Cardiovascular handgrip responses during treadmill exercise: randomized pilot trial

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Background: The isometric exercise performed using the handgrip (HG) acutely promotes elevation of systolic blood pressure (SBP) and diastolic blood pressure (DBP), and in a non-consensual manner among articles elevation or maintenance of heart rate (HR). Currently, although there is a vast literature on the hemodynamic effects of interval training and isometric exercise with HG alone, there is still no consistent evidence of such adjustments occurring in the association between the two. Therefore, the objective of this study was to describe the acute hemodynamic responses found only during interval training and when combined with isometric contraction with HG.

Methods: This is a pilot study of a crossover clinical trial. Seven male volunteers, aged 24 ± 3.9 years, underwent three protocols on an ergometric treadmill, with a 3-minute warm-up at 30% of heart rate reserve (HRR), four sprints 2 minutes at 50% HRR and active intervals at the same speed as the warm-up. Randomization was carried out in a simple random manner. The protocols were classified according to the use of HG during sprints, as follows: PI = without HG; PII = HG 30% of handgrip strength (HGS) and PIII = 60% of HGS). Variations (Δ) in HR, double product (DP), SBP and DBP were evaluated.

Results: The presence of HG did not change HR behavior, but it increased DP (PI: 10,472±2,539 *vs.* PII: 12,217±1,933 *vs.* PIII: 13,369±3,089) through SBP, which in PI had a plateau behavior of 15±22.2 mmHg, while PII varied with an average of 41±12.2 mmHg and PIII 47±11.1 mmHg, in the 4th sprint. DBP fell in PI with 12±13.2 mmHg, while PII and PIII showed an drop of 0±19.6 and 6±13.0 mmHg in the last sprint, respectively.

Conclusions: The use of HG during interval training directly modulates hemodynamic variables, promoting an increase in SBP elevation, attenuation of the drop in DBP and an increase in DP, without an increase in HR.

Trial Registration: RBR-78fhyrf. Available in https://ensaiosclinicos.gov.br/rg/RBR-78fhyrf

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Introduction

Interval training, used consistently for cardiovascular conditioning, stimulates hemodynamic adjustments necessary to maintain adequate cardiac output (1). Hemodynamic adjustments include increasing systolic blood pressure (SBP) and heart rate (HR) and maintaining or reducing diastolic blood pressure (DBP) (2). Unlike interval training, isometric exercise, specifically using handgrip (HG), acutely promotes the elevation of SBP and DBP, and in a way not consensual between the article's, elevation or maintenance of HR (3).

It is interesting to note that although there is a vast literature on the hemodynamic adjustments caused by interval training (4) and isometric exercise with HG (3,5), when considered independently, there is still no consistent evidence of such adjustments occurring in the association between these two exercises. Some hypotheses can be

Highlight box

Key findings

• Isometric contraction with handgrip (HG) is capable of raising systolic blood pressure (SBP) and attenuating the drop in diastolic blood pressure (DBP) during interval training. The increase in SBP determined a greater double product when HG was part of the intervention, however, the use of the HG did not interfere with the heart rate (HR) behavior.

What is known and what is new?

- Until now, the hemodynamic response to exercise with HG had only been experienced in static activities, with isolated exercise and in them there was an increase in SBP and DBP without clear interference on HR.
- This manuscript is a pioneer in the experience of isometric contraction during dynamic activity on a treadmill. This mechanistic view allowed a thorough assessment of the joint effect of the interventions and their cardiovascular response.

What is the implication, and what should change now?

• The outcomes observed in young adults will be tested in a larger population and, if the findings are confirmed, new studies could take this strategy as a basis for managing specific populations, serving, for example, as a strategy to increase cardiac work in elderly people with conditions that make it impossible to increase cardiovascular stress with increased speed. raised, such as the summative effect of interventions on SBP, with a consequent increase in cardiac work. Given the mechanoconstrictive characteristic of isometric HG exercise, it is also possible to expect a response capable of attenuating the drop in blood pressure during the treadmill. The answers to these implicit questions are topics that can directly impact populations of healthy individuals, interested in improving cardiovascular fitness, as well as individuals who have cardiovascular dysfunctions and who also seek to improve their functional capacity, but they cannot increase cardiovascular stress through treadmill speed.

Therefore, the objective of this study is to describe the hemodynamic effects caused by the use of the HG during interval training. To this end, this pilot study was first designed, which will serve as the methodological and executive basis of a future study with a larger number of volunteers. We present this article in accordance with the CONSORT reporting checklist (available at https://atm. amegroups.com/article/view/10.21037/atm-24-59/rc).

Methods

Study design

This is a pilot study of a mechanistic crossover clinical trial, approved by the Ethics and Research Committee of Bahia Adventist College, with CAAE: 44262121.2.0000.0042, conducted in accordance with the Declaration of Helsinki (as revised in 2013) and registered in the Registry of Clinical Trials (REBEC) with code RBR-78fhyrf. Data collections were carried out at Bahia Adventist College, in an airconditioned room with a temperature of 23 °C.

Sample selection

Seven young adults were selected, aged between 18 and 30 years, classified as active or irregularly active by the International Physical Activity Questionnaire (IPAQ)-Short Version (6). Individuals were excluded if, based on the preliminary health assessment, they presented limiting conditions reported in the medical history questionnaire or who were classified as high risk for cardiovascular diseases, with two or more risk factors, in accordance with the

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guidelines for exercise testing and its prescription from the American College of Sports Medicine (4). Individuals who reported the pre-existence of musculoskeletal disorders that made it impossible to carry out the protocol were also excluded.

During the study, the guidelines on research with human beings in Resolution 466/12 of the National Health Council were observed. All volunteers were informed about the study proposal, risks, benefits and the care necessary for participation. The volunteers signed the Free and Informed Consent Form and were alerted about the possibility of interrupting participation in the research at any time, free of charge.

Outcomes

The following variables were chosen as primary outcomes for this research: HR, SBP, DBP and double product (DP = HR × SBP). The variations (Δ) of sprints in relation to rest (sprint – rest) were considered.

Used tools

During the study, the following equipment was used:

- Welmy brand digital scale, with maximum capacity of 200 kg and stadiometer with 0.1 cm accuracy measured by the National Institute of Metrology (INMETRO);
- Pollar H10 HR monitor (Polar Electro Oy, Kempele, Finland) (7);
- ✤ 3MTM Littimann[®] classic IIITM Stethoscope (3M do Brasil Ltda, Sumaré, SP, Brazil);
- Welch sphygmomanometer Allyn DS44-11BR Durashock (Welch Allyn, Barueri, SP, Brazil);
- ♦ EKO CORE MD[™] auscultation amplifier (Eko Devices, Inc., Berkeley, CA, USA), capable of amplifying sound up to 40 times, real-time transmission and audio recording via Bluetooth;
- Jamar[®] Hydraulic Hand Dynamometer (Model J00105, Lafayette Instrument Company, Lafayette, IN, USA) (8);
- HG device (Brother Medical, Jiangsu, China) with a load of 5 to 40 kg;
- Athletic Extreme 3260T 18 km/h treadmill (Athletic Comercio LTDA, São Paulo, SP, Brazil).

Screening

At the first meeting, a physical-clinical assessment was carried out, filling in sociodemographic data and then a physical examination with measurement of height, body mass, HR at rest, blood pressure at rest [following the recommendations of the American Heart Association (9)] and handgrip strength (HGS) (8).

Determination of HGS

To determine HGS, the recommendations of the American Society of Hand Therapists were followed, using the Jamar[®] hydraulic dynamometer (8). The volunteer was instructed to remain seated and perform a maximum palmar contraction with the elbow flexed at 90° and the forearm in a neutral position. Three attempts were made on each hand, with a 1-minute rest between attempts. The highest values on each side were recorded, and the limb with the lowest value was selected to calculate the load used during the intervention protocol.

Physical stress test

An incremental physical effort test was applied on the Athletic Extreme 3260T 18 km/h treadmill to determine the speed necessary for the volunteer to reach the light and moderate training zones, with 30% and 50% of HR reserve, respectively. HR was monitored in real time by the Polar H10 HR monitor (7) and the target HR zones were determined by the Karvonen equation: resting HR + (maximum HR – resting HR) × % intensity. The maximum HR predicted for the calculation was determined by equation 220 – age (10).

Sample randomization

After the screening assessment, volunteers were randomized to the order in which they would participate in each of the three exercise protocols. Each protocol was carried out seven days apart. Randomization was carried out in a simple random manner. At the end of the screening assessment, each volunteer drew a number from 1 to 3 from a black bag containing three numbered balls. Each number represented an exercise protocol to be performed. The volunteer drew the first ball that represented the first protocol to be performed by him and later a second ball that

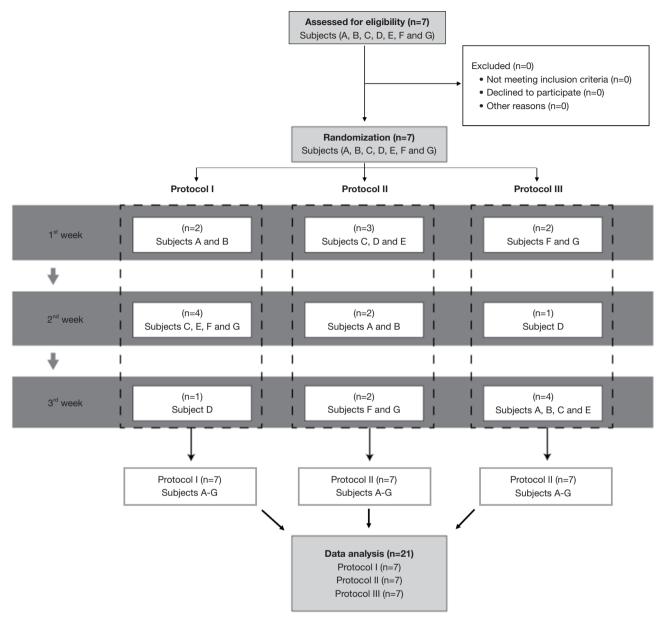


Figure 1 Flow diagram.

represented the second protocol to be performed by him. The remaining ball represented the last protocol. *Figure 1* outlines the randomization process.

Intervention protocols

The protocols were divided as follows: 5 minutes at rest to evaluate HR and BP recording at rest. The volunteer was then taken to the Athletic Extreme 3260T 18 km/h treadmill where he warmed up for 3 minutes at a speed corresponding to 30% of the reserve HR. Subsequently, the participant underwent four 2-minute sprints at a speed corresponding to 50% HR reserve, separated by active intervals of 1 minute at the same speed as the warm-up. In the final 20 seconds of each sprint, systemic blood pressure and HR were measured.

At the end of the 4th sprint, the cool-down phase began for 2 minutes, the first minute being at the warm-up speed and the following with progressive reduction until the treadmill's minimum speed.

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Figure 2 Stages of the intervention protocols. Heart rate, systolic blood pressure and diastolic blood pressure were measured during stages 1 and 3. In stage 3, the measurement occurred at the end of each sprint. HRR, heart rate reserve; HG, handgrip; HGS, handgrip strength.

Differentiation of protocols

While protocol I followed the coordinates described above, protocols II and III added the performance of bilateral isometric contraction with an HG device during sprints, at loads of 30% and 60% of the HGS obtained in the test. *Figure 2* outlines the stages of the three protocols.

Hemodynamic monitoring during the intervention

During the intervention protocols, HR measurements were monitored in real-time using a Polar H10 HR monitor (7) and the highest value at the end of each stage was recorded (see *Figure 2*).

To measure BP, some precautions were taken, such as maintaining the Welch sphygmomanometer with a cuff attached to the volunteers left arm (during the entire intervention) and a watch supported on an external metal support, in order to avoid mechanical shock with the device. Thinking about increasing the quality of auscultation during the treadmill, the Littimann[®] classic IIITM stethoscope was used in conjunction with the EKO CORE MDTM amplifier, which allowed amplification of the auscultation sound by 40 times, mitigating possible measurement errors due to noise from the treadmill.

At the time of measurement, the volunteers were asked to maintain running speed and rest their left arm on the evaluator's shoulder, thus remaining with the limb extended and relaxed in a position of approximately 90° for shoulder flexion, with forearm and hand in supination. Furthermore, for protocols using HG, volunteers were instructed to interrupt the isometric contraction only on the left side, handing over the device at the time of BP measurement. To determine BP values, the evaluator inflated the sphygmomanometer cuff while performing auscultation with an average pressure increase of 20 mmHg/s; upon noticing the absence of sounds, he inflated another 20 mmHg and then opened the valve allowing the air to escape with a pressure reduction of 10 mmHg/s.

Statistical analysis

As this was a pilot study, only descriptive analysis of the data was carried out. Continuous variables were described as mean and standard deviation and median and quartile range. HR, SBP, DP and DBP data were presented based

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on Δ : sprint moment – rest moment.

Results

Seven male individuals were evaluated, two of which were classified as active and five as irregularly active by the International Physical Activity Questionnaire (IPAQ) (6).

Table 1 Anthropometric and clinical characteristics of the sample (n=7)

Variables	Mean ± SD
Age (years)	24±3.9
BMI (kg/m²)	24±2.1
Resting HR (bpm)	77±12.0
Resting SBP (mmHg)	123±9.3
Resting DBP (mmHg)	81±8.2
Right HGS (kgf)	39±8.2
Left HGS (kgf)	36±6.6

BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HGS, handgrip strength; SD, standard deviation.

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Anthropometric characteristics and other physical examination data are presented in *Table 1*.

Table 2 presents a description of the mean and median HR Δ in the four sprints in each protocol. It is noteworthy that the average Δ increases between the first and last sprints in all protocols, with emphasis on an increase of 20 bpm from the 1st to the 4th sprint in protocol III. The Median follows the same pattern with the exception of protocol II.

Table 3 presents the description of the mean and median SBP Δ in the four sprints in each protocol. It is noteworthy that the average Δ , unlike the HR behavior, does not increase between the first and last sprints in protocol I (without HG). An increase in SBP is observed between the 1st and 4th sprints only in protocols II and III (with HG). The median presents a controversial behavior in protocol I, where Δ reduces over time (1st to 4th sprints). When observing the median of protocols II (HG at 30%), there is similarity between the variation of the mean, with 8 mmHg between the first and fourth sprint and in protocol III (HG at 60%), there is a variation of the median almost twice as large (30 mmHg) than the average (17 mmHg).

Table 4 shows the behavior of the variations of the DP in average and median in the four sprints in each protocol. It is noteworthy that the mean and median of Δ increase

Table 2 Description of HR at baseline and variations (Δ) during sprints for each protocol

Protocols	Moment	Average HR (bpm)	SD	Median HR (bpm)	1 st IQR	3 rd IQR
Protocol I (n=7) (treadmill without HG)	Baseline	77	12.0	80	65	85
	Δ 1 st sprint	53	15.4	48	45	64
	$\Delta 2^{nd}$ sprint	58	15.5	54	52	66
	$\Delta 3^{\rm rd}$ sprint	62	14.7	57	56	73
	$\Delta 4^{th}$ sprint	67	13.8	65	62	77
Protocol II (n=7) (treadmill with HG 30% HGS)	Baseline	78	13.8	77	65	70
	Δ 1 st sprint	48	12.5	47	40	56
	$\Delta 2^{nd}$ sprint	54	14.2	53	44	64
	$\Delta 3^{\rm rd}$ sprint	56	18.4	47	41	73
	$\Delta 4^{th}$ sprint	56	14.8	49	45	67
Protocol III (n=7) (treadmill with HG 60% HGS)	Baseline	75	7.3	77	93	79
	Δ 1 st sprint	52	4.7	50	49	57
	$\Delta 2^{nd}$ sprint	61	7.5	56	56	67
	Δ 3 rd sprint	64	9.0	58	57	71
	$\Delta 4^{th}$ sprint	72	12.2	70	64	80

Δ = sprint HR – baseline HR. HR, heart rate; HG, handgrip; HGS, handgrip strength; SD, standard deviation; IQR, interquartile range.

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Protocols	Moment	Average SBP (mmHg)	SD	Median SBP (mmHg)	1 st IQR	3 rd IQR
Protocol I (n=7) (treadmill without HG)	Baseline	123	9.3	120	114	130
	Δ 1 st sprint	14	18.0	20	8	26
	$\Delta 2^{nd}$ sprint	15	20.9	19	3	30
	Δ 3 rd sprint	15	22.2	10	1	28
	$\Delta 4^{th}$ sprint	14	23.3	6	1	28
Protocol II (n=7) (treadmill with HG 30% of HGS)	Baseline	115	12.7	114	107	130
	Δ 1 st sprint	27	6.7	26	23	31
	$\Delta 2^{nd}$ sprint	28	10.1	32	20	36
	Δ 3 rd sprint	41	12.2	46	40	48
	$\Delta 4^{th}$ sprint	35	12.3	34	27	43
Protocol III (n=7) (treadmill with HG 60% of HGS)	Baseline	116	5.2	119	110	120
	Δ 1 st sprint	30	21.3	20	20	40
	$\Delta 2^{nd}$ sprint	46	17.6	34	33	60
	Δ 3 rd sprint	41	14.6	30	30	55
	$\Delta 4^{th}$ sprint	47	11.1	50	40	55

Table 3 Description of S	BP at baseline and variations (Δ)	during sprints for each protocol

 Δ = sprint SBP – baseline SBP. SBP, systolic blood pressure; HG, Handgrip; HGS, handgrip strength; SD, standard deviation; IQR, interquartile range.

Table 4 Description	of the DP at baseline an	d variations (Δ) duri	ng sprints for each	n protocol

Protocols	Moment	Average double product	SD	Median double product	1 st IQR	3 rd IQR
Protocol I (n=7)	Baseline	9,407	1,219	9,720	8,540	10,400
(treadmill without HG)	Δ 1 st sprint	9,046	2,305	8,920	8,155	10,405
	$\Delta 2^{nd}$ sprint	9,440	1,400	9,682	8,420	10,040
	Δ 3 rd sprint	10,121	1,826	9,660	8,900	10,705
	$\Delta 4^{th}$ sprint	10,472	2,539	9,604	8,495	11,865
Protocol II (n=7) (treadmill with HG 30% of HGS)	Baseline	8,990	2,159	8,470	6,527	10,830
	Δ 1 st sprint	9,664	1,257	9,770	9,628	10,192
	$\Delta 2^{nd}$ sprint	11,054	1,973	11,290	9,738	12,595
	Δ 3 rd sprint	12,217	1,933	13,073	10,780	13,110
	$\Delta 4^{th}$ sprint	11,988	1,459	12,410	11,385	12,984
Protocol III (n=7) (treadmill with HG 60% of HGS)	Baseline	8,704	986	8,690	7,700	9,240
	$\Delta 1^{st}$ sprint	9,643	2,486	8,990	7,650	11,215
	$\Delta 2^{nd}$ sprint	13,044	2,370	13,180	12,015	14,426
	Δ 3 rd sprint	12,244	2,211	13,010	11,346	13,585
	$\Delta 4^{th}$ sprint	13,369	3,089	11,961	11,795	14,950

 Δ = double product of sprint – baseline double product. DP, double product; HG, handgrip; HGS, handgrip strength; SD, standard deviation; IQR, interquartile range.

Table 5 Description of DBP at baseline and variations (Δ) during sprints of each protocol

Protocols	Moment	Average DBP (mmHg)	SD	Median DBP (mmHg)	1 st IQR	3 rd IQR
Protocol I (n=7) (treadmill without HG)	Baseline	81	8.1	80	71	90
	Δ 1 st sprint	-11	11.9	-10	-17	0
	$\Delta 2^{nd}$ sprint	-12	17.5	-10	-11	-1
	Δ 3 rd sprint	-16	16.2	-10	-17	-10
	$\Delta 4^{th}$ sprint	-12	13.2	-10	-11	-6
Protocol II (n=7) (treadmill with HG 30% of HGS)	Baseline	78	9.5	80	70	90
	Δ 1 st sprint	-1	17.2	10	-15	10
	$\Delta 2^{nd}$ sprint	-6	20.5	0	-17	6
	Δ 3 rd sprint	-1	19.9	0	-15	10
	$\Delta 4^{th}$ sprint	0	19.6	0	-17	11
Protocol III (n=7) (treadmill with HG 60% of HGS)	Baseline	80	5.9	80	78	82
	Δ 1 st sprint	-2	13.9	0	-7	7
	$\Delta 2^{nd}$ sprint	1	9.6	3	-6	10
	Δ 3 rd sprint	2	14.2	3	-6	14
	$\Delta 4^{th}$ sprint	6	13.0	11	-4	16

 Δ = sprint DBP – baseline DBP. DBP, diastolic blood pressure; HG, handgrip; HGS, handgrip strength; SD, standard deviation; IQR, interquartile range.

in all protocols from the 1st to the 4th sprint, especially in protocols II and III, in which HG was used, with a more substantial increase in protocol III.

In *Table 5*, the behavior of the variations of DBP was observed, described as mean and median in the four sprints in each protocol. It is noteworthy that both the mean and the median of Δ present negative values in protocol I, which reflects a reduction in DBP during exercise without HG, which practically assume null values (close to zero) in protocol II (HG with 30% of HGS) and finally assume positive values in protocol III (HG with 60% of HGS).

Discussion

The main findings of this pilot study suggest that isometric contraction with HG during interval training promotes an increase in the elevation of values (above what isolated interval training promotes) of SBP, DBP and DP, without exerting any action on HR. The time of exposure to interval training increases HR and DP independently of the isometric contraction with HG. Furthermore, the data also allows us to hypothesize the existence of a joint effect of isometric contraction with HG and the time of exposure to interval training, an effect that leads to an increase in DP.

Taking a more detailed approach to the results, a pseudocontradiction can be observed. The data suggest that HG does not have an additive effect on HR elevation. Table 2 demonstrates that the increase in HR deltas occurs over time of exposure to interval training, regardless of the increase in isometric contraction with HG. It would be expected that the imposition of an "extra" load (isometric contraction of upper limbs) would result in an increase in HR (11), however, this was not identified. Such an observation may suggest a physiological pseudocontradiction. However, this result can be explained by the Anrep effect, which in healthy hearts acts primarily by increasing myocardial ventricular contractility in the face of increased afterload, independent of the Franklin-Starling law (12). This effect triggers an improvement in ventricular ejection and a consequent increase in cardiac output, regardless of the need to increase HR (12).

Another result that deserves to be highlighted is that the use of HG during interval training abrogates the drop in DBP. During isolated interval training (protocol I) a drop in DBP is observed, while protocols with HG present similar DBP values (protocol II) and even higher than those

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at rest (protocol III), as seen in *Table 5*. Physiologically, interval training stimulates the production of nitric oxide, which favors arterial vasodilation and culminates in the maintenance or reduction of DBP (13). Especially during low and moderate intensity cyclical exercises, such as the one applied in this work, this is the most expected result (drop or maintenance of DBP). This was clearly observed in *Table 5*, in which the DBP delta values were negative in Protocol I. However, in Protocols II and III in which HG was used, the DBP deltas were close to zero (Protocol II) or positive (Protocol III). This result suggests that the isometric contraction of the upper limbs motivated by the use of HG leverages mechanical vasoconstriction in such a way that it reverses the expected drop in DBP.

Making a brief clinical application of this effect, it can be hypothesized that the use of HG during interval training results in an increase in myocardial perfusion, since the DBP value is one of the main physiological variables (not the only one) that determine cardiac perfusion (14). In a simplistic way, if we were to consider only DBP, its reduction culminates in a decrease and its increase in increase in myocardial perfusion (14). This physiological hypothesis suggested here may ultimately lead to improved cardiac function in patients with coronary artery disease (CAD) undergoing interval training. If the use of HG during interval training really optimizes coronary perfusion without impacting significant" increases in cardiac work (increase in DP), it would be plausible to infer that fewer ischemic events or consequences derived from ischemia would be observed in these patients during this type of exercise.

Finally, the increase in DP with the use of HG is expected, but the reason for this increase was not obvious. From what has been presented here, the increase in DP with the use of HG is due exclusively to the increase in SBP, that is, without the influence of HR (Tables 2,3). However, although it seems contradictory, the DP also increased due to the influence of HR, but not due to the effect of HG. In this case, the time of exposure to exercise causes an increase in HR from the first to the fourth sprints (Table 2), and this results in an increase in DP due to the factor time of exposure to exercise, as observed in Table 4. The as mentioned above, when observing that in Table 3 no increase in SBP was identified over time (SBP increased only with the use of HG). In short, this implies the hypothesis that the DP increases both due to the increase in SBP resulting from the use of HG, and due to the increase in HR due to the time factor of exposure to exercise.

Concluding this more detailed analysis of the results, it is realistic to say that, although promising and consistent, the data presented here should be viewed with caution. That's why, these are findings derived from a pilot study with a small number of volunteers. It is possible, therefore, that when the work is carried out with a number of volunteers that reaches sample sufficiency, the data assumption may differ from what was presented in this study.

Conclusions

Isometric contraction with HG during interval training directly modulates hemodynamic variables, promoting an increase in the increase in SBP, attenuation of the drop in DBP and an increase in the DP. The combined intervention does not promote an increase in HR when compared to isolated interval training.

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Footnote

Reporting Checklist: The authors have completed the CONSORT reporting checklist. Available at https://atm. amegroups.com/article/view/10.21037/atm-24-59/rc

Trial Protocol: Available at https://atm.amegroups.com/ article/view/10.21037/atm-24-59/tp

Data Sharing Statement: Available at https://atm.amegroups. com/article/view/10.21037/atm-24-59/dss

Peer Review File: Available at https://atm.amegroups.com/ article/view/10.21037/atm-24-59/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm. amegroups.com/article/view/10.21037/atm-24-59/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was

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conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics and Research Committee of Bahia Adventist College, with CAAE: 44262121.2.0000.0042 and informed consent was obtained from all individual participants.

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