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Cycling training effects on fat metabolism blood parameters

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# 1 Cycling training effects on fat metabolism blood parameters.

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## 7 Abstract

*Background:* study the acute and middle term (4 weeks training) effect of cycling training on fat
blood hematological parameters, urine, fatigue, and general health in recreational well-trained
cyclists.

11 *Methods:* 19 cyclists undergone 5 blood sampling: 1) before and after an incremental maximal ramp

12 test 7 days before day 0 (D-0); 2) before and after 1 hour exhaustion trial test at baseline (D-0) and

13 after 28 days of training (D-28). Age 34,5 years ( $\pm 9,5$ ); weight 74,87 kg ( $\pm 6,6$ ); height 177,3 cm

14 (±5,2); BMI 26,3 (±4,9); VO2max 53,75 ml/kg/min (±6,01); km week 314,7 Km (±137,1).

15 *Results:* Acute effect was strong elevating WBC from 6,27±2,34 10<sup>3</sup> /ul to 9,01±3,63 10<sup>3</sup> /ul, an 16 increase in LDL and Total CHOL, in this respect, existing literature is controversial. No changes in 17 body weight or blood pressure was observed after 1 month of regular training albeit lipid profile 18 significantly improved, as well as GOT.

*Conclusions:* effect of a short incremental bout of exercise was to temporary elevated all the blood parameters except MCH and MCHC. A month of intensive training (km week:  $314,7 \text{ Km} \pm 137,1$ ) significantly improved blood lipids profile with no permanent effect on WBC, blood pressure or body weight, but improved post effort lactate concentration and fatigue perception. Hematuria is confirmed to be a rare occurrence in recreational cyclists. Data can be useful for training monitoring and comparisons with similar groups of athletes, where there is a lack of information in literature and for comparing exercise effects.

26 Key words: exercise tolerance, hematological test, lactate, urinalysis, cycling.

27

#### 28 Introduction

Recreational cycling is a wide practiced recreational sport and with aging, the risk of cardiovascular 29 diseases increase. One of the main aims of aerobic exercise is to lowering cardiovascular risk 30 through improving blood lipids profile<sup>1</sup>. However, is not completely clear the effect of continuous 31 exercise in lowering blood lipoproteins and even the effect on other blood and urine parameters in 32 recreational sportsmen and surprisingly, there are very few studies on the topic with sometime 33 conflicting results in the athletic populations<sup>2</sup>. In addition, few studies examined the alterations in 34 many common or critical laboratory parameters in controlled (lab) conditions, using standardized 35 and controlled protocols. Despite its diffusion, it exist few studies on the acute and long time effect 36 of cycling in improving fat profiles of recreational cyclists, a very large category of practioners. At 37 our knowledge, it exist only one study on the acute effect of cycling on blood lipids, that show after 38 90 min of cycling at 50% of VO<sup>2</sup>peak, an increase in LDL and HDL cholesterol and a decrease in 39 triglycerides (TRI), with no changes in total cholesterol<sup>3</sup>. The efficacy of different low volume (30-40 40 minutes, 3 times per week at 50% of HRR-heart rate reserve-for 12 weeks) training regimes in 41 middle age male recreational bikers (+60 years old)<sup>4</sup> has been studied. Results show positive effects 42 on body weight reduction and systolic and diastolic blood pressure (83±7 mmHg vs 80±5 mmHg 43 and  $140\pm8$  mmHg vs  $135\pm11$  mmHg), CHOL, (216,3\pm4 mg/dl at baseline vs  $210.9\pm4.3$  mg/dl) and 44 LDL (120,6±4,2 mg/dl vs 116,9±4,7 mg/dl). Effect of aerobic exercise on lowering blood pressure 45 has also been observed in non-athletic population undergone training <sup>5</sup> while metanalysis showed a 46 significant effect of aerobic training in comparison to no effect of resistance training in decreasing 47 plasma lipids of 0,10 -0,8 mg/dl in general population. Middle term decrease of CHOL, LDL, and 48 TRI, with an increase in HDL of 0.05 mg/dl was also observed <sup>6</sup>. Other studies in young soccer 49 players<sup>7</sup> showed a middle term a decrease in CHOL from 155,6±25,7 mg/dl to 151,5±8,7 mg/dl, in 50 TRI from 88,4±41,7 mg/dl to 87,9 ±43,79 mg/dl, in LDL from 89,9±20,9 mg/dl to 86,2±10,11 51 mg/dl and in HDL-C from 51,4±8,4 mg/dl to 50,78±5,11 mg/dl. A meta-analytic study<sup>8</sup>, evidenced 52 high-intensity aerobic training resulted only in the improvement of high-density lipoprotein 53 cholesterol. However, an explanation of these dissimilar results can be the non-homogeneity in the 54 exercise load, kind of exercise, and level of participants. One acute effect of the exercise bout on the 55 immune response was strong elevating white blood cells (WBC) from  $6.27\pm2.34*10^3/ul$  to 56 9,01 $\pm$ 3,63 $\times$ 10<sup>3</sup>/ul. When comparing prerace specimens with those within 4 hours after the marathon 57 in 32 runners, WBC counts increased  $(5.5 \pm 0.2 \times 10^3/\text{ul to } 17.4 \pm 1.5 \times 10^3/\text{ul})^9$  (Siegel, et al., 2001)<sup>10</sup>. 58 Hemoglobin depletion in urine is also a rare finding after effort<sup>11</sup>. Transaminases (GOT and GPT) 59 has been indicated as markers of liver (GPT) and muscle (GOT) damage <sup>12</sup>, thus is important to 60 assess their behavior with exercise. Few information exist in literature about the changes in other 61

62 common blood and urine markers immediately after effort<sup>3</sup>. Our aims were to assess the acute (after 63 a maximal short trial) and middle term (4 weeks, after 1 hour exhaustive endurance trial), 64 modifications of hematological blood and urine parameters in recreational well trained recreational 65 cyclist. We also want to assess the middle term effect of endurance training on basic health 66 parameters of recreational cyclists, as rest heart rate, blood pressure and fatigue perception.

67

# 68 Materials and methods

19 amateur male well trained cyclists undergone 5 blood sampling: 1) before and after an 69 incremental maximal ramp test till exhaustion 7 days before day 0 (D-0); 2) before and after 1 hour 70 exhaustion trial test at baseline (D-0) and after 28 days of training (D-28). Mean age of the subjects 71 was 34,5 years (±9,5), body weight 74,87 kg (±6,6), height 177,3 cm (±5,2), BMI 26,3 (±4,9), 72 VO<sup>2</sup>max 53,75 ml/kg/min (±6,01), km per week 314,7 km (±137,1). The subjects can be classified 73 as "recreational road cyclists" <sup>13</sup> and ethical committee clearance was granted by Ethical committee 74 75 of University of Bologna. The subjects filled a questionnaire aimed at assessing alcohol and caffeine usage, protein, carbo and fats and water consumption. Reported diet did not change over 76 77 the observation period. Clinical anamnesis was performed in order to exclude any past or ongoing pathology, which could interfere with the trials and the absence of drugs intake. VO<sub>2</sub> max 78 (ml/kg/min) was assessed one week prior the D-0, with an incremental ramp test on the 79 cycloergometer (Lode Corival, The Nederlands) with ramp of 20 watts per minute until exhaustion. 80 The VO<sub>2</sub>max was measured during 3 min at max intensity in the exhaustion trial. After the 81 incremental test and the exhaustion trial, blood lactate (mM/L) was measured at 3, 6 and 9 minutes, 82 and the highest value (usually observed at 6 minute) was retained. 83

84 Fatigue protocol at the cycloergometer consisted of a light warm up followed by: 10 min at 70% of  $VO^2max$ ; 9 minutes at 50%  $VO^2max + 1$  min at 90%  $VO^2max x$  4, ending with 3 min at  $VO^2max$  for 85 a total of 60 min exhaustion test. Every 10 minutes the subjects were asked to rate the effort using 86 the Borg RPE scale<sup>14</sup>. Heart rate was measured during the 1-hour bout and in the 10 subsequent 87 minutes. Lactate was measured at 3, 6, and 9 min post effort, after the VO2max test (MAX) and 88 after the fatigue protocols. In order to assess the intensity of the effort, the subjects were asked to 89 rank the effort in the last 10 minutes of the exhaustion on a Borg 20 visual analog scale. The subject 90 practiced the same protocol on road at least three times per week for the subsequent 28 days. Urine 91 92 sampling were taken twice at rest before trials at D-0 and D-28. The following biochemical 93 parameters were measured on fasting blood sample, collected, frozen and processed with standard laboratory methods. Parameters have been corrected for plasma volume changes. The parameters 94

that was collected refer to White blood cell (WBC, 10<sup>3</sup>/ul), a general marker of inflammation, Red 95 Blood Cell concentration (RBC, 10<sup>6</sup>/ul), Hemoglobin (HGB, g/dl) and Hematocrite (HCT, %). 96 Also, we considerate the Mean Corpuscular Volume (MCV, fl), Mean Content of Hemoglobin 97 (MCH, pg), Mean Concentration of Hemoglobin Content (MCHC, g/dl), Red Blood Cell 98 99 Distribution wide (RDW, %), Glicemy (mg/dl). Moreover, we measured a few parameters of organ function, as GPT (U/L), a marker used for liver and muscle damage; Transaminases YGT and GOT, 100 marker of muscle damage<sup>12</sup>. Finally HDL-Cholesterol (mg/dl), total Cholesterol (CHOL, mg/dl), 101 Triglycerides (TRI, mg/dl) and LDL-Cholesterol (LDL, mg/dl). 102

The following biochemical parameters were measured on urine: specific weight, pH, Albumin, 103 Sugar, Urobiline, Bilirubin, hemoglobin, Nitrates, Acetone, Leucocitary Esterase, deposits, 104 leucocytes. In order to avoid hemoconcentration, subjects have been kept constantly hydratated 105 106 checking they can drink water ab libitum during the 1-hour bout. Blood pressure was measured at rest 5 and 10 minutes after the cessation of the exhaustion trials at D-0 and D-28. Blood pressure at 107 108 rest was in normal range for all the subjects according to their age. Statistical analysis was performed using a T test for paired samples with IBM-SPSS v.20 software, with significance level 109 110 set at 0,5%. All the subjects participating in the study gave their informed consent. Clearance was given by the University of Bologna ethical committee. 111

112

#### 113 **Results**

Body weight remained unchanged over the 28 days in total group (74,87±6,48 kg D-0, 74,64±6,19 114 kg D-28). VO<sup>2</sup>max measured during the last bout of 3 minutes at VO<sup>2</sup>max during the fatigue 115 protocol, improved significantly between D-0 and D-28 (49,8±9,93 ml/kg/min vs. 59,8± 8,7 116 117 ml/kg/min; p = 0,22). In the incremental test performed one week before D-0, VO2max was 53,75 ±6,01 ml/kg/min. Heart rate measured at minute 10 of the recovery, significantly decrease from D-0 118 to D-28 (105 vs 99, p = 0.04). Blood pressure (mm/Hg) at 10 minutes post exercise didn't change 119 significantly between D-0 and D-28 (max and min: 114±16 mm/Hg and 82±13 mm/Hg vs 120±14 120 mm/Hg and  $82\pm11$  mm/Hg). 121

Urine. Specific weight at D-0 was  $1013,6 \pm 5,9$  mg and  $1016\pm 9,8$  mg at D-28, without any statistically significant differences and pH remained unchanged too ( $5,85\pm0,76$  and  $5,7\pm0,76$ ) as well as albumin, sugar, bilirubin, hemoglobin, nitrates, acetone, leucocytary esterase, microscopic deposits, leucocytes, which remained absent, while urobiline remained stable at 0.2 mg/dl after 1 month training. Only one subject showed a presence in urine of HGB at 1 month confirm that hematuria is a relatively rare post exercise finding as previously observed<sup>11</sup>. The results of Borg's

- 128 test indicate a close value to maximal intensity effort: D 0 19±1,5 and D 28 19±1 score points,
- 129 without any significant difference. The higher value for lactate was retained and is reported in Table
- 130 I. Maximum lactate concentration significantly decreased between D-0 and D-28.
- 131 [Table I]
- 132 Results for hematological parameter prior and after the incremental test are reported in Table II.
- 133 [Table II]
- 134 All parameters significantly increased except MCH and MCHC.
- Results for hematological parameters at D-0 and D-28 in the pre-trial conditions are shown in TableIII.
- 137 [Table III]
- 138 In the graph 1, are shown the effects of the 28 days of training.
- 139 [Graph 1]
- 140 Discussion

141 All parameters show an increase pre/post trial except MCH and MCHC. This result can indicate a lack of water shift into the erythrocytes during the trial, as demonstrated before<sup>15</sup>: we can 142 hypothesize the "ab libitum" water drinking during the trials, is not enough to guarantee water shift 143 144 in the erythrocytes, which have a limited capacity to store water, not connected with water ingestion. We find an acute increase in LDL and CHOL, contrary to other studies in marathoners 145 who found a decrease in LDL, and no change in cholesterol<sup>16</sup>, but according to another study which 146 showed similar transient results in cyclists<sup>17</sup>. While on the long term the decrease of HDL, LDL and 147 CHOL are in accord with others studies in young soccer players<sup>7</sup>. They show a decrease in CHOL 148 from 155,6±25,7 mg/dl to 151,5±8,7 mg/dl, in LDL from 89,9±20,9 mg/dl to 86,2±10,11 mg/dl and 149 in HDL from 51,4±8,4 mg/dl to 50,78±5,11 mg/dl. One acute effect of the exercise bout on the 150 immune response was strong elevating WBC from 6,27±2,34\*10^3/ul to 9,01±3,63\*10^3/ul, 151 according to other studies <sup>9</sup> which compared prerace specimens with those within 4 hours after the 152 marathon in 32 runners who measured an increased WBC ( $5.5 \pm 0.2$  to  $17.4\pm1,5$ ). We observed a 153 significant increase of HGB and HCT only the acute measurements, while other studies in 154 marathoners didn't observed hematocrit and hemoglobin changes<sup>10</sup>. Contrary to findings after a 155 marathon run<sup>18</sup>, in our study RBC and MCV increased, this seems a characteristic of cycling effort. 156 Depending on the study, hematocrit decreased<sup>18</sup>,<sup>19</sup> not changed<sup>20</sup> or increased<sup>10,21</sup> after a marathon. 157 Besides water loss, the increase in hematocrit can be attributed to the breaking of red blood cells in 158

the foot's plantar circulatory bed during running<sup>22</sup> and even if this is not the case of cycling, a certain degree of compression on the plantar surface of the feet is present. In addition, the controversial observations in previous studies showing an RBC (hemoconcentration) increase, hypothesized it can be due to differences in fluid intake and environmental conditions (e.g. hot). In our study, where subjects were allowed (and controlled) to drink ab libitum, we observed a significant acute increase in all hemoglobin parameters.

We observed on a long term a positive effect on the lipid profile and glycemic indexes, as 165 confirmed in previous studies<sup>2</sup>. The acute increase of WBC observed pre-post trial at D-0, was not 166 present after one month of training, being transitory. Probably a longer period is necessary to 167 observe a lasting effect on WBC. It was observed<sup>23</sup> an increase in WBC, which is an important 168 indicator of immune function, after 1 year of training in recreational soccer players. Mean 169 hemoglobin content also show an increase on the long term. GOT, MCV and MCH showed a 170 significant difference at 1 month. Being GOT a marker of muscle damage<sup>12</sup>, a reduction in this 171 parameter, indicate a better state of training. 172

# 173 Conclusions

Recreational cycling population is an at risk group for cardiovascular disease, mainly due to 174 overweight. Accumulation of arterial cholesterol and triglycerides is a co-factor in heart diseases. 175 For this reason and because is a low impact activity, cycling is highly recommended to middle aged 176 men. Aging is also associated with increased visceral fat and body weight. There are few data in the 177 literature about normal hematological values for this population of recreational sportsmen, and 178 contradictory results on the changes of basic hematological parameters with training, despite the 179 large diffusion of this recreational activity. In our study, we observed an acute post exercise 180 increase of GLI, TRI, HDL-C and LDL-C and CHOL, WBC, and hematocrit, differently from other 181 studies who show an increase in LDL, HDL and a decrease in TRI and no acute changes in CHOL 182 in runners<sup>3</sup>. Endurance trials of running and cycling seems thus to have a different acute effect on 183 acute blood fat turnover. On the long term, we observed a decrease in plasma CHOL, HDL and 184 185 LDL, and a slight increment in the MCV and MHC, without any significant change in the others blood parameters. We did not observe, except in one case, hematuria, confirming it is a rare event 186 as previously observed<sup>11</sup>. Metanalysis studies<sup>8</sup> show that high-intensity aerobic training results in 187 improvement only in high-density lipoprotein cholesterol in normal population. However, when 188 189 considering athletic populations, the improvement affects all the lipid profile, and we confirm this finding. This result can be ascribed to the strong biochemical reactions triggered by training. We 190 191 provided the evidence that recreational cycling has a beneficial long-term effect on the lipid profile in a different way respect to running, even if we did not observed any positive effect on further 192

blood pressure reduction or fatigue perception, probably because our subjects have reached their optimal values. Cycling seems to differ from running in the effect on hematological parameters. The data provided can also be useful for the comparison with other groups of trained recreational cyclists. A limitation of the present study is a lack of control on the diet of the subjects during the study period, which could have affected some hematological parameters. Further studies on recreational cyclists, should clarify the effective exercise protocols able to improve the lipid profile.

199

#### 200 References

- Sarzynski MA, Burton J, Rankinen T, Blair SN, Church TS, Després JP, et. al. The effects
   of exercise on the lipoprotein subclass profile: A meta-analysis of 10 interventions.
   Atherosclerosis. 2015; 243(2), 364-372.
- Foran SE, Lewandrowski KB, Kratz A. Effects Of Exercise On Laboratory Test Results.
   Laboratory medicine, 2003; 10(34), 736-742.
- Søndergaard E, Poulsen MK, Jensen MD, Nielsen S. Acute changes in lipoprotein
   subclasses during exercise. Metabolism. 2014; 63(1), 61-68.
- Paoli A, Pacelli QF, Moro T, Marcolin G, Neri M, Battaglia G, et. al. Effects of highintensity circuit training, low-intensity circuit training and endurance training on blood pressure and lipoproteins in middle-aged overweight men. Lipids Health Disease. 2013; 12:131.
- 5. Halbert JA, Silagy CA, Finucane P, Withers RT, Hamdorf PA, Andrews GR. The
  effectiveness of exercise training in lowering blood pressure: a meta-analysis of randomized
  controlled trials of 4 weeks or longer. Journal of Human Hypertension. 1997; 11:641-649.
- Halbert JA, Silagy CA, Finucane P, Withers RT, Hamdorf PA. Exercise training and blood
   lipids in hyperlipidemic and normolipidemic adults: a meta-analysis of randomized,
   controlled trials. European Journal of Clinical Nutrition. 1999; 53:514-522.
- Jastrzebska M, Kaczmarczyk M, Suárez AD, Sánchez GFL, Jastrzebska J, Radziminski L, et
   al. Hematological Parameters and Blood Plasma Lipid Profile in Vitamin D Supplemented
   and Non-Supplemented Young Soccer Players Subjected to High-Intensity Interval
   Training. The Journal of Nutritional Science and Vitaminology. 2017; 63(6), 357-364.
- Tambalis K, Panagiotakos DB, Kavouras SA, Sidossis LS. Responses of blood lipids to
   aerobic, resistance, and combined aerobic with resistance exercise training: a systematic
   review of current evidence. Angiology, 2009; 60(5), 614-632.

- Siegel AJ, Stec JJ, Lipinska I, Van Cott EM, Lewandrowski KB, Ridker PM et. al. Effect of marathon running on inflammatory and hemostatic markers. American Journal of Cardiology. 2001; 88(8), 918-920, A9.
- 10. Adner MM, Gembarowicz R, Casey J, Kelley R, Fortin R, Calflin K, et. al. Point-of Care Biochemical Monitoring of Boston Marathon Runners: A Comparison of Prerace and
   Postrace Controls to Runners Requiring On-site Medical Attention. Point of Care: The
   Journal of Near-Patient Testing & Technology, 2002; 1(4)237–240.
- 232 11. Gilli P, De Paoli Vitali E, Tataranni G, Farinelli A. Exercise-induced urinary abnormalities
  233 in long distance runners. International Journal of Sports Medicine, 1984; 05(5), 237-240.
- 234 12. Banfi G, Colombini A, Lombardi G, Lubkowska A. Metabolic markers in sports medicine.
  235 Advances In Clinical Chemistry, 2012; 56:1-54.
- Priego QJI, Kerr ZY, Bertucci WM, Carpes FP. The categorization of amateur cyclists as
  research participants: findings from an observational study. Journal of Sports Sciences.
  2018; 36:2018-2024.
- 239 14. Borg G. Physical training, perceived exertion in physical work. Lakartidningen, 1970;
  240 67(40), 4548-4557.
- 15. Böning D, Tibes U, Schweigart U. Red cell hemoglobin, hydrogen ion and electrolyte
  concentrations during exercise in trained and untrained subjects. European Journal of
  Applied Physiology, 1976; 35(4), 243-249.
- 16. Ketelhut RG, Ketelhut K, Messerli FH, Badtke G. Fitness in the fit: Does physical
  conditioning affect cardiovascular risk factors in middle-aged marathon runners? European
  Heart Journal, 1996; 17(2), 199-203.
- 247 17. Cullinane E, Siconolfi S, Saritelli A, Thompson PD. Acute decrease in serum triglycerides
  248 with exercise: is there a threshold for an exercise effect? Metabolism. 1982; 31(8), 844-847.
- 18. Kratz A, Lewandrowski KB, Siegel AJ, Chun KY, Flood JG, Van Cott EM, et. al. Effect of
  marathon running on hematologic and biochemical laboratory parameters, including cardiac
  markers. American Journal Of Clinical Pathology. 2002; 118(6), 856-863.
- 252 19. Casoni I, Borsetto C, Cavicchi A, Martinelli S, Conconi F. Reduced hemoglobin
  253 concentration and red cell hemoglobinization in Italian marathon and ultramarathon runners.
  254 International Journal of Sports Medicine, 1985; 6(03), 176-179.

- 20. Wells CL, Stern JR, Hecht LH. Hematological changes following a marathon race in male
  and female runners. European Journal of Applied Physiology and Occupational Physiology.
  1982; 48(1), 41-49.
- 258 21. Kraemer RR, Brown BS. Alterations in plasma volume-corrected blood components of
   259 marathon runners and concomitant relationship to performance. European Journal of
   260 Applied Physiology, 1986; 55(6), 579-584.
- 261 22. Mairbäurl H. Red blood cells in sports: effects of exercise and training on oxygen supply by
  262 red blood cells. Frontiers in Physiology. 2013; 4:332.
- 263 23. Seneczko F. White blood cell count and adherence in sportsmen and non-training subjects.
  264 Acta physiologica Polonica. 1983; 34:601-610.
- 265 Notes
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MAX	G-0	G-28

 Total group
 14,63±3
 9,65±2,74
 7,35±3\*

Tab. 1. Max Blood lactate (mM/L) measured during the ramp test, after exhaustion test at baseline (G-0), (G-1) and after 28 days (G-28) of training, in all subjects \* p = 0,14;

271

272

Index	PRE	POST	p value
GLI	80,95±15,59	98,30±14,79	0,001*
YGT	19,79±7,33	21,21±6,77	0,000*
GOT	26,20±8,10	34,45±8,95	0,000*
GPT	23,40±11,13	40,80±15,16	0,000*
HDL	62,14±15,45	67,84±18,45	0,000*

CHOL	182,55±22,47	190,70±33,55	0,093
TG	81,20±49,67	84,70±32,95	0,269
LDL	88,64±16,89	94,86±21,23	0,010*
RBC	4,85±0,39	5,11±0,35	0,000*
HGB	14,77±0,92	15,36±0,85	0,000*
HCT	45,11±3,08	47,31±2,61	0,000*
MCV	92,71±4,34	92,79±4,35	0,356
MCH	30,39±1,53	30,13±1,34	0,016*
RDW	13,26±0,80	13,3±30,82	0,104
WBC	6,27±2,34	9,01±3,63	0,000*

273

Tab 2. Ematochemistry PRE-POST 1 hour intensive cycling on the cycloergometer in the total sample.(G 0).

275 GLI: Glicemia, mg/dl; YGT, GOT and GPT: Transamynases, U/L; HDL: HDL-Cholesterol, mg/dl; CHOL:

total Cholesterol, mg/dl; TRI: Tryglicerides mg/dl; LDL: LDL-Cholesterol, mg/dl.RBC : Red Blood Cell,

277 10^6/ul ; HGB: Hemoglobin, g/dl; HCT: Hematocrit , %; MCV Mean Corpuscular Volume, fl; MCH: Mean

278 Content of Hemoglobin, pg; MCHC: Mean Concentration of Hemoglobin Content, g/dl; RDW: Red Blood

279 Cell Distribution Wide, %; WBC : White blood cell, 10<sup>3</sup>/ul. \* significantly different

280				
281		G-0	G-28	Sig. T
282				
283	GLIC	80,95±19,59	73,95±19,84	0,141
284				
285	YGT	19,79±7.33	19,35±7.09	0,818
286				
287	GOT	26,20±8,10	24,30±6,42	0,122
288				
289	GPT	23,40±11,13	22,30±5,69	0,55
290				
291	HDL	61,14±15,45	58,52±15,12	0,095*
292				
293	CHOL	182,55±22,47	164,00±40,78	0,065*
294				
295	TRI	81,2±49,67	87,25±64,96	0,817
296				

297 LDL 88,64±16,89 83,32±17,33 0,083\* 298 299 RBC 4,84±0,37  $4,86\pm0,42$ 0,827 300 301 HGB 14,71±0,91 14,79±1,15 0,888 302 HCT 303 44,96±3,06 45,13±3,56 0,952 304 305 MCV 92,52±4,30 93,04±4,44 0.09\* 306 307 MHC 30,3±1,51 30,49±1,51 0,462 308 309 MCHC 32,75±0,57 32,77±0,64 0,87 310 311 RDW 13,29±0,79 0,969 13,26±0,86 312 313 WBC 6,27±2,34 6,01±1,46 0,604

314

315 Tab. 3. Ematochemical parameters at G0 and G28, in the pre-TGal condition. \* significantly different

316 GLI: Glicemia, mg/dl; YGT, GOT and GPT: Transamynases, U/L; HDL: HDL-Cholesterol, mg/dl; CHOL: total Cholesterol, mg/dl;

**317** TRI: Tryglicerides mg/dl; LDL: LDL-Cholesterol, mg/dl.RBC : Red Blood Cell , 10<sup>6</sup>/ul ; HGB: Hemoglobin, g/dl; HCT:

318 Hematocrite, %; MCV Mean Corpuscular Volume, fl; MCH: Mean Content of Hemoglobin, pg; MCHC: Mean Concentration of

Hemoglobin Content, g/dl; RDW: Red Blood Cell Distribution Wide, %; WBC : White blood cell, 10^3/ul. \* significantly different

320