 20% improvement in the lipid profile of the treated obese mice
373 and humans. Contrasting with the present study, they obtained an increase in plasma
374 triglycerides (20%).

375 The current research revealed improvements in insulin sensitivity and glucose
376 intolerance in SL 0.8 compared to the counterpart control group (10%). Recent findings by
377 Takagi et al. 2021, demonstrated that ACC2 inhibition resulted in decreased skeletal muscle
378 long-chain acyl-CoA pools and total intramyocellular lipid content, as well as insulin resistance,
379 using either ACC2 knockout mice or the ACC2 inhibitor. The ACC enzyme has two distinct
380 domains of action. Cytosolic ACC1 generates a pool of malonyl-CoA used by fatty acid
381 synthase to generate palmitate. Mitochondrial ACC2, provides a local pool of malonyl-CoA to
382 allosterically inhibit carnitine palmitoyl transferase 1 (CPT1). This enzyme transports medium-
383 and long-chain fatty acids into the mitochondria for β-oxidation (Bates et al. 2020).

384 Inhibition of ACC2 may result in greater accumulation of mitochondrial acetyl-CoA and
385 activation of pyruvate carboxylase, which promotes increased hepatic gluconeogenesis
386 (Lambrecht and Tacke 2020). This could explain both the improvement in the lipid and

387 glycemic profile, as well as the more oxidative state in treated animals, shown in the present
388 study. In addition, Kim et al. 2017 showed in human clinical trials, that single oral doses of
389 another ACC inhibitor, MK-4074 at 30 and 100 mg/kg, increased plasma total ketones, a
390 biomarker for liver fatty acid oxidation. Despite some experimental limitations of the current
391 study, we suggest that the expression of some enzymes and metabolic pathways should be
392 investigated to elucidate the reasons behind the mechanism underlying these responses.

393 Although the administration of clodinafop-propargyl was not effective to reduce the fat
394 pad stores, it improved glucose metabolism and biochemical markers of liver dysfunction.
395 Taken together, the current study suggests that clodinafop-propargyl, an ACC inhibitor was
396 able to inhibit lipid metabolism, and it holds clear potential for new therapeutic approaches to
397 treat obesity and metabolic syndrome. These findings support the rationale for new studies
398 involving basic and clinical investigations with a scientific translation approach needed to fill
399 the gaps related to human outcomes.

400 **Conflict of Interest**

401 The authors declare that the research was conducted in the absence of any commercial or
402 financial relationships that could be construed as a potential conflict of interest.

403 **Author Contributions**

404 MC, LPJS, SP, PCFM, and WDS contributed to the design of the study project, data analysis,
405 and manuscript writing. MC, LPJS, SP, MVGR, AROF, SRR, CBZ, ACHS, and NCL
406 contributed to theperformance of the experiments and data analysis. LPJS, MVGR, AROF, JFC,
407 PCFM, and WDS helped in interpreting the results. All authors approved the final version of
408 the manuscript.

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