Long-term consumption of artificial sweeteners does not affect cardiovascular health and survival in rats

Satvinder K Guru [1], **Ying Li** [1], **Olga V Savinova** [1], **Youhua Zhang** Corresp. [1]

[1] Department of Biomedical Sciences, New York Institute of Technology College of Osteopathic Medicine, Old Westbury, New York, United States

Corresponding Author: Youhua Zhang
Email address: yzhang49@nyit.edu

Background. Recent epidemiological cohort studies have suggested that consumption of artificial sweeteners (AS) is associated with adverse cardiovascular events and mortality. However, these population association studies cannot establish a causal relationship. In this study we investigated the effect of long-term (1-year) consumption of AS (Equal and Splenda, 2 commonly used AS) on cardiovascular health and survival in rats.

Methods. Adult Sprague-Dawley rats (both sexes, 4-5 months old) were randomized into the following 3 groups: control (n=21), AS Equal (n=21) and Splenda (n=18). In the AS groups, Equal or Splenda was added to the drinking water (2-packets/250ml), while drinking water alone was used in the control rats. The treatment was administered for 12 months. Cardiovascular function and survival were monitored in all animals.

Results. It was found that rats in the AS groups consistently consumed more sweetened water than those in the control group. AS did not affect body weight, non-fasting blood cholesterol, triglycerides, blood pressure or pulse wave velocity. There were no significant differences in left ventricular wall thicknesses, chamber dimension, cardiac function or survival. AS did not affect heart rate or atrial effective refractory period. However, rats in both Equal and Splenda groups had prolonged PR intervals (63±5ms in Equal, 68±6 ms in Splenda, vs 56±8ms in control, p<0.05) and a tendency of increased atrial fibrillation inducibility.

Conclusion. Long-term consumption of AS does not affect cardiovascular structure, function or survival but may cause some electrophysiological abnormalities with prolonged PR intervals and a tendency of increased atrial fibrillation inducibility in rats.

1 **Long-term Consumption of Artificial Sweeteners Does Not Affect**

2 **Cardiovascular Health and Survival in Rats**

4 Satvinder K. Guru, Ying Li, Olga V. Savinova, Youhua Zhang

5 Department of Biomedical Sciences, New York Institute of Technology College of Osteopathic

6 Medicine, Old Westbury, NY 11791, USA

9 **Corresponding author:**

10 Youhua Zhang, MD, PhD

11 Department of Biomedical Sciences

12 New York Institute of Technology College of Osteopathic Medicine

13 Northern Boulevard, P.O. Box 8000, Old Westbury, NY 11568-8000, USA

14 Email: yzhang49@nyit.edu

15 **Abstract**

16 **Background.** Recent epidemiological cohort studies have suggested that consumption of

17 artificial sweeteners (AS) is associated with adverse cardiovascular events and mortality.

18 However, these population association studies cannot establish a causal relationship. In this study

19 we investigated the effect of long-term (1-year) consumption of AS (Equal and Splenda, 2

20 commonly used AS) on cardiovascular health and survival in rats.

21 **Methods.** Adult Sprague-Dawley rats (both sexes, 4-5 months old) were randomized into the

22 following 3 groups: control (n=21), AS Equal (n=21) and Splenda (n=18). In the AS groups,

23 Equal or Splenda was added to the drinking water (2-packets/250ml), while drinking water alone

24 was used in the control rats. The treatment was administered for 12 months. Cardiovascular

25 function and survival were monitored in all animals.

26 **Results.** It was found that rats in the AS groups consistently consumed more sweetened water

27 than those in the control group. AS did not affect body weight, non-fasting blood cholesterol,

28 triglycerides, blood pressure or pulse wave velocity. There were no significant differences in left

29 ventricular wall thicknesses, chamber dimension, cardiac function or survival. AS did not affect

30 heart rate or atrial effective refractory period. However, rats in both Equal and Splenda groups

31 had prolonged PR intervals (63±5ms in Equal, 68±6 ms in Splenda, vs 56±8ms in control,

32 $p<0.05$) and a tendency of increased atrial fibrillation inducibility.

33 **Conclusion.** Long-term consumption of AS does not affect cardiovascular structure, function or

34 survival but may cause some electrophysiological abnormalities with prolonged PR intervals and

35 a tendency of increased atrial fibrillation inducibility in rats.

36

Introduction

Sugar intake is associated with weight gain, type 2 diabetes mellitus, coronary heart disease, stroke and total mortality.[1] Sugar-sweetened beverages (SSB) are the single largest source of added sugar in the US diet.[2, 3] Artificial sweeteners, with their intense sweetness and virtually no calories, are approved by the U.S. Food and Drug Administration (FDA) as sugar substitutes, commonly used in foods and beverages. Currently, there are 8 different artificial sweeteners approved by the FDA, including aspartame, sucralose, etc.[4] Artificial sweeteners are thought to be metabolically inert, contain only a few to no calorie and don't raise blood sugar levels. The American Heart Association and American Diabetes Association have given a cautious nod to the use of artificial sweeteners in place of sugar to combat obesity, metabolic syndrome, and diabetes mellitus.[5] In 2018, the American Heart Association advised that short-term replacement of SSB with beverages containing low-calorie sweeteners, including artificially sweetened beverages (ASB), may be an effective and realistic approach to calorie reduction and weight loss in some adults.[6]

Since artificial sweeteners are approved by the FDA, they are generally considered to be safe for consumption. However, the safety of long-term use of artificial sweeteners is still a concern. For example, there is a concern about aspartame's carcinogenic potential, despite no evidence to conclusively prove it.[7] There are also concerns that artificial sweeteners could affect glucose metabolism, body weight, diabetes,[8, 9] and gut microflora,[10, 11] etc. Recent epidemiological studies have shown an association between consumption of artificial sweeteners and adverse cardiovascular events - in particular ischemic stroke, coronary heart disease, and all-cause mortality.[1, 12, 13] It is interesting that the association of ASBs intake and cardiovascular disease mortality was found mostly in women, but not in men.[1] It should be noted that these

60 epidemiological cohort studies were unavoidably affected by confounding and bias (e.g.,

61 different types of ASBs and personal choice of the ASBs), thus could not establish a definite

62 causal relationship. Only evidence from randomized controlled trials (the gold-standard in

63 clinical research) can prove or disprove the cause-effect relationship.

64 Understandably, long-term randomized controlled trial involving a large population is very

65 difficult, if not impossible, to conduct. Currently there is no report of randomized controlled

66 trials testing whether long-term consumption of artificial sweeteners causes adverse

67 cardiovascular effects in humans. We hypothesized that long-term consumption of artificial

68 sweeteners may adversely affect cardiovascular health and survival. Accordingly, this study was

69 designed to test the above hypothesis and provide experimental evidence whether long-term (1-

70 year, equivalent to 30 human years) consumption of artificial sweeteners (Equal and Splenda, 2

71 commonly used artificial sweeteners) causes adverse cardiovascular effects and survival in

72 animals.

73 **Materials & Methods**

74 **Study groups**

75 There were 2 groups of adult (both sexes, 4-5month-old, borne in-house) Sprague-Dawley rats.

76 The first group of 33 rats was randomized into the artificial sweetener Equal (main ingredient:

77 aspartame) group (n=21) and control group (n=12) in a 2:1 manner. The second group of 27 rats

78 was randomized similarly in a 2:1 manner into the artificial sweetener Splenda (main ingredient:

79 sucralose) group (n=18) and the control group (n=9). As a result, the study consisted of the

80 following 3 groups: (1) control group (n=21), (2) artificial sweetener Equal (aspartame) group

81 (n=21), and (3) artificial sweetener Splenda (sucralose) group (n=18). Either Equal or Splenda

82   was added to the drinking water at a concentration of 2 packets per cup (8 Oz) of water (a high

83   concentration for human consumption recommended by the manufactures) in the respective

84   groups. Note that both Equal and Splenda are commercial mixtures containing other ingredients

85   besides the main ingredient aspartame or sucralose respectively. The calculated aspartame

86   concentration was about 300mg/L in Equal group, and the sucralose concentration was about

87   100mg/L in Splenda group. Control animals drank only drinking water. Both Equal and Splenda

88   were purchased from market. The treatment was administered for 12 months. Body weight and

89   water consumption were monitored before the treatment and at 2 weeks, 2 months, 4 months, 6

90   months, 9 months and 12 months after the treatment. At the end of the treatment, pulse wave

91   velocity (an index of arterial stiffness) and cardiac echocardiogram were taken, together with

92   invasive blood pressure, left ventricular hemodynamic measurement, and cardiac

93   electrophysiological tests. Non-fasting blood samples were also taken at the end of the

94   experiment for blood glucose and lipids analysis.

95   The use of the animals in this study was approved by the Institutional Animal Care and Use

96   Committee (IACUC) at the New York Institute of Technology College of Osteopathic Medicine

97   (IACUC approved protocol number: 2019-YZ-02) and was in accordance with the Guide for the

98   Care and Use of Laboratory Animals. The animals were housed in our institutional animal care

99   facility, fed with standard rat chow (Laboratory Rodent Diet-5001, LabDiet, St. Louis, MO, with

100   calories from protein 28.903%, fat 13.606% and carbohydrates 57.491%), kept on a 12-hour

101   light/dark cycle with food and water available *ad libitum*.

102   **Pulse wave velocity measurement**

103   As an index of arterial stiffness, pulse wave velocity (PWV) was measured at the end of the 12-

104   month treatment. Rats were anesthetized with isoflurane (maintained at 1.5%) and PWV was

105   measured utilizing an ECG-gated kilohertz visualization (EKV) on a VisualSonics Vevo 3100

106   platform equipped with a 40-MHz transducer (Fujifilm, Toronto, Canada). Time-resolved and

107   averaged ultrasound data was collected from the longitudinal view of the left carotid artery at

108   3000 frame/sec rate. Data were analyzed using VevoLab analysis software equipped with a

109   VevoVasc module as previously reported.[14]

110   **Echocardiographic measurement**

111   Echocardiographic measurement was taken just before the terminal experiments of

112   hemodynamic study and electrophysiological test. VisualSonics Vevo 3100 platform was used

113   coupled with an ultrasound transducer probe (25 MHz), as we have reported previously.[15]

114   Echocardiograms were obtained from the short axis (at the papillary muscle level) and the long

115   axis view of the left ventricle (LV) under isoflurane anesthesia (maintained at 1.5%). We

116   determined LV wall thickness and chamber dimensions in systole and diastole from short-axis

117   views using 2-dimensionally-targeted M-mode echocardiograms. The following parameters were

118   measured: LV anterior wall thickness in end-systole and end-diastole, LV systolic and diastolic

119   internal diameters, LV posterior wall thickness in end-systole and end-diastole, and LV

120   fractional shortening. The left atrial (LA) diameter was determined at the aortic valve level from

121   the parasternal long-axis view.

122   **Invasive blood pressure and left ventricular hemodynamic measurement**

123   After echocardiographic measurements and just prior to cardiac electrophysiology and atrial

124   fibrillation (AF) inducibility test, each rat had a 1.9F Scisense pressure catheter (Transonic

125   Scisense, London, Ontario, Canada) inserted into the LV chamber through the right carotid

126   artery, as previously described.[16] Placement of the catheter in the LV was verified by the LV

127   pressure curve displayed in the data acquisition system (LabScribe). A 20-minute period was

128   given for stabilization prior to the hemodynamic data collection. Based on the acquired LV

129   pressure recording, the following LV hemodynamic data were calculated: LV systolic pressure

130   (LVSP), LV end-diastolic pressure (LVEDP), positive change in LV pressure over time (LV

131   +dP/dt), negative change in LV pressure over time (LV -dP/dt), and Tau (left ventricular

132   relaxation time constant).

133   After completing the LV pressure recording, blood pressure was recorded by withdrawing the

134   catheter from the LV to the ascending aorta.

135   **Cardiac electrophysiology and atrial fibrillation inducibility test**

136   *In vivo* cardiac electrophysiology and atrial fibrillation (AF) inducibility tests were performed in

137   all animals immediately after LV hemodynamic measurements, as previously described.[16, 17] Rats

138   were placed at supine position under isoflurane anesthesia (maintained at 1.5%). An octopolar

139   Millar electrophysiology catheter (1.6F, EPR-802, Millar Instruments, In., Houston, Texas) was

140   advanced into the right atrium (RA) through right jugular vein. The catheter has 8 poles with 3

141   pairs of electrodes recording RA electrocardiograms and one pair for pacing. Standard ECG lead

142   II and 3 RA ECG were recorded using a PowerLab data acquisition system (ADInstruments,

143   Colorado Springs, CO).

144   Atrial effective refractory period (ERP) was determined using standard S1S2 pacing protocol

145   (basic cycle length at 150ms, at x3 the atrial threshold). Atrial ERP was defined as the longest

146   coupling intervals that did not capture the atria. The ERP was determined first by shortening of

147   S2 interval in a 5-ms decrement and then by a 1-ms step. AF inducibility was tested with burst

148   pacing containing 200 impulses at 100 Hz. Each rat received burst pacing 5 times and the

149   subsequent AF duration after each burst pacing was documented. AF was defined as rapid

150   irregular atrial activations with varying electrocardiographic morphology lasting $\geq$ 0.5 seconds,

151   as reported previously.[16] AF duration was calculated based on the average duration of AF over 5

152   burst pacing trials.

153   **Blood glucose and lipids assay**

154   Rat Glucose Assay kit (catalog # 81693) was used to quantify levels of glucose in non-fasted

155   plasma according to the manufacturer's recommended protocol (Crystal Chem, Elk Grove

156   Village, IL).

157   Total cholesterol, triglycerides, and HDL-cholesterol were determined using specific reagents

158   obtained from Pointe Scientific (Canton, MI) in plasma collected before the animals were

159   euthanized. Samples were analyzed using corresponding manual protocols in a 96-well format

160   using a plate reader (BioTek Instruments, Winooski, VT). Non-HDL cholesterol was calculated

161   by subtracting HDL cholesterol from total cholesterol.

162   **Statistical analysis**

163   Data are expressed as mean ± SD where appropriate. Normally distributed data such as body

164   weight and cardiac functional parameters among the 3 groups were first evaluated using one-way

165   analysis of variance (ANOVA) followed by post hoc analysis with Bonferroni correction for

166   multiple comparisons if needed. Chi-square test was used to compare the AF incidence. Because

167   the AF duration data were not normally distributed, they are expressed as median, first and third

168   quartile (Q1-Q3) values. A nonparametric Kruskal-Wallis test followed by Mann-Whitney U

169   tests were used to compare the AF duration data. Kaplan-Meier survival analysis with Log-rank

170   (Mantel-Cox) test was used to compare the survival curves among the 3 groups. Statistical

171   analysis was performed using GraphPad Prism 8.2.1 software. A p<0.05 was accepted as

172   statistically significant.

173   **Results**

174   **Body weight, water consumption, and survival**

175   There were no significant differences in body weights among the 3 groups at baseline (before the

176   treatment) and during the 1-year treatment in both female and male rats (Figure 1A and B. Note

177   that the body weight of males was heavier than females and both male and female rats gained

178   weight as they grew).

179   After administration of artificial sweeteners, rats in both Equal and Splenda groups consistently

180   consumed more sweetened water than those in the control group (Figure 1C), indicating that the

181   animals liked drinking sweetened water. Based on the amount of water consumed, the calculated

182   aspartame intake ranged from 60-78mg/kg/d, and sucralose intake ranged from 18-22mg/kg/d in

183   rats with respective treatments during the 1-year experiment. Both sweeteners exceeded the FDA

184   acceptable daily intake (ADI, aspartame 50mg/kg/d, sucralose 5 mg/kg/d) for human

185   consumption.[4] For reference, the daily amounts of aspartame and sucralose intake were

186   equivalent to 20-50 (12-oz)-cans of diet soda (the aspartame and sucralose contents in diet soda

187   were from Wikipedia[18]) per day for a 60-kg (132-pound) person.

188   At the end of the experiment, 16/21 rats in the control group, 15/21 rats in the Equal group and

189   10/18 rats in the Splenda group survived. The survival was not significantly different among the

190   3 groups (Figure 1D), indicating that the treatments did not affect survival. It should be pointed

191 out that all rats that died were males except 1 female rat in the Equal group. Thus, the overall

192 survival rate at the end of the 12-month experiment was higher in females (29/30 rats or 96.7%

193 in females versus 12/30 rats or 40% in males, p<0.001), indicating that female rats lived longer

194 than males, as expected.

195 **Blood pressure and pulse wave velocity**

196 Both systolic (139±26mmHg in the control, 142±10mmHg in the Equal, and 146±17mmHg in

197 the Splenda group, ANOVA test, F=0.4751, P=0.6256) and diastolic (90±20mmHg in the

198 control, 90±8mmHg in the Equal, and 94±15mmHg in the Splenda group, ANOVA test,

199 F=0.3483, P=0.7082) blood pressures were similar among the 3 groups (Figure 2A). The pulse

200 wave velocity was also similar among the 3 groups (2.72±0.54m/s in the control, 2.83±0.45m/s

201 in the Equal, and 3.13±0.76m/s in the Splenda group, ANOVA test, F=1.386, P=0.2636, Figure

202 2B).

203 **Echocardiographic parameters**

204 The left ventricular wall thickness, chamber dimension and function were similar among the 3

205 studied groups (Table 1).

206 **LV hemodynamic parameters**

207 There were no differences in the LV hemodynamic parameters among the 3 groups except

208 LVEDP. LVEDP was slightly but statistically higher in the Splenda group (Table 2). However,

209 the value of LVEDP was still in the normal range.

210 **Electrophysiology and AF inducibility**

211 The PR interval (AV conduction time) was significantly increased in both Equal (63±5ms) and

212 Splenda (68±6ms) groups compared with that in the control (56±8ms) animals (ANOVA test,

213 F=10.84, P=0.0002). Atrial effective refractory period (ERP, 43±13ms in the control, 37±6ms in

214 the Equal, and 39±7ms in the Splenda group, ANOVA test, F=1.619, P=0.2114) was not

215 significantly different among the 3 groups (Figure 3).

216 Figure 4 (A, B) shows the original ECG (lead II) and right atrial electrocardiograms (RA) traces

217 during AF inducibility test. Panel A shows an example that burst pacing did not induce AF,

218 while an example that AF was induced immediately after the burst pacing is shown in Panel B.

219 The AF inducibility was higher in both Equal and Splenda groups compared with that in the

220 control group, but the difference did not reach statistical significance (Chi-square test, P=0.1092,

221 Figure 4C). The AF duration similarly showed an increased tendency in both Equal and Splenda

222 groups, but the difference was not statistically significant (Kruskal-Wallis test, P=0.1795, Figure

223 4D).

224 **Blood lipids and glucose level**

225 There were no significant differences in blood triglycerides (142±99mg/dl in control,

226 123±95mg/dl in Equal, and 152±110mg/dl in Splenda group, AVOVA test, F=0.2811,

227 P=0.7565), total cholesterol (189±89mg/dl in control, 121±63mg/dl in Equal, and 163±118

228 mg/dl in Splenda group, AVOVA test, F=2.069, P=0.1403), HDL-cholesterol (120±46 mg/dl in

229 control, 81±25 mg/dl in Equal, and 102±77 mg/dl in Splenda group, AVOVA test, F=2.446,

230 P=0.1005), or non-HDL cholesterol (71±69 mg/dl in control, 45±56mg/dl in Equal, and 45±39

231 mg/dl in Splenda, AVOVA test, F= 1.011, P= 0.3736) levels among the 3 groups at the end of 1-

232 year treatment (Figure 5).

233   The non-fasting blood glucose level was similar in the control group (310±72mg/dl) and the

234   Equal group (308±82mg/dl, ANOVA test, F= 4.418, P= 0.0188, control vs Equal P>0.05), but

235   slightly lower in the Splenda group (230±57mg/dl, P<0.05 versus Control).

236   **Discussion**

237   **Major findings**

238   In this study we administered artificial sweeteners in drinking water to avoid other potential

239   variables, such as soda, caffeine and other active substances existed in various diet drinks. This

240   design allowed us to directly compare consumption of artificial sweeteners with those without

241   artificial sweeteners. Our results indicate that rats liked to drink artificially sweetened water. The

242   daily water consumption was consistently higher in both Equal and Splenda groups than that in

243   the control group (Figure 1C). The long-term (1-year) consumption of artificial sweeteners did

244   not significantly affect body weight, blood lipids, blood pressure, arterial stiffness (reflected in

245   PWV), left ventricular wall thickness, chamber dimension, or cardiac function. Artificial

246   sweeteners did not affect heart rate or atrial effective refractory period. However, the artificial

247   sweeteners caused some electrophysiological abnormalities with a prolonged PR interval and a

248   tendency of increased atrial fibrillation (AF) inducibility in rats. The survival was not

249   significantly affected by the 1-year treatments.

250   **Artificial sweeteners and adverse cardiovascular health**

251   Artificial sweeteners are generally recognized as safe to pass the FDA approval. However, their

252   health concerns have not been completely cleared. A report indicated that drinking diet

253   beverages with artificial sweeteners was associated with an increased risk of heart attack and

254   stroke.[12] The study was conducted in a cohort of postmenopausal U.S. women from the Women's

255   Health Initiative Study, a multicenter longitudinal study of the health of 93 676 postmenopausal

256   women. It was found that women who consumed an average of ≥2 ASB per day (≥24

257   ounces/day) had an elevated risk of stroke, coronary heart disease, and all-cause mortality

258   compared with those who consumed <1 ASB per week. In a separate study,[1] associations

259   between consumption of ASB and risks of total and cause-specific mortality were examined

260   among 37,716 men from the Health Professional's Follow-up study and 80,647 women from the

261   Nurses' Health study who were free from chronic diseases at baseline. After adjusting for major

262   diet and lifestyle factors, consumption of ASB was associated with total and cardiovascular

263   disease mortality in the highest intake category only. In cohort-specific analysis, ASB was

264   associated with mortality in women from Nurses' Health Study but not in men from Health

265   Professionals Follow-up Study,[1] suggesting a sex difference in ASB induced adverse

266   cardiovascular effects. Along this line, sexes and species differences in artificial sweeteners'

267   effects have also been reported.[19, 20]

268   A recent prospective cohort study found that both artificially sweetened beverages and sugary

269   drinks were associated with increased cardiovascular disease risk.[13] These findings certainly

270   raised concerns about the adverse effects of artificial sweeteners on cardiovascular health.[21]

271   However, these findings are consistent with some, but not all, previous reports [22-24]. In addition,

272   these observational cohort studies are prone to confounding and bias. For example, participants

273   consumed various diet beverages, which contain different kinds of artificial sweeteners and other

274   active substances, making it difficult to isolate the effect of artificial sweeteners (needless to say

275   a particular artificial sweetener). In addition, participants might have other bias in choosing

276   ASBs. Thus, these cohort studies cannot establish a causal effect. Data from randomized

277   controlled trials are needed to prove or disprove the causal effect and currently there is no

278   randomized controlled trial available examining the long-term artificial sweetener consumption

279   on cardiovascular health in humans. There are a few randomized controlled trials available that

280   reported the effect of artificial sweeteners on body weight, blood lipids, glucose and blood

281   pressure,[25-27] but with few participants and short duration, the confidence in the reported results

282   is limited.[28]

283   To our knowledge this is the first study specifically designed to examine the effect of long-term

284   consumption of artificial sweeteners on cardiovascular health in an animal model. In this study,

285   artificial sweeteners were administered in drinking water, which avoided many active substances

286   and potential confounding existed in ASBs. We found that long-term consumption of artificial

287   sweeteners did not affect body weight, blood pressure, arterial stiffness, cardiac function,

288   chamber dimension, and mortality. However, artificial sweeteners caused some

289   electrophysiological abnormalities, such as a prolonged PR interval and a tendency for increased

290   AF inducibility in rats. AF is the most common, clinically significant arrhythmia and the number

291   of AF patients is expected to increase worldwide.[29] AF is a known risk factor for increased

292   stroke and cardiovascular mortality.[29] If artificial sweeteners could cause electrophysiological

293   alterations leading to AF, then long-term consumption of artificial sweeteners would contribute

294   to increased arrhythmias and stroke in patients, as suggested in the cohort studies.[1] However, the

295   increase of AF was not statistically significant in this study. This could be due to a relatively

296   small sample size at the end of the experiment owing to high mortality in male rats. Thus, we

297   think that the increased AF tendency found in the treated rats is important and deserves further

298   investigation both experimentally and clinically in patients.

299   Our results indicate that long-term consumption of artificial sweeteners did not significantly

300   affect left ventricular hemodynamics, except that Splenda caused a slight but statistically

301 significant elevation in LVEDP. Despite the fact that the LVEDP value was still in the normal

302 range, it may indicate early signs of impaired LV relaxation. Risdon et al[30] have reported that

303 consumption of a mixture of sucralose/acesulfame potassium impaired vascular endothelial

304 function in adult rats. These results are interesting and if confirmed, they could help to explain

305 the increased cardio-metabolic risk reported by the epidemiological cohort studies.[1, 12, 13]

306 **Comparison with previous reports**

307 In a double-blind randomized study, it was found that consumption of aspartame for 13-weeks

308 did not have a meaningful effect on body weight, plasma triglyceride or cholesterol levels in

309 young adults.[25] Our results revealed that 1-year consumption of artificial sweeteners did not

310 affect body weight or blood lipid levels in rats, consistent with the report conducted in young

311 adults. Similarly, in another 13-week, double-blind study conducted in apparently healthy

312 children and adolescents, consumption of aspartame did not have clinically significant

313 differences in laboratory parameters compared with those taking sucrose.[26]

314 A meta-analysis of randomized controlled trials found that consumption of nonnutritive

315 sweeteners did not increase blood glucose levels.[27] The glycemic impact of nonnutritive

316 sweeteners consumption did not differ by the types of nonnutritive sweeteners but to some extent

317 varied by participants' age, body weight, and diabetic status. A recent study in humans suggested

318 that consuming sucralose-sweetened beverages with a carbohydrate (maltodextrin) impaired

319 insulin sensitivity, but consuming either sucralose or carbohydrate alone did not affect insulin

320 sensitivity.[31] Our data indicate that long-term consumption of artificial sweeteners in rats did not

321 increase blood glucose levels, compared with controls. In the current study blood glucose levels

322 were similar in the control and the Equal groups, but slightly lower in the Splenda group,

323   suggesting that long-term consumption of different types of artificial sweeteners may affect

324   blood glucose levels differently.

325   It was reported that acute intraperitoneal injection of aspartame could decrease blood pressure in

326   spontaneously hypertensive rats.[32] However, in the current study, we found that long-term

327   consumption of artificial sweeteners (including Equal, which contains aspartame) did not affect

328   blood pressure levels or arterial stiffness. These discrepancies could be caused by the dosages,

329   route of administration, duration of the treatment or the animal conditions (spontaneously

330   hypertensive rats versus normal control rats). As mentioned already, there is a report that

331   consumption of a mixture of sucralose/acesulfame potassium impaired vascular endothelial

332   function in rats.[30] However, in this study we did not observe significant changes in blood

333   pressure and arterial stiffness in the Splenda and Equal treated animals.

334   A systematic review and meta-analysis summarized current evidence of artificial sweeteners on

335   body weight.[33] There were only 7 randomized controlled trials (the gold standard in clinical

336   research), involving 1003 participants, with a median follow-up of 6 months. There were 30

337   cohort studies (405 907 participants; median follow-up 10 years). In the included randomized

338   controlled trials, nonnutritive sweeteners had no significant effect on body mass index. In the

339   included cohort studies, consumption of nonnutritive sweeteners was associated with a modest

340   increase in body mass index. The data from the randomized controlled trials did not show

341   consistent effects of nonnutritive sweeteners on other measures of body composition. In the

342   cohort studies, consumption of nonnutritive sweeteners was associated with increases in weight,

343   waist circumference, and a higher incidence of obesity, hypertension, metabolic syndrome, type

344   2 diabetes and cardiovascular events,[33] indicating a discrepancy in findings from randomized

345   controlled trials and cohort studies. Another meta-analysis of randomized controlled trials

346   indicated that substituting low calorie sweeteners for their regular-calorie versions resulted in a

347   modest weight loss and therefore may be a useful dietary tool to improve compliance with

348   weight loss or weight maintenance plans.[34] There is also a report suggesting that different low-

349   calorie sweeteners may have distinct effect on body weight.[35] Our results indicate that long-term

350   consumption of artificial sweeteners did not affect body weights in rats.

351   **Study limitations**

352   During the 1-year experiment, the status of the animals was checked daily and recorded by our

353   animal care facility personnel. The date of animal death (when it happened) was recorded.

354   However, due to the COVID-19 lockdown in the early part of 2020 (when most deaths

355   occurred), campus access was limited and autopsy for the dead animals was not performed.

356   Thus, the exact cause of death for the animals was not known. Nevertheless, the survival of the

357   rats in the artificial sweetener groups was not significantly different from that in the control

358   group.

359   Blood glucose level was measured from non-fasted plasma samples taken at the end of the

360   terminal experiments and the values in all groups (including the controls) were higher than

361   typically seen in non-fasted rats. It is reported that surgical stress and anesthesia can elevate

362   blood glucose level.[36] The extensive surgical procedures at the terminal experiments

363   (hemodynamic measurements, electrophysiological tests, and open chest blood draw) might

364   contribute to the elevated blood glucose levels in these non-diabetic rats.

365   Our data indicate that artificial sweeteners caused some electrophysiological abnormalities, such

366   as a prolonged PR interval and a tendency for increased AF inducibility in rats. However, our

367   data do not permit to delineate the mechanism(s) by which the artificial sweeteners caused these

368   electrophysiological abnormalities.

369   Finally, due to the nature of an animal study, the findings from this study cannot be directly

370   extrapolated to humans. Thus, human studies are still needed to verify these findings.

371

372   **Conclusion**

373   Contrary to the hypothesis that long-term consumption of artificial sweeteners may adversely

374   affect cardiovascular health and survival, consumption of artificial sweeteners did not affect

375   body weight, blood lipids, blood pressure, cardiovascular structure, function, or survival in rats.

376   However, artificial sweeteners caused some electrophysiological abnormalities, such as a

377   prolonged PR interval and a tendency for increased AF inducibility in rats. Considering that AF

378   is a known risk factor for stroke and other adverse cardiovascular events, further studies are

379   warranted to clarify this issue and to determine whether artificial sweeteners lead to similar

380   electrophysiological alternations in humans.

381   **Acknowledgement:** The authors would like to thank Jeanne Quidore-Jermann, AAS, Senior

382   Animal Care Technician, and Sandra Kahler at the New York Institute of Technology College of

383   Osteopathic Medicine animal care facility for their expert care, monitoring, and administration of

384   the artificial sweeteners to the animals during the experiment.

385   **Conflict of Interests:** There are no conflicts of interest in connection with this article.

References:

386

387   1.   Malik VS, Li Y, Pan A, De Koning L, Schernhammer E, Willett WC and Hu FB. Long-

388   Term Consumption of Sugar-Sweetened and Artificially Sweetened Beverages and Risk of

389   Mortality in US Adults. *Circulation*. 2019;139:2113-2125.

390   2.   Hu FB and Malik VS. Sugar-sweetened beverages and risk of obesity and type 2

391   diabetes: epidemiologic evidence. *Physiol Behav*. 2010;100:47-54.

392   3.   Drewnowski A and Rehm CD. Consumption of added sugars among US children and

393   adults by food purchase location and food source. *Am J Clin Nutr*. 2014;100:901-7.

394   4.   US Food and Drug Administration (FDA). Additional Information about High-Intensity

395   Sweeteners Permitted for Use in Food in the United States. *https://wwwfdagov/food/food-

396   additives-petitions/additional-information-about-high-intensity-sweeteners-permitted-use-food-

397   united-states* (accessed 1/18/2022).

398   5.   Gardner C, Wylie-Rosett J, Gidding SS, Steffen LM, Johnson RK, Reader D,

399   Lichtenstein AH. Nonnutritive sweeteners: current use and health perspectives: a scientific

400   statement from the American Heart Association and the American Diabetes Association.

401   *Circulation*. 2012;126:509-19.

402   6.   Johnson RK, Lichtenstein AH, Anderson CAM, Carson JA, Despres JP, Hu FB, Kris-

403   Etherton PM, Otten JJ, Towfighi A, Wylie-Rosett J. Low-Calorie Sweetened Beverages and

404   Cardiometabolic Health: A Science Advisory From the American Heart Association.

405   *Circulation*. 2018;138:e126-e140.

406   7.   Mallikarjun S and Sieburth RM. Aspartame and Risk of Cancer: A Meta-analytic

407   Review. *Arch Environ Occup Health*. 2015;70:133-41.

408   8.   Pepino MY, Tiemann CD, Patterson BW, Wice BM and Klein S. Sucralose affects

409   glycemic and hormonal responses to an oral glucose load. *Diabetes Care*. 2013;36:2530-5.

410   9.   Choudhary AK. Aspartame: Should Individuals with Type II Diabetes be Taking it? *Curr*

411   *Diabetes Rev*. 2018;14:350-362.

412   10.   Abou-Donia MB, El-Masry EM, Abdel-Rahman AA, McLendon RE and Schiffman SS.

413   Splenda alters gut microflora and increases intestinal p-glycoprotein and cytochrome p-450 in

414   male rats. *Journal of toxicology and environmental health Part A*. 2008;71:1415-29.

415   11.   Pang MD, Goossens GH and Blaak EE. The Impact of Artificial Sweeteners on Body

416   Weight Control and Glucose Homeostasis. *Front Nutr*. 2020;7:598340.

417   12.   Mossavar-Rahmani Y, Kamensky V, Manson JE, Silver B, Rapp SR, Haring B,

418   Beresford SAA, Snetselaar L and Wassertheil-Smoller S. Artificially Sweetened Beverages and

419   Stroke, Coronary Heart Disease, and All-Cause Mortality in the Women's Health Initiative.

420   *Stroke; a journal of cerebral circulation*. 2019;50:555-562.

421   13.   Chazelas E, Debras C, Srour B, Fezeu LK, Julia C, Hercberg S, Deschasaux M and

422   Touvier M. Sugary Drinks, Artificially-Sweetened Beverages, and Cardiovascular Disease in the

423   NutriNet-Sante Cohort. *Journal of the American College of Cardiology*. 2020;76:2175-2177.

424   14.   Di Lascio N, Kusmic C, Stea F and Faita F. Ultrasound-based Pulse Wave Velocity

425   Evaluation in Mice. *J Vis Exp*. 2017; (120): 54362. doi: 10.3791/54362.

426   15.   Zhang Y, Dedkov EI, Lee B, Li Y, Pun K and Gerdes AM. Thyroid hormone replacement

427   therapy attenuates atrial remodeling and reduces atrial fibrillation inducibility in a rat myocardial

428   infarction-heart failure model. *Journal of cardiac failure*. 2014;20:1012-9.

429   16.   Zhang Y, Dedkov EI, Teplitsky D, Weltman NY, Pol CJ, Rajagopalan V, Lee B and

430   Gerdes AM. Both hypothyroidism and hyperthyroidism increase atrial fibrillation inducibility in

431   rats. *Circulation Arrhythmia and electrophysiology*. 2013;6:952-9.

432   17.   Delfiner MS, Nofi C, Li Y, Gerdes AM and Zhang Y. Failing Hearts Are More

433   Vulnerable to Sympathetic, but Not Vagal Stimulation-Induced, Atrial Fibrillation-Ameliorated

434   with Dantrolene Treatment. *Journal of cardiac failure*. 2018;24:460-469.

435   18.   Wikipedia. Diet drink-Amount of artificial sweeteners in diet soft drinks.

436   *https://enwikipediaorg/wiki/Diet_drink* *(accessed 1/18/2022)*.

437   19.   Marshall AT, Liu AT, Murphy NP, Maidment NT and Ostlund SB. Sex-specific

438   enhancement of palatability-driven feeding in adolescent rats. *PloS one*. 2017;12:e0180907.

439   20.   Sclafani A, Bahrani M, Zukerman S and Ackroff K. Stevia and saccharin preferences in

440   rats and mice. *Chem Senses*. 2010;35:433-43.

441   21.   Gardener H and Elkind MSV. Artificial Sweeteners, Real Risks. *Stroke; a journal of*

442   *cerebral circulation*. 2019;50:549-551.

443   22.   Gardener H, Rundek T, Markert M, Wright CB, Elkind MS and Sacco RL. Diet soft drink

444   consumption is associated with an increased risk of vascular events in the Northern Manhattan

445   Study. *J Gen Intern Med*. 2012;27:1120-6.

446   23.   Pase MP, Himali JJ, Beiser AS, Aparicio HJ, Satizabal CL, Vasan RS, Seshadri S and

447   Jacques PF. Sugar- and Artificially Sweetened Beverages and the Risks of Incident Stroke and

448   Dementia: A Prospective Cohort Study. *Stroke; a journal of cerebral circulation*. 2017;48:1139-

449   1146.

450   24.   de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC and Hu FB. Sweetened

451   beverage consumption, incident coronary heart disease, and biomarkers of risk in men.

452   *Circulation*. 2012;125:1735-41, S1.

453   25.   Knopp RH, Brandt K and Arky RA. Effects of aspartame in young persons during weight

454   reduction. *J Toxicol Environ Health*. 1976;2:417-28.

455   26.   Frey GH. Use of aspartame by apparently healthy children and adolescents. *J Toxicol*

456   *Environ Health*. 1976;2:401-15.

457   27.   Nichol AD, Holle MJ and An R. Glycemic impact of non-nutritive sweeteners: a

458   systematic review and meta-analysis of randomized controlled trials. *Eur J Clin Nutr*.

459   2018;72:796-804.

460   28.   Toews I, Lohner S, Kullenberg de Gaudry D, Sommer H and Meerpohl JJ. Association

461   between intake of non-sugar sweeteners and health outcomes: systematic review and meta-

462   analyses of randomised and non-randomised controlled trials and observational studies. *BMJ*.

463   2019;364:k4718.

464   29.   January CT, Wann LS, Calkins H, Chen LY, Cigarroa JE, Cleveland JC, Jr., Ellinor PT,

465   Ezekowitz MD, Field ME, Furie KL, Heidenreich PA, Murray KT, Shea JB, Tracy CM and

466   Yancy CW. 2019 AHA/ACC/HRS Focused Update of the 2014 AHA/ACC/HRS Guideline for

467   the Management of Patients With Atrial Fibrillation: A Report of the American College of

468   Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the

469   Heart Rhythm Society in Collaboration With the Society of Thoracic Surgeons. *Circulation*.

470   2019;140:e125-e151.

471   30.   Risdon S, Meyer G, Marziou A, Riva C, Roustit M and Walther G. Artificial sweeteners

472   impair endothelial vascular reactivity: Preliminary results in rodents. *Nutr Metab Cardiovasc*

473   *Dis*. 2020;30:843-846.

474   31.   Dalenberg JR, Patel BP, Denis R, Veldhuizen MG, Nakamura Y, Vinke PC, Luquet S

475   and Small DM. Short-Term Consumption of Sucralose with, but Not without, Carbohydrate

476   Impairs Neural and Metabolic Sensitivity to Sugar in Humans. *Cell metabolism*. 2020;31:493-

477   502 e7.

478   32.   Kiritsy PJ and Maher TJ. Acute effects of aspartame on systolic blood pressure in

479   spontaneously hypertensive rats. *J Neural Transm*. 1986;66:121-8.

480   33.   Azad MB, Abou-Setta AM, Chauhan BF, Rabbani R, Lys J, Copstein L, Mann A,

481   Jeyaraman MM, Reid AE, Fiander M, MacKay DS, McGavock J, Wicklow B and Zarychanski

482   R. Nonnutritive sweeteners and cardiometabolic health: a systematic review and meta-analysis of

483   randomized controlled trials and prospective cohort studies. *CMAJ*. 2017;189:E929-E939.

484   34.   Miller PE and Perez V. Low-calorie sweeteners and body weight and composition: a

485   meta-analysis of randomized controlled trials and prospective cohort studies. *Am J Clin Nutr*.

486   2014;100:765-77.

487   35.   Higgins KA and Mattes RD. A randomized controlled trial contrasting the effects of 4

488   low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *Am J*

489   *Clin Nutr*. 2019;109:1288-1301.

490   36.   Duggan EW, Carlson K and Umpierrez GE. Perioperative Hyperglycemia Management:

491   An Update. *Anesthesiology*. 2017;126:547-560.

492

Echocardiographic measurements

HR: heart rate, LVAWd: left ventricular (LV) anterior wall thickness in diastole, LVAWs: LV anterior wall thickness in systole, LVDd: Left ventricular diameter in diastole, LVDs: Left ventricular diameter in systole, LVPWd: LV posterior wall thickness in diastole, LVPWs: LV posterior wall thickness in systole, FS: fractional shortening, LA: left atrial diameter. P>0.05 for all parameters.

1 **Table 1. Echocardiographic measurements**

	HR (bpm)	LVAWd (mm)	LVAWs (mm)	LVDd (mm)	LVDs (mm)	LVPWd (mm)	LVPWs (mm)	FS (%)	LA (mm)
Control	271±40	1.6±0.2	2.7±0.3	7.3±0.9	3.7±0.9	1.6±0.2	2.8±0.3	49.6±7.3	3.8±0.9
Equal	261±20	1.6±0.2	2.6±0.2	6.9±0.7	3.5±0.7	1.6±0.2	2.7±0.2	49.6±6.1	4.0±0.7
Splenda	260±31	1.5±0.4	2.5±0.5	7.7±0.9	4.1±0.9	1.5±0.4	2.6±0.5	46.6±6.3	4.2±0.6

2 HR: heart rate, LVAWd: left ventricular (LV) anterior wall thickness in diastole, LVAWs: LV

3 anterior wall thickness in systole, LVDd: Left ventricular diameter in diastole, LVDs: Left

4 ventricular diameter in systole, LVPWd: LV posterior wall thickness in diastole, LVPWs: LV

5 posterior wall thickness in systole, FS: fractional shortening, LA: left atrial diameter. $P>0.05$ for

6 all parameters.

LV hemodynamic measurements

LVSP: left ventricular systolic pressure, LVEDP: LV end-diastolic pressure, +dp/dt, maximal positive change in pressure over time; −dp/dt, maximal negative change in pressure over time. Tau: Left ventricular relaxation time constant. *P<0.05 versus Control.

1   **Table 2. LV hemodynamic measurements**

	LVSP (mmHg)	LVEDP (mmHg)	+dp/dt (mmHg/s)	-dt/dt (mmHg/s)	Tau (ms)
Control	135±25	2.5±2.2	8059±2028	-6864±1991	13.7±2.7
Equal	143±11	3.2±1.9	8056±888	-7322±814	13.1±1.6
Splenda	146±19	6.1±2.9*	7804±1005	-6817±579	14.5±1.4

2   LVSP: left ventricular systolic pressure, LVEDP: LV end-diastolic pressure, +dp/dt, maximal

3   positive change in pressure over time; −dp/dt, maximal negative change in pressure over time.

4   Tau: Left ventricular relaxation time constant. *P<0.05 versus Control.

Figure 1

Body weight, water consumption and survival.

(A) Body weight in female rats. (B) Body weight in male rats. (C) Water consumption. (D) survival. Note that data are presented based on treatments with inclusion of both sexes, except as specified in A and B. *p<0.05 versus both Equal and Splenda groups.
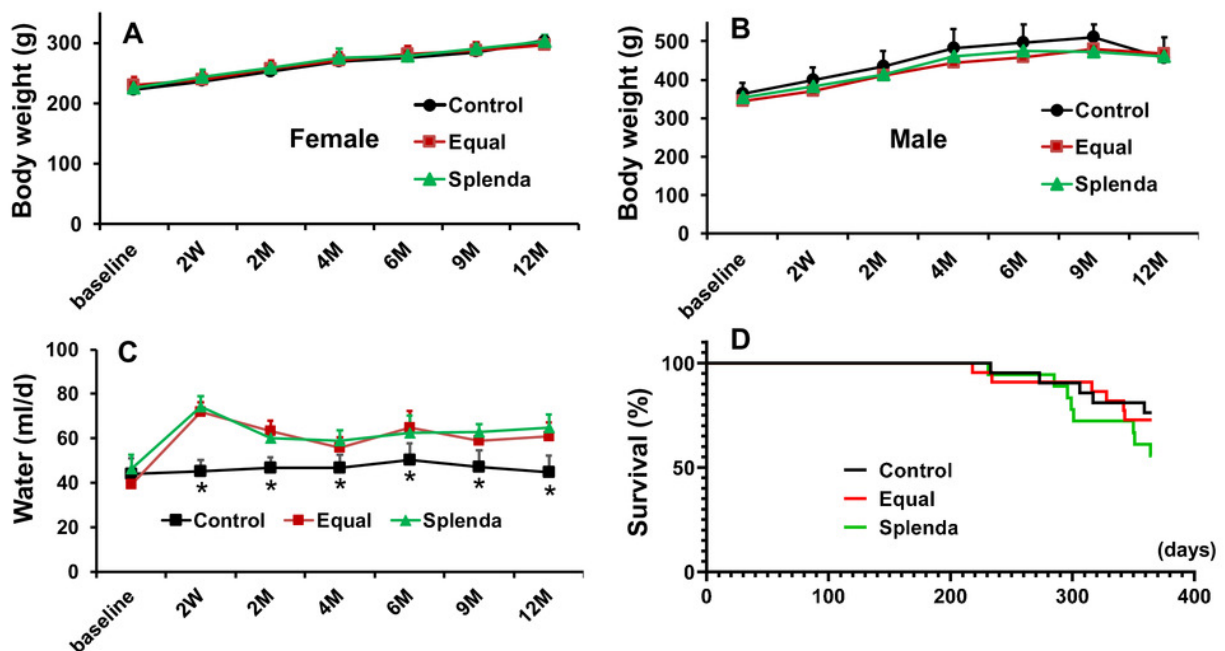
Figure 1

Figure 2

Blood pressure (BP) and pulse wave velocity (PWV).

A: Systolic (SBP) and diastolic (DBP) blood pressure. B: PWV. No statistical differences were found in all parameters.
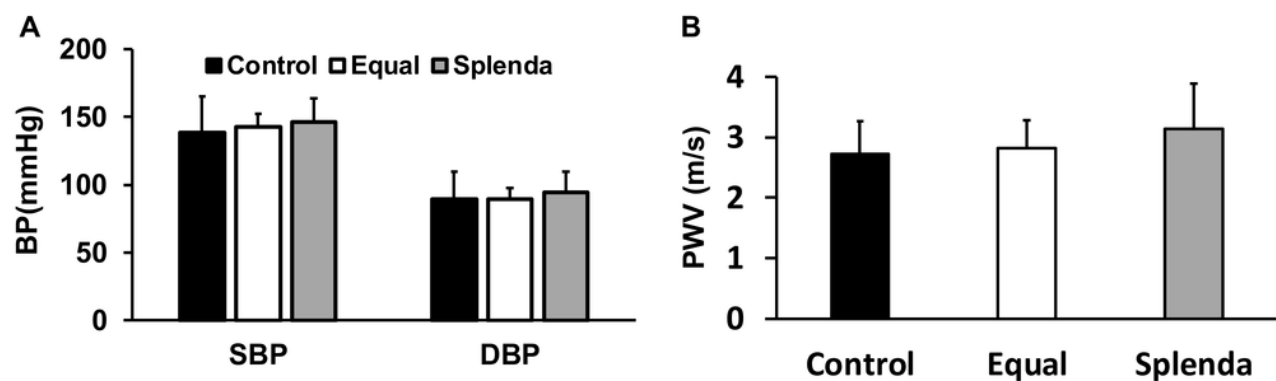
Figure 2

Figure 3

Atriventricular conduction time (AVCT or PR interval) and atrial effective refractory period (ERP).

*p<0.05 versus Control group.
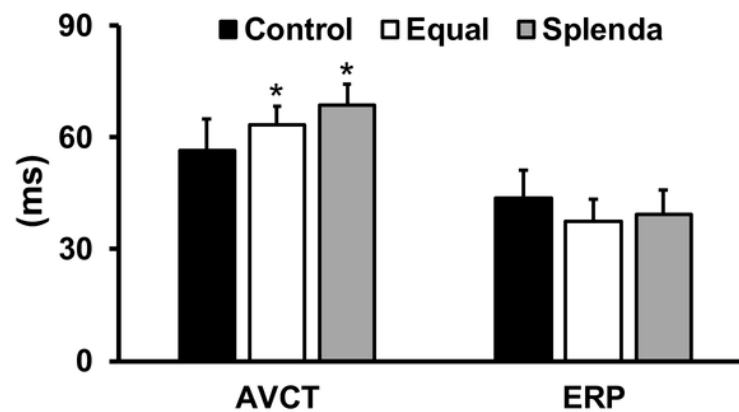
Figure 3

Figure 4

Atrial fibrillation inducibility and duration

(A) Original ECG traces show an example that burst pacing did not induce AF. (B) shows an example that AF was induced immediately after the burst pacing. (C) The AF inducibility was higher in both Equal and Splenda groups compared with that in the control group, but the difference did not reach statistical significance (Chi-square test, P=0.1092). (D) The AF duration similarly showed an increased tendency in both Equal and Splenda groups, but the difference was not statistically significant (Kruskal-Wallis test, P=0.1795).
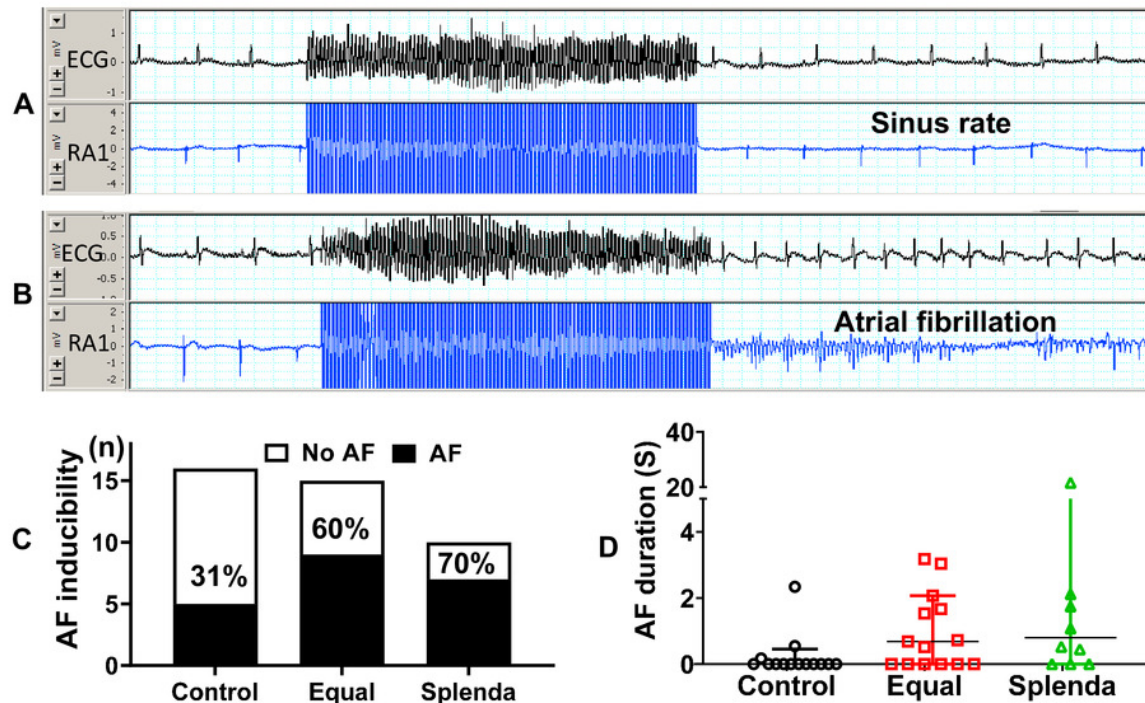
Figure 4

Figure 5

Blood lipids levels.

A: Triglycerides. B: Total cholesterol. C: HDL cholesterol. D: Non-HDL cholesterol. No statistical differences were found in all parameters.

Figure 5