ARCHIVES OF THE TURKISH SOCIETY OF CARDIOLOGY

Official journal of the TURKISH SOCIETY OF CARDIOLOGY

Effect of Sacubitril/Valsartan and Dapagliflozin on Cardiac Functions and Exercise Capacity of Rats

Sacubutril/Valsartan ve Dapagliflozin'in Sıçanların Kardiyak Fonksiyonları ve Egzersiz Kapasiteleri Üzerine Etkisi

ABSTRACT

Objective: Recent studies have demonstrated the positive effects of sacubitril/valsartan and dapagliflozin on cardiac prognosis and performance. These drugs have the potential to be misused as doping agents by professional athletes. This study aimed to evaluate the effects of sacubitril/valsartan and dapagliflozin on athletic performance.

Methods: In this study, swimming performance was assessed in three groups of rats divided into control, sacubitril/valsartan, and dapagliflozin groups. Echocardiography, weight, and rotarod performance were also evaluated during follow-up.

Results: In comparisons between the sacubitril/valsartan and control groups, a statistical difference was observed in the 13th, 19th, and 20th swimming sessions. For total and median swimming times, the *P* values were 0.115 and 0.015, respectively. In comparisons between the dapagliflozin and control groups, a statistical difference was observed starting from the 10th swimming session, with *P* values of < 0.001 for both total and median swimming times. In a three-group analysis, statistical differences were observed from the ninth swimming session until the end of the experiment. Additionally, rotarod results showed a significant difference for both sacubitril/valsartan and dapagliflozin compared to baseline ($P < 0.001$ and $P = 0.011$, respectively).

Conclusion: This study demonstrated a limited positive effect of sacubitril/valsartan on athletic performance, while the impact of dapagliflozin on athletic performance was particularly significant.

Keywords: Athletic performance, dapagliflozin, doping agents, performance enhancing, sacubitril/valsartan, sports cardiology

ÖZET

Amaç: Son yıllarda yapılan çalışmalar sakubitril/valsartan ve dapagliflozinin kardiyak prognoz ve performans üzerine olumlu etkilerini ortaya koymuştur. Bu ilaçlar potansiyel olarak profesyonel sporcular tarafından doping maddesi olarak suistimal edilebilir. Çalışmamızda sakubitril/valsartan ve dapagliflozinin atletik performans üzerine etkilerini değerlendirdik.

Yöntem: Araştırmada üç grup sıçanın yüzme performansları kontrol, sakubitril/valsartan ve dapagliflozin gruplarına ayrılarak değerlendirildi. Ayrıca takip sırasında ekokardiyografi, ağırlık ve rotarod verileri değerlendirildi.

Bulgular: Sakubitril/valsartan ve kontrol gruplarının karşılaştırılmasında 13., 19. ve 20. yüzme seanslarında istatistiksel olarak farklılık görülürken, toplam ve median yüzme süreleri karşılaştırıldığında *P* değerleri 0,115 ve 0,015 olarak belirlendi. Dapagliflozin ve kontrol gruplarının karşılaştırılmasında 10. yüzme seansından itibaren istatistiksel olarak fark gözlenirken, toplam ve median yüzme süreleri karşılaştırıldığında *P* değerleri < 0,001 ve < 0,001 olarak belirlendi. Üçlü analizde 9. yüzme seansından deney sonuna kadar istatistiksel olarak farklılık görüldü. Ayrıca sakubitril/valsartan ve dapagliflozin için rotarod sonuçlarında başlangıca kıyasla istatistiksel bir fark gözlendi (sırasıyla $P < 0,001$ ve 0,011).

Sonuç: Çalışmamız sakubitril/valsartanın atletik performans üzerinde sınırlı olumlu etkisini gösterdi. Dapagliflozinin atletik performans üzerindeki etkisinin daha anlamlı düzeyde olduğu gözlemlenmiştir.

Anahtar Kelimeler: Atletik performans, dapagliflozin, doping ajanları, performans artırıcı, sakubitril /valsartan, spor kardiyolojisi

ORIGINAL ARTICLE
KLİNİK ÇALIŞMA

Akın Torun[1]
Şahhan Kılıç[1]
Süha Asal[1]
Mert Babaoğlu[1]
Samet Yavuz[1]
Cumaali Demirtaş[2]
Serkan Dilmen[3]
Ahmet Lutfullah Orhan[1]
Mehmet Uzun[1]

[1]*Department of Cardiology, Sultan II. Abdulhamid Han Training and Research Hospital, İstanbul, Türkiye*
[2]*Veterinary Medicine, University of Health Sciences Hamidiye Health Sciences Institute, İstanbul, Türkiye*
[3]*Department of Cardiology, Izmir Bayraklı City Hospital, İzmir, Türkiye*

Corresponding author:
Akın Torun
✉ torunakin@hotmail.com

Received: July 29, 2024
Accepted: October 21, 2024

Cite this article as: Torun A, Kılıç Ş, Asal S, et al. Effect of Sacubitril/Valsartan and Dapagliflozin on Cardiac Functions and Exercise Capacity of Rats. *Turk Kardiyol Dern Ars.* 2025;53(1):13-21.

DOI:10.5543/tkda.2024.74353

Available online at archivestsc.com. Content of this journal is licensed under a Creative Commons Attribution – NonCommercial-NoDerivatives 4.0 International License.

The term "athlete's heart" refers to the physiological adaptations of the heart to repetitive exercise. Different physiological adaptation mechanisms may develop depending on the type of exercise performed.[1] Elite athletes aim to optimize cardiac fitness through training to achieve the best athletic performance. Training involves a systematic process in which athletes undergo regular and repetitive exercise stimuli to induce changes aligned with specific goals. These goals may include delaying fatigue onset, enhancing power generation, strengthening motor coordination, or reducing injury risk. The limits of sports performance have been debated and speculated upon for a considerable time. However, recent years have seen a noticeable plateau in sports performance, indicating that further improvement in individuals' physical capabilities may be limited.[2] Therefore, performance-enhancing substances are increasingly scrutinized within elite sports. The World Anti-Doping Agency (WADA) updates its list of prohibited substances and methods annually due to the vast array of chemicals and the constant introduction of new designer drugs on the market. One of the most critical aspects of sports performance is, undoubtedly, cardiac function, which raises significant concerns about the use of certain heart medications as doping agents. The inclusion of trimetazidine on WADA's Prohibited List as a doping substance in 2014 can be considered the beginning of this trend.[3,4] In recent years, the combination of sacubitril and valsartan, as well as dapagliflozin, has gained attention in the literature for its efficacy in improving cardiac performance and its positive prognostic effect on heart failure.[5,6] These medications could potentially be misused as doping substances among elite athletes. Therefore, this study aimed to investigate the effects of sacubitril/valsartan and dapagliflozin on athletic performance.

Materials and Methods

A swimming experiment was designed in rats to objectively measure exercise capacity, as this method is suitable and reliable for such assessments. In other methodologies, rats may resist exercise, but the forced swimming test is advantageous in this regard. Male Sprague-Dawley rats were obtained from the Animal Center. Based on a G*Power 3.1 analysis, it was determined that a minimum of six rats per group would be necessary to achieve the desired experimental power. Consequently, the study was designed to include a total of 24 animals, with approval obtained from Hamidiye University of Health Sciences Animal Experiments Local Ethics Committee (Approval Number: 05, Date: 19.10.2023).

The rats were housed in a controlled environment with a 12-hour light/dark cycle, maintained at a temperature of 25 °C and a relative air humidity of 40%. They had ad libitum access to water and food and were allowed unrestricted movement in a laboratory animal room for at least one week prior to the experiment. Three-month-old rats were then randomly assigned to one of three groups. At the beginning of the study, the Rota-Rod test was used to measure motor coordination and antifatigue ability in the rats. The Rota-Rod apparatus consists of a cylindrical rod (3 cm in diameter) divided into five tracks, each 6 cm wide, equipped with an infrared detector and connected to a computer. Rats were positioned on the horizontally oriented, rotating rod to ensure there were no differences between groups.

In the first group, a dose of 60 mg/kg/ml of sacubitril/valsartan was administered daily via oral gavage. In the second group, a dose of 1.5 mg/kg/ml of dapagliflozin was administered daily via oral gavage. Commercial formulations of both drugs were used. The third group served as the control. The researchers administering the drugs and the researchers conducting the swim test were separate individuals, with the latter being blinded to group assignments.

Initially, before the experiment, each group underwent swimming training in the water tank for three days. Each rat was placed in a separate water tank. There were eight tanks in total, and the work was repeated in three groups. The square prism water tank measured 40 x 40 cm with a height of 50 cm. The water temperature was maintained at 28 °C throughout the swimming process. The endurance of each rat was assessed by measuring the swimming time from the start of the activity until exhaustion, defined by uncoordinated movements and the inability to resurface within 7 seconds. The maximum swimming time (in seconds) of each rat was recorded daily. In addition, echocardiography (ECHO) and heart rate measurements were performed for each rat at the beginning of the experiment, on the 15th day, and at the end of the experiment (Figure 1). The study was conducted on weekdays, with weekends designated as rest periods for the animals to prevent overuse.

Ethical approval was obtained from Hamidiye University of Health Sciences Animal Experiments Local Ethics Committee (Approval Number: 05, Date: 19.10.2023), and the study was conducted in accordance with the Declaration of Helsinki. Artificial intelligence-supported technologies were not used in the production of this work.

Statistical Analysis

The data analysis was conducted using the Statistical Package for the Social Science (SPSS) version 27.0 (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as median (minimum-maximum) values. The Kruskal-Wallis test was used to assess differences between groups. In post hoc intergroup pairwise analyses, the Mann-Whitney U test was applied with Bonferroni correction. The Friedman test was used to determine intragroup differences. The Wilcoxon test was applied, with Bonferroni correction used for post hoc intragroup pairwise comparisons. The significance level for statistical analyses was set at $P < 0.05$.

Results

A total of 24 rats, divided into three groups of eight rats each, were observed throughout the experiment. No differences were observed between the groups when comparing echocardiography parameters at baseline. Table 1 shows the echo parameters of the control group on days 0, 15, and 30, Table 2 shows the parameters for the dapagliflozin group, and Table 3 shows those for the sacubitril/valsartan group.

ABBREVIATIONS

ATP	Adenosine triphosphate
ECHO	Echocardiography
EF	Ejection fraction
SGLT-2	Sodium-Glucose Cotransporter-2
T2DM	Type 2 diabetes mellitus
WADA	World Anti-Doping Agency

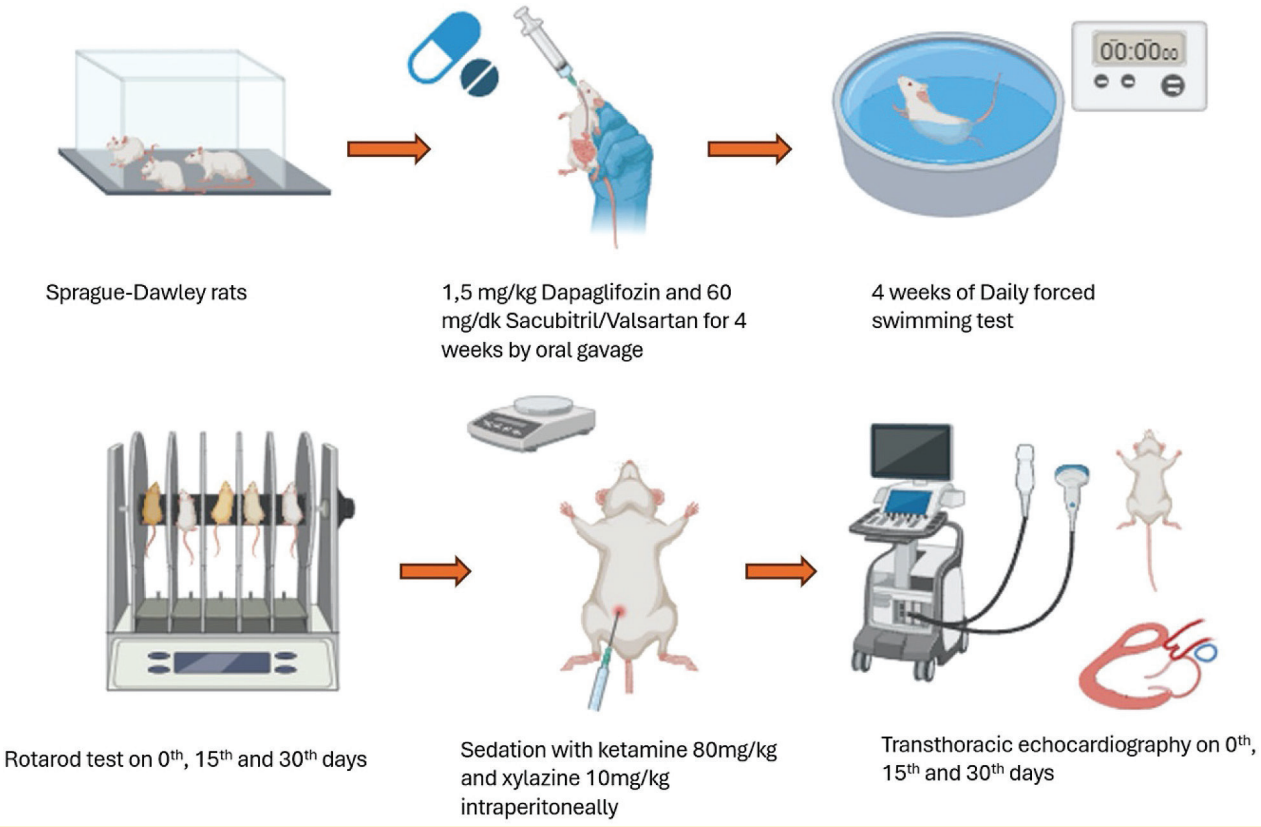

Figure 1. Study design.

Table 1. Follow-up of Echocardiography Parameters of the Control Group

Echo Parameters	Day 0	Day 15	Day 30	*P**
Heart rate (beats/min)	245 (223-261)	231 (208-254)	238.5 (221-266)	**0.010**
IVSD (mm)	1.9 (1.8-2)	1.9 (1.8-2)	1.95 (1.8-2)	**0.022**
IVSS (mm)	2.7 (2.6-2.8)	2.7 (2.7-2.9)	2.75 (2.7-3)	**0.006**
LVSD (mm)	7.4 (7.1-8.3)	7.45 (7.0-8.2)	7.4 (6.9-8.1)	**0.003**
LVDD (mm)	5.2 (4.5-6.1)	5.1 (4.5-6)	5.05 (4.4-6.1)	**0.034**
LVPWD (mm)	1.9 (1.8-2.0)	1.9 (1.8-2.0)	2 (1.8-2.1)	0.280
LVPWS (mm)	2.7 (2.5-2.7)	2.7 (2.5-2.8)	2.7 (2.6-2.7)	0.646
AoS (mm)	3.6 (3.5-3.7)	3.65 (3.4-3.7)	3.7 (3.5-3.9)	0.059
AoD (mm)	3.2 (3.1-3.3)	3.1 (3-3.2)	3.1 (3.1-3.3)	0.401
Aortic strain (%)	12.9 (12.12-16.12)	15.62 (9.67-23.3)	17.15 (12.5-22.58)	0.179
AV Vmax (m/s)	1.29 (1.1-1.54)	1.41 (1.18-1.52)	1.49 (1.18-1.83)	0.115
PV Vmax (m/s)	0.77 (0.63-0.91)	0.80 (0.69-1.05)	0.88 (0.73-1.14)	0.250
EF	69 (60-74)	70 (60-74)	68 (57-74)	0.325
F shortening	32.9 (26.5-36.6)	33.1 (26.8-36.4)	32 (24.6-36.2)	0.325
EDV	0.468 (0.374-0.598)	0.432 (0.359-0.577)	0.424 (0.343-0.556)	**0.003**
ESV	0.147 (0.095-0.237)	0.138 (0.095-0.226)	0.134 (0.089-0.237)	**0.034**
Cardiac output (mL/min)	80 (65-88)	70 (60-78)	69 (58-82)	**0.010**
LV mass (g)	1.94 (1.87-1.97)	1.96 (1.89-1.99)	1.96 (1.92-2.02)	**0.024**

*Friedman Test. AoD, Aortic Diastole; AoS, Aortic Systole; AV, Aortic Valve; EDV, End Diastolic Volume; EF, Ejection Fraction; ESV, End Systolic Volume; F, Fractional; IVSD, Interventricular Septum Diastolic; IVSS, Interventricular Septum Systolic; LV, Left Ventricle; LVDD, Left Ventricle Diastolic Diameter; LVPWD, Left Ventricle Posterior Wall Diastolic; LVPWS, Left Ventricle Posterior Wall Systolic; LVSD, Left Ventricle Systolic Diameter; PV, Pulmonary Valve.

Table 2. Follow-up of Echocardiography Parameters of the Dapagliflozin Group

Echo Parameters	Day 0	Day 15	Day 30	*P**
Heart rate (beats/min)	243 (225-255)	221 (184-253)	240 (210-282)	0.197
IVSD (mm)	1.9 (1.7-2)	1.8 (1.8-1.9)	1.85 (1.7-2)	0.368
IVSS (mm)	2.7 (2.4-2.8)	2.75 (2.2-2.9)	2.75 (2.4-2.9)	0.268
LVSD (mm)	8 (6.6-8.1)	7.85 (6.7-8.0)	7.9 (7.2-8.3)	0.191
LVDD (mm)	5.6 (4.1-6)	5.45 (3.8-5.8)	5.5 (4-6.1)	**0.042**
LVPWD (mm)	1.9 (1.7-2)	1.9 (1.8-2)	1.95 (1.9-2.1)	**0.012**
LVPWS (mm)	2.6 (2.6-2.8)	2.7 (2.6-2.8)	2.7 (2.3-2.9)	0.229
AoS (mm)	3.6 (3.4-3.9)	3.55 (3.4-4)	3.6 (3.5-3.8)	0.882
AoD (mm)	3.1 (3.1-3.4)	3.1 (2.9-3.4)	3.1 (3-3.3)	0.891
Aortic strain (%)	13.8 (9.6-16.1)	13.1 (12.1-20.6)	15.6 (9.1-20)	0.748
AV Vmax (m/s)	1.43 (1.14-1.56)	1.38 (1.19-1.68)	1.54 (1.37-1.99)	0.417
PV Vmax (m/s)	0.74 (0.58-0.91)	0.92 (0.74-1.25)	0.89 (0.77-1.04)	**0.05**
EF	67 (56-79)	66 (61-81)	69 (58-82)	0.648
F shortening	30.8 (24-40.5)	30.5 (27.5-43.3)	30.8 (25.3-44.4)	0.648
EDV	0.53 (0.30-0.55)	0.50 (0.31-0.53)	0.51 (0.39-0.59)	0.191
ESV	0.18 (0.07-0.22)	0.16 (0.06-0.20)	0.17 (0.07-0.23)	**0.042**
Cardiac output (mL/min)	82.7 (51.8-95)	72 (52.7-91)	79 (65-94)	0.197
LV mass (g)	1.93 (1.84-1.97)	1.96 (1.82-1.99)	1.93 (1.84-2.02)	0.119

*Friedman Test. AoD, Aortic Diastole; AoS, Aortic Systole; AV, Aortic Valve; EDV, End Diastolic Volume; EF, Ejection Fraction; ESV, End Systolic Volume; F, Fractional; IVSD, Interventricular Septum Diastolic; IVSS, Interventricular Septum Systolic; LV, Left Ventricle; LVDD, Left Ventricle Diastolic Diameter; LVPWD, Left Ventricle Posterior Wall Diastolic; LVPWS, Left Ventricle Posterior Wall Systolic; LVSD, Left Ventricle Systolic Diameter; PV, Pulmonary Valve.

Table 3. Follow-up of Echocardiography Parameters of the Sacubitril/Valsartan Group

Echo Parameters	Day 0	Day 15	Day 30	*P**
Heart rate (beats/min)	244 (213-262)	230 (184-266)	232 (219-252)	0.325
IVSD (mm)	1.9 (1.8-2)	1.9 (1.8-2.1)	1.9 (1.8-1.9)	0.202
IVSS (mm)	2.7 (2.6-2.8)	2.6 (2.4-2.9)	2.7 (2.6-2.8)	0.878
LVSD (mm)	7.55 (7.1-8.2)	7.55 (7.2-8.2)	7.55 (7.3-8.4)	0.779
LVDD (mm)	5.3 (4.6-5.9)	5.3 (4.6-5.7)	4.6 (4-5.5)	0.061
LVPWD (mm)	1.9 (1.8-2)	1.9 (1.8-2)	1.9 (1.7-2)	0.186
LVPWS (mm)	2.7 (2.6-2.8)	2.75 (2.6-2.8)	2.7 (2.6-2.8)	0.417
AoS (mm)	3.55 (3.4-3.7)	3.5 (3.3-3.7)	3.55 (3.4-3.9)	**0.013**
AoD (mm)	3.1 (3-3.3)	3.05 (2.9-3.2)	3.1 (2.9-3.2)	0.167
Aortic strain (%)	12.7 (9.6-16.6)	12.9 (10-20.6)	15.8 (9.6-25.8)	0.227
AV Vmax (m/s)	1.39 (1.14-2)	1.49 (1.34-1.61)	1.67 (1.52-2.23)	**0.030**
PV Vmax (m/s)	0.73 (0.6-1)	0.8 (0.66-1.25)	1.09 (0.89-1.72)	**0.010**
EF	64 (59-72)	67 (60-75)	74 (69-83)	**0.002**
F shortening	29.3 (25.9-35.2)	31.3 (26.6-37.8)	36.9 (32.9-45.2)	**0.002**
EDV	0.45 (0.37-0.57)	0.45 (0.39-0.57)	0.45 (0.41-0.62)	0.779
ESV	0.15 (0.11-0.21)	0.16 (0.11-0.19)	0.11(0.7-0.17)	0.061
Cardiac output (mL/min)	71 (62-85)	75 (52-101)	80 (66-111)	**0.034**
LV mass (g)	1.94 (1.89-1.99)	1.93 (1.84-2.02)	1.96 (1.92-1.97)	0.891

*Friedman Test. AoD, Aortic Diastole; AoS, Aortic Systole; AV, Aortic Valve; EDV, End Diastolic Volume; EF, Ejection Fraction; ESV, End Systolic Volume; F, Fractional; IVSD, Interventricular Septum Diastolic; IVSS, Interventricular Septum Systolic; LV, Left Ventricle; LVDD, Left Ventricle Diastolic Diameter; LVPWD, Left Ventricle Posterior Wall Diastolic; LVPWS, Left Ventricle Posterior Wall Systolic; LVSD, Left Ventricle Systolic Diameter; PV, Pulmonary Valve.

Table 4. Follow-up Data of the Weights of the Groups and Comparison Between Groups

Group	Day 0	Day 15	Day 30	*P***
Control	430 (370-450)	405 (350-430)	385 (330-400)	**0.002**
Dapagliflozin	420 (390-470)	380 (360-430)	361 (344-407)	**<0.001**
Sacubitril/Valsartan	435 (380-460)	397 (340-435)	377 (319-410)	**<0.001**
P*	0.996	0.889	0.826	

*Kruskal-Wallis test. **Friedman test.

Table 5. Rotarod Follow-Up Data of the Groups and Comparison Between Groups

Group	Day 0	Day 15	Day 30	*P***
Control	31 (14-131)	67.5 (27-129)	67.5 (38-131)	0.115
Dapagliflozin	43.5 (17-103)	60 (41-132)	88 (38-148)	**0.011**
Sacubitril/Valsartan	45 (30-70)	74 (58-103)	89 (75-157)	**<0.001**
P*	0.750	0.652	0.278	

*Kruskal-Wallis test. **Friedman test.

Table 6. Comparison of Swimming Times of Groups

Swimming Session (seconds) Median (Min-Max)	Control Group	Dapagliflozin Group	Sacubitril/Valsartan Group	*P**
1	188 (165-297)	203 (171-293)	203 (182-244)	0.671
2	339 (290-510)	345 (270-590)	394 (288-420)	0.655
3	728 (688-910)	808 (654-950)	767 (555-954)	0.777
4	1154 (966-1245)	1180 (945-1365)	1194 (765-1362)	0.760
5	1269 (1100-1330)	1402 (1189-1490)	1370 (1143-1613)	0.072
6	1532 (1214-1974)	1722 (1600-1910)	1827 (1464-2026)	0.165
7	1680 (1430-2050)	1906 (1653-2170)	1764 (1385-2184)	0.68
8	1746 (1480-2496)	2170 (1790-2450)	1866 (1685-2252)	0.52
9	1889 (1478-2210)	2144 (1986-2387)	2044 (1800-2387)	**0.003**
10	1955 (1551-3455)	2724 (2625-2939)	2085 (1686-2533)	**0.009**
11	2110 (1810-2280)	2407 (2190-3001)	2238 (2022-2464)	**<0.001**
12	2261 (2054-2320)	2787 (2620-3160)	2354 (2104-2773)	**<0.001**
13	2103 (2037-2276)	3055 (3004-3274)	2350 (2247-2656)	**<0.001**
14	2304 (2060-2474)	3037 (2798-3345)	2677 (2136-2950)	**<0.001**
15	2299 (2100-2382)	2630 (2229-3141)	2435 (2110-2187)	**0.039**
16	2314 (2245-2405)	2710 (2340-3312)	2407 (2135-2173)	**0.011**
17	2371 (2214-2472)	3006 (2360-3140)	2480 (2179-2895)	**0.046**
18	2372 (2256-2492)	3372 (2480-4130)	2397 (2284-2980)	**0.001**
19	2343 (2272-2424)	3500 (2630-4003)	2470 (2320-29559)	**<0.001**
20	2382 (2300-2467)	3580 (2914-4212)	2591 (2457-3002)	**<0.001**
Total Time	35654 (32315-38332)	45406 (39839-49157)	38105 (34423-42696)	**<0.001**
Median	1782 (1615-1916)	2270 (1991-2457)	1905 (1721-2134)	**<0.001**

*Kruskal-Wallis test.

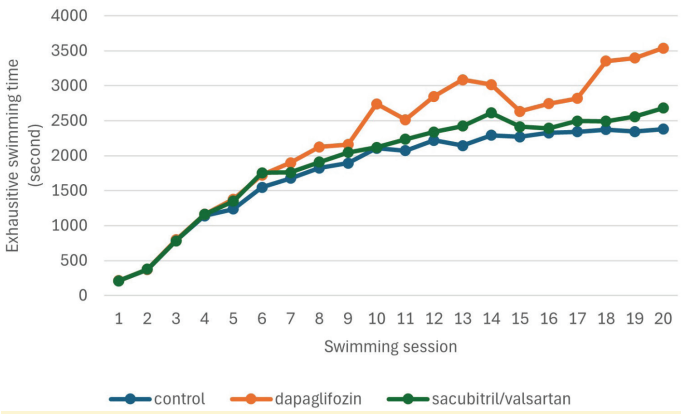

Figure 2. Improvement in median swimming times across groups during the experiment.

When comparing baseline data, there was no significant difference in the rotarod performance or weight of rats. Table 4 shows the change in the rats' weight over the course of the experiment, and Table 5 presents the rotarod follow-up results.

Table 6 presents the median swimming times for each group throughout the swimming sessions. Starting from the ninth swimming session, a noticeable difference in swimming times emerged between the groups. Figure 2 displays the median swimming performance of the three groups. Table 7 compares the sacubitril/valsartan group with the control group, while Table 8 compares the dapagliflozin group with the control group.

Discussion

Instances of doping and other forms of cheating have been documented throughout sports history. The use of performance-enhancing drugs in sports is a serious issue. The use of such substances in professional sports and competitions has significantly damaged the reputations of numerous athletes worldwide and poses a threat to their health.[7] The pharmaceutical industry has expanded significantly in the past decade, alongside advancements in technology. This growth has led to key developments, particularly in cardiac medications. These recently introduced cardiac drugs may enhance athletic performance and, therefore, may be misused as doping agents. Therefore, our study aimed to examine the impact of

Table 7. Comparison of Swimming Times of the Control Group and Sacubitril/Valsartan Group

Swimming Session (seconds) Median (Min-Max)	Control Group	Sacubitril/Valsartan Group	*P**
1	188 (165-297)	203 (182-244)	0.431
2	339 (290-510)	394 (288-420)	0.528
3	728 (688-910)	767 (555-954)	0.344
4	1154 (966-1245)	1194 (765-1362)	0.462
5	1269 (1100-1330)	1370 (1143-1613)	0.115
6	1532 (1214-1974)	1827 (1464-2026)	0.172
7	1680 (1430-2050)	1764 (1385-2184)	0.600
8	1746 (1480-2496)	1866 (1685-2252)	0.248
9	1889 (1478-2210)	2044 (1800-2387)	0.141
10	1955 (1551-3455)	2085 (1686-2533)	0.401
11	2110 (1810-2280)	2238 (2022-2464)	0.115
12	2261 (2054-2320)	2354 (2104-2773)	0.172
13	2103 (2037-2276)	2350 (2247-2656)	**0.002**
14	2304 (2060-2474)	2677 (2136-2950)	0.46
15	2299 (2100-2382)	2435 (2110-2187)	0.093
16	2314 (2245-2405)	2407 (2135-2173)	0.462
17	2371 (2214-2472)	2480 (2179-2895)	0.401
18	2372 (2256-2492)	2397 (2284-2980)	0.462
19	2343 (2272-2424)	2470 (2320-29559)	**0.009**
20	2382 (2300-2467)	2591 (2457-3002)	**0.002**
Total Time	35654 (32315-38332)	38105 (34423-42696)	0.115
Median	1782 (1615-1916)	1905 (1721-2134)	0.115

*Mann-Whitney U test.

Table 8. Comparison of Swimming Times of the Control Group and Dapagliflozin Group

Swimming Session (seconds) Median (Min-Max)	Control Group	Dapagliflozin Group	*P**
1	188 (165-297)	203 (171-293)	0.462
2	339 (290-510)	345 (270-590)	0.916
3	728 (688-910)	808 (654-950)	0.793
4	1154 (966-1245)	1180 (945-1365)	0.636
5	1269 (1100-1330)	1402 (1189-1490)	0.027
6	1532 (1214-1974)	1722 (1600-1910)	0.059
7	1680 (1430-2050)	1906 (1653-2170)	0.027
8	1746 (1480-2496)	2170 (1790-2450)	0.046
9	1889 (1478-2210)	2144 (1986-2387)	0.021
10	1955 (1551-3455)	2724 (2625-2939)	**0.012**
11	2110 (1810-2280)	2407 (2190-3001)	**0.003**
12	2261 (2054-2320)	2787 (2620-3160)	**<0.001**
13	2103 (2037-2276)	3055 (3004-3274)	**<0.001**
14	2304 (2060-2474)	3037 (2798-3345)	**<0.001**
15	2299 (2100-2382)	2630 (2229-3141)	0.027
16	2314 (2245-2405)	2710 (2340-3312)	**0.003**
17	2371 (2214-2472)	3006 (2360-3140)	0.027
18	2372 (2256-2492)	3372 (2480-4130)	**0.001**
19	2343 (2272-2424)	3500 (2630-4003)	**<0.001**
20	2382 (2300-2467)	3580 (2914-4212)	**<0.001**
Total Time	35654 (32315-38332)	45406 (39839-49157)	**<0.001**
Median	1782 (1615-1916)	2270 (1991-2457)	**<0.001**

*Mann-Whitney U test.

dapagliflozin and sacubitril/valsartan—known for their significant symptomatic relief and favorable prognosis, especially in heart failure—on sports performance.

The mechanisms by which Sodium-Glucose Cotransporter-2 (SGLT-2) inhibitors improve exercise tolerance in patients with type 2 diabetes mellitus (T2DM) combined with heart failure (HF) are multifactorial and complex, and they have not yet been fully elucidated. Current evidence suggests that these mechanisms are linked to the effects of SGLT-2 inhibitors on increasing hematocrit and erythropoietin levels to enhance oxygen delivery, improving mitochondrial fatty acid oxidation in skeletal muscle, promoting weight loss, and increasing ketone body synthesis. Additionally, SGLT-2 inhibitors facilitate a shift in energy metabolism from glucose to fatty acid oxidation, which is then utilized by the heart.[8]

Both drugs demonstrated the ability to improve athletic performance, with the effect being significantly more prominent for dapagliflozin. Dapagliflozin alters cardiac energy metabolism. It may increase the production and utilization of ketone bodies, which serve as more energy-efficient metabolic substrates for cardiac muscle fibers.[9] It can alleviate oxidative stress in cardiomyocytes and positively impact mitochondrial functions by enhancing mitochondrial fatty acid oxidation in skeletal muscle. Additionally, dapagliflozin promotes fatty acid oxidation over glucose in the heart[10] and increases adenosine triphosphate (ATP) production in heart muscle by 30%. It also inhibits the sympathetic nervous system, reduces blood pressure, and lowers pulmonary resistance and pressure, likely exerting these effects through the kidneys.[11] All of these actions may contribute to the impact of dapaglobulin on athletic performance.

The effect of exercise on the heart can be observed more quickly in rats.[12] The changes seen in the echocardiography data of the control group regarding the left ventricle are consistent with the development of an athlete's heart. This demonstrates the methodological reliability of the experiment. In both treatment groups, there was a significant increase in antegrade flow of the pulmonary artery compared to baseline, which may be related to an improvement in right ventricular function. Another notable finding is the increase in ejection fraction (EF) observed in the sacubitril/valsartan group, an effect also seen in heart failure patients.[13] The impact of dapagliflozin on athletic performance was assessed independently of its effect on EF.

There was a decrease in the weight of the rats in all three groups due to intense exercise throughout the experiment. However,

there was no significant difference in weight loss between the groups. The decrease in weight is related to both the direct effect of physical activity on energy expenditure and the increase in metabolic rate during the resting period.[14] The rats were not under any calorie restriction and had unrestricted access to food. If the experimental period were extended, a plateau in their weight could potentially be observed.[15]

We observed an increase in rotarod performance compared to baseline data in both medication groups, though there was no significant difference compared to the control group. The adaptation of the rats to the rotarod test should not be overlooked. While a statistical difference was observed in the medication groups, the increase in the control group was not statistically significant. These results may serve as the foundation for more comprehensive studies, as the positive effects of dapagliflozin and sacubitril/valsartan on rotarod performance may provide an advantage in skill-based sports (e.g., golf, table tennis, shooting, curling, bowling, etc.). [16]

The primary objective of our investigation was to assess improvements in athletic performance. After similar results were observed during the first nine swimming sessions, a significant difference in the athletic performance of rats receiving both medications became apparent. The effect of sacubitril/valsartan was generally comparable to that of the control group, with slightly better outcomes. Sacubitril/valsartan, an angiotensin receptor-neprilysin inhibitor, enhances the natriuretic peptide system by inhibiting the neprilysin enzyme and blocks the renin-angiotensin-aldosterone system by inhibiting the angiotensin II receptor. Evidence indicates that it improves mortality and reduces hospitalization rates in individuals with heart failure caused by impaired left ventricular systolic function.[17] Additionally, sacubitril/valsartan augments the effects of bradykinin, substance P, and adrenomedullin, which are other hormones that may contribute to the medication's cardiac efficacy.[18] The positive effect of sacubitril/valsartan on athletic performance may be related to increased cardiac efficiency and modest improvements in left ventricular function. However, this effect was observed to be limited, with only a relative increase in athletic performance.

The effect of dapagliflozin was notably observed. Sodium-glucose cotransporter-2 inhibitors reduce the risk of hospitalization for heart failure in patients with either preserved or reduced ejection fraction. However, the specific hemodynamic mechanisms responsible for these benefits are not yet fully understood.[5] Research conducted on rats has demonstrated that dapagliflozin exerts a vasodilatory effect on the thoracic aorta, which depends on potassium channel voltage.[19] This finding indicates a direct effect on vascular cells in both acute and chronic treatment.[20] Therefore, it is reasonable to observe performance improvement in the initial periods and progressing over time. Another possible mechanism of action may involve its positive effects on microvascular and endothelial activity.[21] SGLT-2 inhibitors, which are currently the only treatment for heart failure with preserved EF, may have additional, as-yet-unexplored capabilities for enhancing cellular myocardial efficiency.

Our study indicates that dapagliflozin has a clear doping effect on athletic performance. Future studies examining its mechanism of action may provide clearer insights into this effect.

Conclusion

Advancements in cardiac treatment research have provided novel and effective therapeutic options. The heart, central to high-level athletic performance, and related medications can be misused as doping agents. Our study demonstrated a limited positive effect of sacubitril/valsartan on athletic performance, while the impact of dapagliflozin on athletic performance was particularly significant.

Ethics Committee Approval: Ethics committee approval was obtained from Hamidiye University of Health Sciences Animal Experiments Local Ethics Committee (Approval Number: 05, Date: 19.10.2023).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – A.T., Ş.K., S.A., M.U.; Design – A.T., Ş.K., C.D., S.D., A.L.O.; Supervision – A.L.O., Ş.K., M.U.; Materials – S.A., M.B., S.Y., C.D.; Data Collection and/or Processing – Ş.K., S.A., M.B., S.Y., C.D.; Analysis and/or Interpretation – Ş.K., S.A., M.B., S.Y.; Literature Review – A.T., S.D.; Writing – A.T.; Critical Review – S.D., A.L.O., M.U.

Use of AI for Writing Assistance: Artificial intelligence-supported technologies were not used in the production of this work.

Conflict of Interests: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding: The authors received no financial support for the research, authorship, and/or publication of this article.

References

1. Torun A, Erdem A, Doğan S, et al. Comparison of the effects of resistance, aerobic and mixed exercise on athlete's heart. *J Sports Med Phys Fitness.* 2024;64:88-93.[CrossRef]
2. Berthelot G, Sedeaud A, Marck A, et al. Has athletic performance reached its peak?. *Sports Med*. 2015;45(9):1263-1271.[CrossRef]
3. Jarek A, Wójtowicz M, Kwiatkowska D, et al. The prevalence of trimetazidine use in athletes in Poland: Excretion study after oral drug administration. *Drug Test Anal*. 2014;6(11-12):1191-1196. [CrossRef]
4. Li YJ, Wang PH, Chen C, Zou MH, Wang DW. Improvement of mechanical heart function by trimetazidine in db/db mice. *Acta Pharmacol Sin*. 2010;31(5):560-569. [CrossRef]
5. Solomon SD, McMurray JJV, Claggett B, et al. Dapagliflozin in heart failure with mildly reduced or preserved ejection fraction. *N Engl J Med*. 2022;387(12):1089-1098.
6. Docherty KF, Vaduganathan M, Solomon SD, McMurray JJV. Sacubitril/Valsartan: Neprilysin inhibition 5 years after PARADIGM-HF. *JACC Heart Fail.* 2020;8(10):800-810. Erratum in: *JACC Heart Fail.* 2020;8(12):1057.[CrossRef]
7. Palmi I, Berretta P, Tini A, Ricci G, Marinelli S. The unethicality of doping in sports. *Clin Ter*. 2019;170(2):e100-e101.
8. Linden MA, Ross TT, Beebe DA, et al. The combination of exercise training and sodium-glucose cotransporter-2 inhibition improves glucose tolerance and exercise capacity in a rodent model of type 2 diabetes. *Metabolism*. 2019;97:68-80.[CrossRef]
9. Voorrips SN, Saucedo-Orozco H, Sánchez-Aguilera PI, De Boer RA, Van der Meer P, Westenbrink BD. Could SGLT2 inhibitors improve exercise intolerance in chronic heart failure?. *Int J Mol Sci*. 2022;23(15):8631.[CrossRef]
10. Herring RA, Parsons I, Shojaee-Moradie F, et al. Effect of dapagliflozin on cardiac function and metabolic and hormonal responses to exercise. *J Clin Endocrinol Metab*. 2023;108(4):888-896.[CrossRef]
11. Peng Y, Qin D, Wang Y, Xue L, Qin Y, Xu X. The effect of SGLT-2 inhibitors on cardiorespiratory fitness capacity: A systematic review and meta-analysis. *Front Physiol*. 2023;13:1081920.[CrossRef]

12. Jiang H, Jia D, Zhang B, et al. Exercise improves cardiac function and glucose metabolism in mice with experimental myocardial infarction through inhibiting HDAC4 and upregulating GLUT1 expression. *Basic Res Cardiol*. 2020;115(3):28.[CrossRef]
13. Solomon SD, Vaduganathan M, L Claggett B, et al. Sacubitril/Valsartan across the spectrum of ejection fraction in heart failure. *Circulation*. 2020;141(5):352-361.[CrossRef]
14. Speakman JR, Selman C. Physical activity and resting metabolic rate. *Proc Nutr Soc*. 2003;62(3):621-634.[CrossRef]
15. Celik O, Yildiz BO. Obesity and physical exercise. *Minerva Endocrinol (Torino)*. 2021;46(2):131-144.[CrossRef]
16. Deacon RMJ. Measuring motor coordination in mice. *J Vis Exp*. 2013;(75):e2609. [CrossRef]
17. Hubers SA, Brown NJ. Combined angiotensin receptor antagonism and neprilysin inhibition. *Circulation*. 2016;133(11):1115-1124. [CrossRef]
18. Singh JSS, Burrell LM, Cherif M, Squire IB, Clark AL, Lang CC. Sacubitril/valsartan: Beyond natriuretic peptides. *Heart*. 2017;103(20):1569-1577.[CrossRef]
19. Salvatore T, Caturano A, Galiero R, et al. Cardiovascular benefits from gliflozins: Effects on endothelial function. *Biomedicines*. 2021;9(10):1356.[CrossRef]
20. Alshnbari AS, Millar SA, O'Sullivan SE, Idris I. Effect of sodium-glucose cotransporter-2 inhibitors on endothelial function: A systematic review of preclinical studies. *Diabetes Ther*. 2020;11(9):1947-1963. [CrossRef]
21. Ma L, Zou R, Shi W, et al. SGLT2 inhibitor dapagliflozin reduces endothelial dysfunction and microvascular damage during cardiac ischemia/reperfusion injury through normalizing the XO-SERCA2-CaMKII-coffilin pathways. *Theranostics*. 2022;12(11):5034-5050. [CrossRef]