

1 **Green tea infusion aggravates nutritional status of the juvenile**
2 **untreated STZ-induced type 1 diabetic rat**

3
4 Short title: Tea worsen diabetic rats' health

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1 **Summary**

2

3 We have described for the first time the potential harmful effects of green tea on the
4 metabolism and body composition of untreated juvenile experimental type 1 diabetic rats.
5 The treatment containing 19.38% of epigallocatechin-3-gallate, its main catechin,
6 increased blood glucose and water intake. It also increased oxygen consumption,
7 enhanced energy expenditure and led to a lipid oxidation tendency in diabetic animals,
8 which worsened the development of body fat in a way significantly more aggravated than
9 diabetes alone. Taken together, our findings indicate that green tea treatment, when
10 provided to juvenile diabetics, increases glycaemia, changes the body composition by
11 reducing fat content and increases oxygen consumption, besides affecting energy
12 expenditure. Therefore, the nutritional status of the juvenile type 1 diabetic rat is
13 aggravated.

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15 **Keywords:** Type 1 diabetes, green tea, body composition, energy expenditure, nutritional
16 status

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1 **1. Introduction**

2 Diabetes mellitus (DM) is a serious heterogeneous metabolic disease with
3 increasing rates of incidence and prevalence worldwide ^{1,2}. It is estimated that about 415
4 million adults have DM, and in 2040 figures are expected to reach 642 million ³. The
5 disease-related complications, especially in type 1 DM (i.e. insufficient production of
6 insulin by the pancreatic beta cells) include micro and macrovascular disturbances ⁴,
7 hepatic damage ⁵, renal ⁶, cardiac ⁷, and neurological impairment ⁸, aside from the poor
8 nutritional status characterized by diabetes. Taken together, these complications become
9 an important cause of morbidity and mortality, with negative impact on life quality,
10 consequently reducing the life expectancy of these individuals ^{1,3}.

11 Insulin therapy is effective and safe for the treatment of type 1 DM ⁹, but it alone
12 does not eliminate the risk of complications from the disease. Therefore, non-
13 pharmacological strategies, such as physical exercises and use of natural compounds with
14 antioxidant polyphenols have been described as a complementary treatment ¹⁰⁻¹⁴. Within
15 this category of compounds, studies have focused on the effect of epigallocatechin-3-
16 gallate (EGCG), a catechin present in large amounts in green tea ¹⁴⁻¹⁶. Due to its potential
17 therapeutic effect and pharmacological action (e.g. antioxidant, antidiabetic, anti-
18 inflammatory and anti-apoptotic properties) described in previous studies ^{15,17-20}, EGCG
19 has been considered as a possible adjuvant in the treatment of diabetes, so as to improve
20 the general health of individuals and, consequently, delay the development of DM
21 complications ^{13,14,21}. It has been suggested that this adjuvant profile is closely correlated
22 with the inhibition of glucose production in hepatocytes ²². In fact, studies showed that
23 green tea catequins suppressed hepatic gluconeogenic activity and activated the 5'-AMP-
24 activated protein kinase (AMPK), which improved insulin signaling pathway and
25 downregulated the genes that encode gluconeogenic enzymes ²²⁻²⁴.

1 Due to the antidiabetic activity described for EGCG and its antioxidant
2 proprieties, various studies have explored the use of tea catechins, isolated or in
3 combination with other drinks with similar proprieties, as an alternative to evaluate its
4 systemic effect on different pathophysiological processes^{15,25}. Despite its relevant effects
5 in most cases, one should consider the thermogenic potential of this kind of substance
6 ^{26,27}. It is well known that green tea polyphenolic compounds also modulate energy
7 metabolism, thus enhancing thermogenesis, fat oxidation and energy expenditure²⁷⁻³⁰.
8 Therefore, caution should be taken, especially regarding the use of these substances to
9 treat some diseases, since this type of response can be potentially noxious and aggravate
10 an already installed pathological process.

11 In type 1 DM, some of the green tea metabolic effects can be harmful to a certain
12 extent³¹⁻³³. Studies with experimental diabetes in rats showed that untreated diabetic
13 animals present an impaired nutritional status and, especially when the disease appears in
14 preadolescent rats or at younger ages, this condition can be aggravated and irreversible
15 ^{34,35}. Thus, the present study aimed to investigate the effects of green tea infusion on the
16 nutritional status of type 1 diabetic rats, considering their feeding and murinometric
17 parameters, body composition and metabolism of their untreated experimental model of
18 type 1 diabetic rat.

19

20 **2. Materials and methods**

21 *2.1. Animals and ethics statement*

22 Eighteen male Wistar rats (30 days old; 82.52 ± 10.83 g) were provided by the
23 Central Animal Laboratory of the Center of Biosciences and Health from the Federal
24 University of Viçosa. The animals were housed in polypropylene cages, in pairs, under
25 controlled conditions of temperature (22 ± 2 °C) and light-dark cycles (12/12h). All

1 animals received food (Presence Alimentos, Paulínea, SP, Brazil) and water *ad libitum*.
2 The use of animals in the research was approved by the Ethics Committee of Animal Use
3 of the Federal University of Viçosa (CEUA/UFV – protocol number 53/2018).

4

5 *2.2. Preparation of green tea infusion*

6 Five different lots of green tea (*Camellia sinensis*) leaves were obtained from
7 Leão[®] - Food and Beverages (Coca-Cola Company[®]). The lots were mixed (1:1) and the
8 infusion was prepared mixing the leaves with warm distilled water (1:40 w/v, 80 °C)³⁶.
9 The mixture remained infused for 20 minutes on a magnetic stirrer. Then, it was filtered
10 through a 0.45 µm porous filter, frozen at -80 °C and lyophilized. The lyophilized samples
11 were resuspended in distilled water at the moment of use.

12

13 *2.3. Determination of total phenolic content*

14 Total phenolic content was determined in triplicates as described before by
15 Singleton and Rossi³⁷ using the Folin-Ciocalteu reagent. To that porpoise, an aliquot of
16 0.6 mL of the lyophilized extract resuspended in distilled water (1:25 w/v) was added to
17 3 mL of the Folin-Ciocalteu reagent. After 6 minutes, 2.4 mL of 7.5% sodium carbonate
18 solution was added and agitated. The tubes were allowed to stand in dark for 1 hour at
19 room temperature. The absorbance was measured at 760nm using an ultraviolet (UV)-
20 spectrophotometer (BEL UV-M51, BEL Photonics, Italy). Different concentrations of
21 gallic acid dissolved in distilled water were used to prepare the calibration curve ($r^2 =$
22 0.9992). The total phenolic content was expressed as milligrams of gallic acid equivalent
23 per gram of lyophilized samples of tea (mg GAE/g GTI).

24

25 *2.4. EGCG analysis*

1 EGCG analysis was performed as described by Kim-Park et al.³⁸, with some
2 modifications. High-performance liquid chromatography (HPLC) (Prominence LC-20A,
3 Shimadzu, Kyoto, Japan), equipped with Diode Arrangement Detector (DAD), LC-20AD
4 pump, SPD-M20A detector, CTO-20A oven and LabSolutions software, was used to
5 determine the EGCG content using a maximal absorption peaks at 272nm. It was used a
6 Vydac C18 (4.6 x 250 mm) column, at 30 °C, with a 5µL injection volume. The mobile
7 phase was composed of water and 2.0% acetic acid (1:1). The infusion lyophilized
8 powder was suspended in methanol before analysis. The mobile phase flow rate was 1.0
9 mL/min and the run time was 15 min. The retention time of EGCG was 4.5 min and the
10 total amount of it was calculated using a standard curve ($r^2 = 0.9967$) developed under
11 the same conditions using an EGCG chemical standard ($\geq 98.0\%$, Sigma Aldrich Inc. -
12 CAS Number 989-51-5. St. Louis, MO, USA).

13

14 *2.4. Antioxidant capacity by the 2,2'-Azinobis-[3-ethylbenzthiazoline-6-sulfonic acid]*
15 *(ABTS) decolorization assay*

16 ABTS radical stabilization by the lyophilized tea extract was determined at a
17 wavelength of 734 nm on an ultraviolet (UV)-spectrophotometer (BEL UV-M51, BEL
18 Photonics, Italy), following the described method by RE et al.³⁹. Different concentrations
19 of trolox dissolved in ethanol (80 %) were used to prepare the calibration curve ($r^2 =$
20 0.9996). The antioxidant capacity by the ABTS method was expressed as µMol of trolox
21 equivalent per gram of lyophilized samples of tea (µMol TE/g GTI).

22

23 *2.5. Antioxidant capacity by ferric reducing antioxidant power (FRAP) assay*

1 The FRAP assay was performed as described before ⁴⁰. An aliquot of the
2 lyophilized extract resuspended in distilled water (1:25 w/v) was added to the FRAP
3 reagent and incubated at 37°C for 30 min. The absorbance was determined at a 595 nm
4 wavelength on an ultraviolet (UV)-spectrophotometer (BEL UV-M51, BEL Photonics,
5 Italy). Different concentrations of ferrous sulphate (FeSO₄) dissolved in distilled water
6 were used to prepare the calibration curve ($r^2 = 0.9985$). The antioxidant capacity by the
7 FRAP method was expressed as μMol of FeSO₄ equivalent per gram of lyophilized
8 samples of tea ($\mu\text{Mol FeSO}_4/\text{g GTI}$).

9

10 *2.6. Experimental design*

11 After seven days of acclimation in the bioterium, six rats were randomly selected
12 to integrate the healthy control group. Type 1 diabetes was induced in 12 rats, after 12 h
13 fasting by a single intraperitoneal (i.p.) injection of streptozotocin (STZ) (Sigma
14 Chemical Co., St, Louis, MO, USA) at a dosage of 60mg/Kg of body weight (BW) diluted
15 in 0.01 M sodium citrate buffer, pH 4.5. The control group received the buffer alone by
16 the same administration route ¹². Two days after the STZ injection, following 12 h fasting,
17 blood samples were collected from the tail vein and glycaemia was measured using a
18 glucometer (Accu-Chek® Performa, Roche LTDA). All animals presented fasting blood
19 glucose levels higher than 250 mg/dL and were included in the study. The hyperglycemic
20 rats were divided into two groups (n = 6, each). Thus, the experimental protocol consisted
21 in three groups: healthy control group (Ctrl, n = 6); diabetic control group (Diabetes, n =
22 6); and the diabetic group treated with the green tea infusion (GTI diabetic, n = 6), which
23 received a 100mg/Kg dosage of GTI, diluted in 0.6mL of water. The control groups
24 received 0.6mL of water alone. All treatments (GTI and water) were administered by
25 gavage, every day, during 42 days.

1 Considering that type 1 diabetes usually appears at young ages⁴¹, the experimental
2 protocol started when the animals were 40 days old and finished when they reached 82
3 days of age, (i.e. from periadolescence to the early adult phase)⁴².

4 After the experimental protocol period, the animals were euthanized by deep
5 anesthesia (sodium thiopental, 60mg/Kg i.p.) followed by cardiac puncture and
6 exsanguination³³.

7

8 *2.7. Blood glucose, body weight and food and water consumption*

9 Fasting blood glucose was measured in blood samples from the tail vein using a
10 glucometer and reactive strips (Accu-Chek® Performa, Roche LTDA. Jaguaré, SP,
11 Brazil). Body weight, water and food consumption were measured using a precision scale
12 (BEL M503, 0.001g, Piracicaba, SP, Brazil). All these parameters were monitored
13 weekly.

14

15 *2.8. Murinometric and feeding parameters*

16 All the murinometric and feeding measurements were calculated as described by
17 Nery et al.⁴³. On the last day of the experimental protocol, the naso-anal length (NAL) of
18 the rats was measured with an inelastic measuring tape ($e = 0.1$ cm) to calculate the
19 following indicators: Lee index (Lee) = $\left[\frac{3\sqrt{BW}}{NAL} \right]$ and Body Mass Index (BMI) = $\frac{BW}{NAL^2}$, where
20 BW refers to the final body weight, and NAL, the naso-anal length. The following feeding
21 indexes were also calculated: Specific Rate of Weight Gain (SRWG) = $\frac{fBW - iBW}{iBW}$, where
22 iBW refers to body weight at the beginning of the experiment and fBW refers to the final
23 BW. Feeding efficiency was indicated by the Coefficient of Feeding Efficiency (CFE)
24 and Weight Gain per Caloric Consumption (WGCC), calculated as follows: CFE =
25 $\frac{fBW - iBW}{tF}$, where tF refers to the total amount of food ingested (g) in the experiment.

1 $WGCC = \frac{fBW - iBW}{tKcal}$, where tKcal stands for the total amount of Kcal ingested in the
2 experiment.

3

4 *2.9. Dual-energy X-ray absorptiometry analysis*

5 Body composition was evaluated under anesthesia (sodium thiopental, 60mg/Kg
6 i.p.) on the 36th day of treatment. The rats were positioned in ventral recumbency on the
7 scan table. All scans were performed using dual-energy X-ray absorptiometry (DXA)
8 (Lunar, DPX, Madison, WI, USA) to evaluate fat (% and g) and lean mass (%). An
9 accelerating voltage of 100 kV with current of 0.188 mA and radiation dose of 10
10 microGy were used for scanning. The Encore v.13 2011 (GE Healthcare Systems,
11 Chicago, IL, USA) software system was used for data analysis. The results were
12 expressed as a mean value.

13

14 *2.10. Calorimetric analysis*

15 The oxygen (O₂) consumption and carbon dioxide (CO₂) production of the
16 experimental animals were measured through gas analyzer (Oxylepto, Harvard
17 Apparatus, Holliston, MA, USA) on the 40th day of treatment, without fasting. To that
18 end, the ambient air was pumped through a metabolic chamber and samples of the
19 extracted air were directed to the gas analyzer (air flow = 1.0 L/min). The Metabolism
20 (Panlab, Barcelona, Spain) software system was used for data analysis. The animals
21 remained for 60 minutes in the metabolic cage mimicking their real conditions in the
22 laboratory for the determination VO₂ (mL/min/Kg^{0.75}) and VCO₂ (mL/min/Kg^{0.75}) at rest.
23 The test was performed with animals from the three experimental groups, concomitantly,
24 from 6 pm to 11 pm ⁴⁴.

1 The respiratory quotient (RQ) and the total 24 h energy expenditure rate (EE)
2 (Kcal/day) were calculated using the following equations: $RQ = VCO_2/VO_2$, where VCO_2
3 refers to the volume of CO_2 produced by the rats and VO_2 , the O_2 volume consumed
4 during the assay; and $EE = (3.815 + (1.232 * RQ)) * VO_2 * 1.44$ is used for energy
5 expenditure.

6

7 *2.11. Statistical analysis*

8 All the results were submitted to the Shapiro-Wilk test for normality assessment.
9 The data expressed as percentage were transformed by angular transformation before the
10 analysis. The results were expressed as mean \pm standard deviation (mean \pm SD) and
11 analyzed using unpaired Student's *t*-test when the variances were equal (by *F* test) and
12 unpaired Student's *t*-test with Welch's correction for data with unequal variances (Ctrl vs
13 Diabetes; Diabetes vs GTI diabetic). Statistical significance was established at $P \leq 0.05$.
14 All tests and graphics were performed using the GraphPad Prism 6.0 statistical software
15 system (GraphPad Software Inc., San Diego, CA, USA).

16

17 **3. Results**

18 *3.1. Green tea infusion phytochemical analysis*

19 The total amount phenolic components in the green tea infusion lyophilized
20 powder was evidenced to be 3.88 ± 2.49 mg GAE/g GTI. The EGCG content, analyzed
21 by HPLC methodology, was shown to be 19.38% of the total GTI content. The extract
22 presented an antioxidant capacity of 3.26 ± 0.06 μ Mol TE/g GTI in the ABTS assay and
23 46.38 ± 4.1 μ Mol $FeSO_4$ /g GTI in the FRAP assay.

24

25 *3.2. Green tea infusion increases glycaemia and favors the polydipsia in diabetic animals*

1 After diabetes induction and subsequent hyperglycaemia confirmation in the
2 experimental animals (i.e. above 250 mg/dL), both diabetic groups maintained high
3 blood glucose levels, which remained above 400 mg/dL, compared with the healthy
4 control group (glucose < 100 mg/dL) in the last four weeks of the experimental protocol.
5 Besides, GTI diabetic rats presented glycemic levels significantly aggravated ($P =$
6 0.0223; Fig. 1 B). Increased glucose levels were consistent with the consequent increment
7 in water consumption in the same experimental groups (Fig. 1 D). The diabetic animals
8 maintained high water consumption compared to the Ctrl group during the entire
9 experiment. In the sixth week, this value was significantly higher for the GTI diabetic
10 group ($P = 0.0296$), compared with the diabetic animals that did not receive the green tea
11 infusion and between the diabetic group compared with the healthy control group ($P <$
12 0.0001; Fig. 1 D).

13 Body weight (Fig. 1 E) presented normal evolution during the six weeks of the
14 experimental protocol for the healthy control group, ranging from 100 to 280g from the
15 first to the sixth week, respectively. In the diabetic animals, this weight gain was severely
16 impaired and no additional weight was registered in these animals during the six weeks
17 of the experiment. This deficiency was observed in the sixth week (Fig. 1 F).. Body
18 weight gain decreased significantly in diabetic animals ($P < 0.0001$), compared to the
19 control. No differences for this variable were observed between the diabetic groups,
20 treated with GTI or not. No statistic differences were observed for food ingestion between
21 the control and the experimental groups (Fig. 1 G and H) during the six weeks of the
22 study.

23

24 3.3. Diabetes changes murinometric and feeding parameters

1 The murinometric parameters indicate that the rats from the diabetic groups
2 remained with similar body proportions, but were different from the healthy control
3 group, as indicated by NAL and the BMI ($P < 0.0001$; Table 1). The Lee index shows
4 that body weight is proportional to NAL in all groups, regardless of the rat size.

5 Food intake did not differ throughout the experimental period and between
6 experimental groups. However, the differences in BW and murinometric parameters can
7 be explained by the feeding efficiency parameters. SRWG, CFE, and consequently
8 WGCC, presented lower values in the diabetic groups ($P < 0.0001$; Table 1F), which
9 indicates decreased efficiency in the conversion of food nutrients into tissue components.

10

11 *3.4. Green tea infusion reduces fat mass gain in diabetic animals*

12 The body composition examination revealed significant differences in the fat mass
13 of the diabetic animals in both percentage levels (%) and absolute amount (g) when
14 compared with the control animals ($P < 0.0001$; Fig. 2 A and B, respectively). Green tea
15 infusion accelerated this response by significantly reducing fat accumulation, which is
16 demonstrated by the relative amount of fat at the end of the experiment ($P = 0.0045$). It
17 reached $9.3 \pm 2.9\%$ of the total body weight with the minimum value of 5.8%. Similar
18 behavior was observed in its absolute amount compared to the untreated diabetic animals
19 ($P = 0.0053$). Consequently, the relative lean mass (Fig. 2 C) represented a major portion
20 of the body of the rats in the Diabetic group ($P < 0.0001$) compared to the Ctrl, and even
21 higher in the GTI diabetic ($P = 0.005$) compared to the Diabetes group.

22

23 *3.5. Green tea infusion elevates the energy expenditure of diabetic animals*

24 The calorimetric analysis revealed modulation in the metabolism of diabetic rats.
25 Increased oxygen consumption was observed in the Diabetes group ($P = 0.0058$; Fig. 3

1 A) resulting in higher energy expenditure throughout the day ($P = 0.0106$; Fig. 3 D). On
2 the other hand, animals treated with the GTI presented higher oxygen consumption and
3 EE, when compared with the diabetic group that received the placebo treatment (water)
4 ($P < 0.05$; Fig. 3 A and D). As shown in Fig. 3 C, the respiratory quotient (RQ) of the
5 diabetic groups was lower, compared to the results of the control group ($P = 0.0010$),
6 which indicates variation in the preferential macronutrient substrate used for energy
7 generation.

8

9 **4. Discussion**

10 Large amount of catechins are found in green tea (i.e. obtained from the *Camellia*
11 *sinensis* L.). Their effects have been extensively explored⁴⁵⁻⁴⁷ due to their potential health
12 benefits in the treatment and prevention of human diseases. Its antidiabetic properties
13 have been proven in previous studies¹⁷⁻¹⁹, but this is the first time that strong evidence is
14 reported that green tea infusion has great impact on glycaemia, body composition,
15 nutritional status and metabolic activity in young streptozotocin-induced diabetic rats.

16 Admittedly, regarding the hypoglycemic effects of green tea and its components,
17 specifically EGCG, important therapeutic potential has been demonstrated under
18 experimental conditions^{1,14,15,48,49}. This, in turn, has been closely related to the potential
19 of this substance to increase insulin activity^{19,50}. However, it was observed that green tea
20 containing a proven amount of 19.38% of EGCG contributed differently in that
21 parameter. The diabetic animals treated with green tea presented considerably higher
22 blood glucose levels compared to the untreated rats. Streptozotocin, used in our study to
23 induce the diabetic condition in the experimental animals, destroys the pancreatic insulin
24 producer beta cells, leading to hyperglycemic condition⁵¹. Thus, a positive relation
25 cannot be attributed to the interaction between tea catechins and insulin. Therefore, it is

1 possible to hypothesize that the maintenance of hyperglycaemia by GTI in our
2 investigation may be related to the differential expression of glucose transporters or
3 alterations in energetic metabolic pathways.

4 Aligned with this perspective, Kobayashi et al.⁵² showed that green tea catechins
5 can inhibit the sodium-dependent glucose transporter 1 (SGLT1) in the brush-border
6 membrane of enterocytes. This *in vitro* study, using brush-border membrane vesicles
7 obtained from the small intestine of healthy rabbits, demonstrates that catechins
8 containing the galloyl radical (epicatechin gallate and EGCG) were shown to bind to
9 SGLT1, rendering the transporter unusable. Due to the inhibition of glucose uptake by
10 the intestine and consequent drop in blood glucose, such an outcome could be
11 encouraging. However, animals with STZ-induced diabetes are able to express another
12 glucose transporter at the brush-border and basolateral membranes of enterocytes, such
13 as transporter GLUT1, which is not expressed on the brush membrane of enterocytes in
14 healthy animals⁵³, and GLUT2, that is inserted in the brush-border membrane when the
15 luminal amount of glucose is still significant⁵⁴⁻⁵⁶. GLUT1 expression, combined with
16 GLUT2 regulation, maintains intestinal glucose uptake, regardless of the inactivation of
17 SGLT1, thus preserving the hyperglycemic condition of the STZ-diabetic animal.

18 It is well established that persistently elevated glycaemia exacerbates the
19 symptoms of type 1 diabetes (i.e. polyuria, polyphagia, polydipsia, extreme fatigue,
20 weight loss despite high food intake), as reviewed by Ullah et al.⁸. Green tea contributed
21 to aggravate these symptoms, since tea in our study reinforces the maintenance of
22 hyperglycaemia. Although polyphagia is also a symptomatic consequence of type 1
23 diabetes⁸, all groups presented similar food consumption throughout the experiment. On
24 the other hand, body weight was compromised in diabetic animals, regardless of GTI
25 intake. In contrast, studies were consistent in showing a positive relationship between

1 green tea consumption and increased body weight gain in untreated experimental type 1
2 diabetes^{13–15}. However, in these studies, diabetes was induced in adult animals already
3 presenting an optimal level of body development. We believe that the fact that diabetes
4 was induced in animals at periadolescent age has caused the poor development and
5 compromised weight gain observed during the six weeks of study.

6 Those differences in body weight, combined with similar levels of food
7 consumption generated lower values of feeding efficiency features in our experimental
8 model. Thus, the specific rate of weight gain as well as the coefficient of feeding
9 efficiency and weight gain per caloric consumption were significantly lower in diabetic
10 groups regardless of green tea consumption. Aligned with this perspective, other studies
11 had already shown clear evidence that confirm these findings^{57–59}. These data reveal
12 reduced efficiency in food nutrient conversion into tissue components, as previously
13 described³⁵. Herrero et al.⁶⁰ attributed this fact to the lack of plasma insulin, which
14 prevents the transport of glucose to insulin-dependent cells (i.e. adipocytes, myocytes and
15 cardiomyocytes), thereby forcing changes in metabolic routes so as to increase fat use.

16 This impairment in weight gain also delayed the body development of the diabetic
17 rats. This finding can be corroborated by both the growth impairment of their naso-anal
18 length and the stagnant fat mass accumulation observed in the X-ray absorptiometry
19 scanning. According to Silva et al.³⁴, when diabetes occurs at young age, it may
20 compromise normal bone development. We did not directly evaluate this tissue, but some
21 studies consistently point out the positive relation between diabetes and poor bone
22 mineral metabolism and consequent impaired animal growth^{34,35}. This condition impacts
23 the rate of bone mineral apposition and decreases the activity of osteoblastic cells, which
24 leads to premature bone growth interruption, with consequent impairment to bone
25 development in murine models of type 1 diabetes induced by STZ or alloxan. These facts

1 consequently impair the length and size development of diabetic animals^{34,35}. We also
2 proved their negative impact on the body composition of diabetic animals, in which
3 impaired fat mass gain was aggravated when green tea was administered.

4 It has been discussed the relation between the EGCG, present in green tea, and
5 increased lipolysis secondary to glucagon secretion^{61,62}. Studies have shown that EGCG
6 is a potent inhibitor of the enzyme catechol-o-methyltransferase (COMT), which
7 degrades norepinephrine^{63,64}. Norepinephrine persistence maintains beta adrenergic
8 stimuli in pancreatic alpha cells, which increases glucagon production and release⁶⁵.
9 Without inhibition by insulin, glucagon stimulates glycogenolysis in the liver until the
10 depletion of the glycogen stocks⁶¹. At this point, glucagon also stimulates
11 gluconeogenesis, leading to the production of glucose from other substrates, such as
12 proteins, besides increasing lipolysis and reducing fat deposits^{61,66}. Although we have
13 not quantified glucagon, these mechanisms can explain the green tea impact on body
14 composition in diabetic animals.

15 This persistent activation of the beta adrenergic stimuli mediated by green tea
16 catechins in the pancreatic alpha cells, as previously described, corroborates the findings
17 of higher oxygen consumption (VO_2) and daily energy expenditure (EE: Kcal/day/Kg^{0.75})
18 in diabetic animals treated with tea in our study. Type 1 diabetes induces higher oxygen
19 consumption by modulating the energetic metabolism and the substrate utilization in
20 energy production^{60,67}. Qualitatively, the respiratory quotient (RQ) indicates the types of
21 energy substrate the animal preferentially consumes. Our control animals presented an
22 RQ ranging between 0.9 and 1.0, which indicates a preference for carbohydrate
23 hydrolysis. On the other hand, diabetic groups presented an RQ between 0.7 and 0.9,
24 which indicates major fat oxidation for energy production⁶⁸⁻⁷⁰. Tea catechins are linked
25 to an improved expression of proteins related to beta oxidation and thermogenic capacity

1 ^{27,71,72}. Both mechanisms require an expanded mitochondrial activity that, in turn, leads
2 to an increased demand of oxygen ^{8,73}. We measured and demonstrated that the diabetic
3 animals consumed more oxygen than the healthy control group. The green tea treatment,
4 in contrast, increased oxygen consumption, which reflected in the daily energy
5 expenditure of the rats treated with tea, maybe due to increased metabolic rate and/or the
6 stimulation of lipolysis, beta oxidation and thermogenesis.

7 Treating young type 1 diabetic animals with green tea or its catechins seems to be
8 a two-way pathway. At first, the use of tea and its molecules with highly antioxidant
9 capacity seems effective against diabetes complications, as exhaustively described by the
10 scientific literature. However, these molecules have other activities. They increase the
11 mobilization and use of fat as energy source by the organism and even stimulate
12 thermogenesis, processes that generate large amounts of reactive oxygen species.
13 Catechins also contribute to maintain the hyperglycaemia by glycogenolysis and
14 gluconeogenesis stimulated by glucagon. The most likely explanation for the lack of
15 hypoglycemic effect of green tea combined with the impaired fat mass gain and increased
16 energy expenditure in this study seems to be the hypothesis of COMT inhibition with
17 consequent prolongation of beta adrenergic pathway stimulation in pancreatic alpha cells
18 and thermogenic adipocytes.

19 In experimental type 1 diabetes not treated with insulin in young animals, the
20 effect of green tea remains controversial. Even with the previously reported beneficial
21 effects, these results are subject to factors such as the age at which the disease is induced.
22 Collectively, we propose that 1) the studied parameters behave differently when observed
23 in animals with type 1 diabetes induced at periadolescence or younger ages, when the
24 disease is aggravated. 2) When diabetes appears at the juvenile ages, the green tea
25 treatment increases glycaemia, changes body composition by reducing the fat content and

1 increases oxygen consumption. It affects energy expenditure and worsens the nutritional
2 status of the young type 1 diabetic rat.

3

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12

13 **Conflict of interest**

14 The authors declare that they have no conflict of interest.

15

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1 **Figure Captions**

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3 **Fig. 1.** Body weight, food and water intake, and glucose levels of male Wistar healthy
4 and diabetic rats treated with green tea infusion. **A** - 12h fasting blood glucose (mg/dL).
5 **C** - Daily average water consumption (mL). **E** - Body weight (g) measured weekly. **G** -
6 Daily average food ingestion (g). The **B, D, F** and **H** graphs represent the same variables
7 featured in the last week of the experiment. Mean \pm SD. In the A, C, E and G graphs, the
8 asterisk (*) indicates that Diabetes group is statistically different from Ctrl, and the hash
9 (#) indicates that GTI diabetic is different from the Diabetes group. The statistical
10 differences are indicated with bars at the B, D, F and H graphs, with the *P* value above
11 the bars. The data were compared (Ctrl vs Diabetes; Diabetes vs GTI diabetic),
12 considering statistical differences when $P \leq 0.05$. (n = 6 animals/group).

13

14 **Fig. 2.** Body composition of male Wistar healthy and diabetic rats treated with green tea
15 infusion. **A** – Relative fat mass (%). **B** – Absolute fat mass (g). **C** – Relative lean mass
16 (%). The data are represented as Mean \pm SD. The statistical differences are indicated with
17 bars in the graphs, with the *p* value above the bars. The data were compared (Ctrl vs
18 Diabetes; Diabetes vs GTI diabetic) considering statistical differences when $P \leq 0.05$. (n
19 = 6 animals/group).

20

21 **Fig. 3.** Calorimetric analysis of male Wistar healthy and diabetic rats treated with green
22 tea infusion. **A** - VO_2 , average volume of oxygen consumed (mL/min/Kg^{0.75}). **B** - VCO_2 ,
23 average volume of carbon dioxide produced (mL/min/Kg^{0.75}). **C** - Respiratory quotient.
24 **D** - Daily energy expenditure (EE) (Kcal/day/Kg^{0.75}). The data are presented as Mean \pm
25 SD. The box represents the interquartile interval with the mean indicated (horizontal line),

1 and the whiskers represent the superior and inferior quartiles. The statistical differences
2 are indicated with bars in the graphs, with the P value above the bars. The data were
3 compared (Ctrl vs Diabetes; Diabetes vs GTI diabetic) considering statistical differences
4 when $P \leq 0.05$. (n = 6 animals/group).

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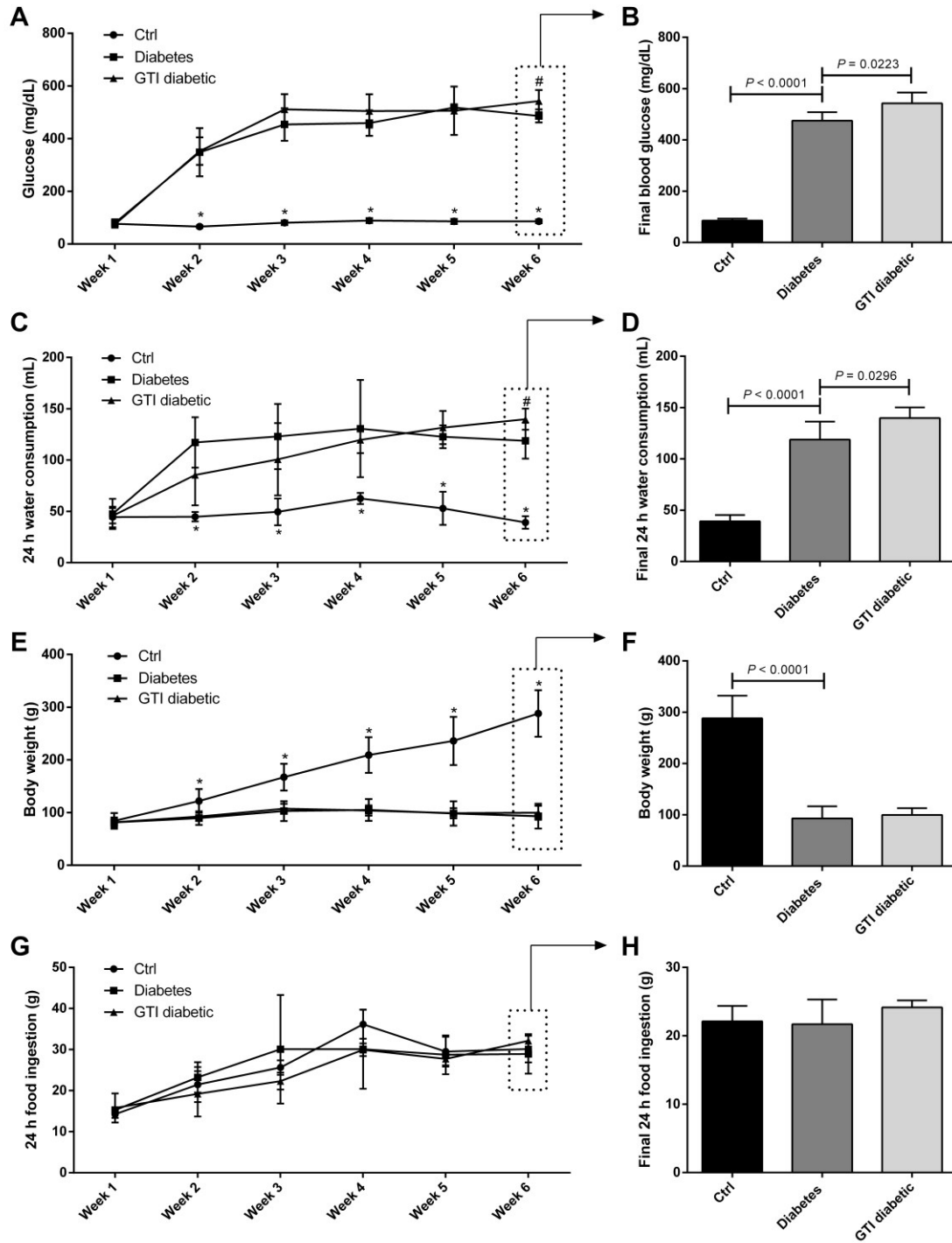
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1 **Table 1:** Murinometric and feeding parameters of male Wistar healthy and diabetic rats
2 treated with green tea infusion

	Ctrl	Diabetes	GTI diabetic
Naso-anal length (cm)	22.29 ± 1.25	15.60 ± 1.34*	16.00 ± 1.27
Lee index (g/cm)	0.29 ± 0.01	0.29 ± 0.01	0.29 ± 0.03
BMI (g/cm ²)	0.55 ± 0.06	0.40 ± 0.06 [#]	0.40 ± 0.09
SRWG (g/Kg)	2.44 ± 0.25	0.15 ± 0.26*	0.23 ± 0.20
CFE (g/g food)	0.185 ± 0.019	0.009 ± 0.018*	0.017 ± 0.015
WGCC (g/Kcal food)	0.048 ± 0.005	0.002 ± 0.004*	0.004 ± 0.004

3 The data (Mean ± SD) were compared (Ctrl vs Diabetes; Diabetes vs GTI diabetic)
4 considering statistical differences when $P \leq 0.05$. (n = 6 animals/group). Asterisk (*)
5 indicates difference between the Ctrl and Diabetes group ($P < 0.0001$), and the hash (#)
6 indicates different means from the same comparison ($P = 0.0049$). BMI – body mass
7 index; SRWG – specific rate of weight gain; CFE – coefficient of feeding efficiency;
8 WGCC - weight gain per caloric consumption.

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Figure 1

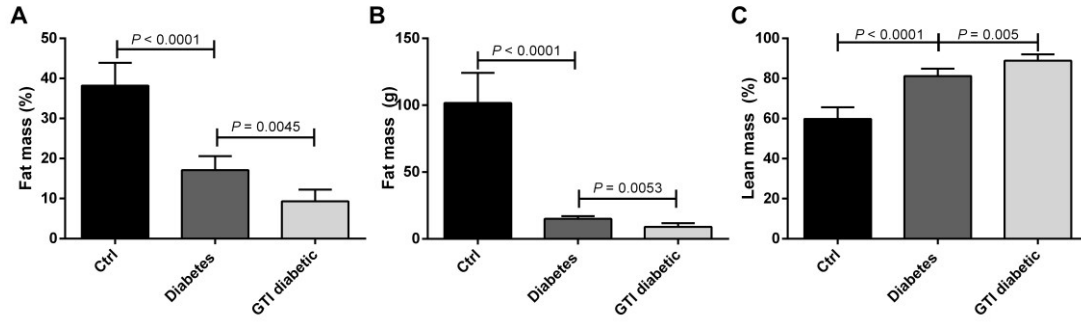
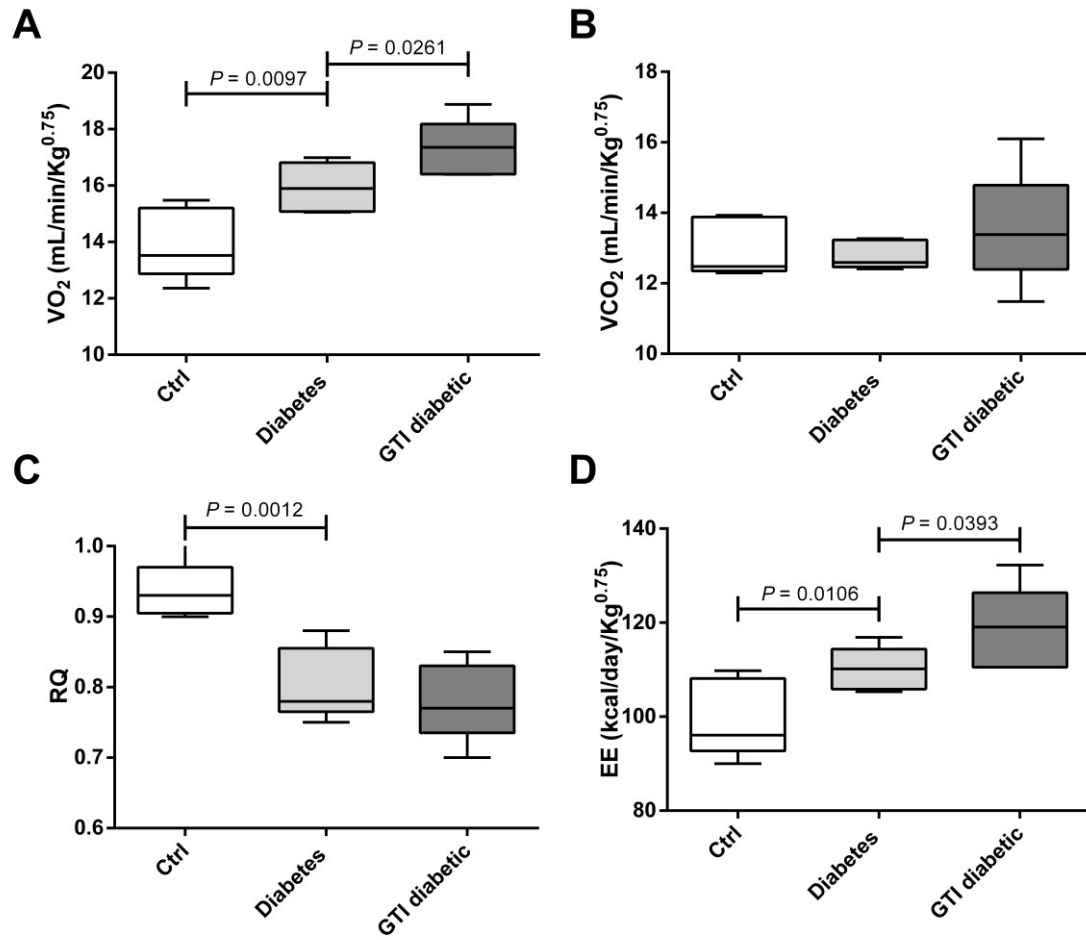


Figure 2

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Figure 3