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Maximal Oxygen Uptake Is Underestimated during Incremental Testing in Hypertensive Older Adults: Findings from the HAEL Study

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ABSTRACT

Purpose: The present cross-sectional study aimed to investigate whether a maximal oxygen uptake ($\text{VO}_{2\text{max}}$) verification phase (VER) could improve the accuracy of a previous graded exercise test (GXT) to assess individual $\text{VO}_{2\text{max}}$ in hypertensive individuals. **Methods:** Thirty-three older adults with hypertension (24 women) taking part in the Hypertension Approaches in the Elderly Study (NCT03264443) were recruited. Briefly, after performing a treadmill GXT to exhaustion, participants rested for 10 min and underwent a multistage VER to confirm GXT results. Individual $\text{VO}_{2\text{max}}$, respiratory exchange ratio (RER), maximal heart rate (HR_{max}), and rating of perceived exertion (RPE) were measured during both GXT and VER tests. Mean values were compared between bouts using paired sample t-tests and $\text{VO}_{2\text{max}}$ was also compared between GXT and VER on an individual basis. **Results:** Testing was well tolerated by all participants. Both absolute ($p=0.011$) and relative ($p=0.014$) $\text{VO}_{2\text{max}}$ values were higher in VER than in GXT. RER ($p<0.001$) and RPE ($p=0.002$) were lower in VER, whereas HR_{max} ($p=0.286$) was not different between the two trials. Individual $\text{VO}_{2\text{max}}$ comparisons revealed that 54.6% of the participants (18/33) achieved a $\text{VO}_{2\text{max}}$ value that was $\geq 3\%$ during VER (mean: 13.5%, range: from +3% to +22.1%, $\text{ES}=0.062$), whereas 87.9% (29/33) of the tests would have been validated as a maximal effort if the classic criteria were employed (i.e. VO_2 plateau or at least two secondary criteria). **Conclusion:** In sedentary older individuals with hypertension, GXT to exhaustion underestimated $\text{VO}_{2\text{max}}$ in more than half of tested participants, even when established but criticized criteria were used to confirm whether a maximal effort was attained. Employing VER after GXT is a quick approach to assist with the verification of an individual's $\text{VO}_{2\text{max}}$.

1 **Maximal Oxygen Uptake Is Underestimated during Incremental Testing in Hypertensive**
2 **Older Adults: Findings from the HAEL Study**

3

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29

30 **Running head:** VO_{2max} verification in hypertensive patients

31

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43 Medicine.

45 **ABSTRACT**

46 **Purpose:** The present cross-sectional study aimed to investigate whether a maximal oxygen uptake
47 ($\text{VO}_{2\text{max}}$) verification phase (VER) could improve the accuracy of a previous graded exercise test
48 (GXT) to assess individual $\text{VO}_{2\text{max}}$ in hypertensive individuals. **Methods:** Thirty-three older adults
49 with hypertension (24 women) taking part in the Hypertension Approaches in the Elderly Study
50 (NCT03264443) were recruited. Briefly, after performing a treadmill GXT to exhaustion,
51 participants rested for 10 min and underwent a multistage VER to confirm GXT results. Individual
52 $\text{VO}_{2\text{max}}$, respiratory exchange ratio (RER), maximal heart rate (HR_{max}), and rating of perceived
53 exertion (RPE) were measured during both GXT and VER tests. Mean values were compared
54 between bouts using paired sample t-tests and $\text{VO}_{2\text{max}}$ was also compared between GXT and VER
55 on an individual basis. **Results:** Testing was well tolerated by all participants. Both absolute
56 ($p=0.011$) and relative ($p=0.014$) $\text{VO}_{2\text{max}}$ values were higher in VER than in GXT. RER ($p<0.001$)
57 and RPE ($p=0.002$) were lower in VER, whereas HR_{max} ($p=0.286$) was not different between the
58 two trials. Individual $\text{VO}_{2\text{max}}$ comparisons revealed that 54.6% of the participants (18/33) achieved
59 a $\text{VO}_{2\text{max}}$ value that was $\geq 3\%$ during VER (mean: 13.5%, range: from +3% to +22.1%, $\text{ES}=0.062$),
60 whereas 87.9% (29/33) of the tests would have been validated as a maximal effort if the classic
61 criteria were employed (i.e. VO_2 plateau or at least two secondary criteria). **Conclusion:** In
62 sedentary older individuals with hypertension, GXT to exhaustion underestimated $\text{VO}_{2\text{max}}$ in more
63 than half of tested participants, even when established but criticized criteria were used to confirm
64 whether a maximal effort was attained. Employing VER after GXT is a quick approach to assist
65 with the verification of an individual's $\text{VO}_{2\text{max}}$. **Keywords:** Exercise testing; Verification phase;
66 Oxygen consumption; Hypertension; Blood pressure

67 **INTRODUCTION**

68 Maximal oxygen uptake ($\dot{V}O_{2\max}$) represents the maximum rate at which the body can transport
69 and utilize oxygen for cellular work (1). This concept was initially introduced by Hill and Lupton
70 (2), and since then $\dot{V}O_{2\max}$ has become one of the most comprehensive measures of
71 cardiorespiratory capacity (3), being considered an important health marker (4) and an independent
72 predictor of all-cause mortality and cardiovascular events (4, 5). The assessment of $\dot{V}O_{2\max}$,
73 however, has been a matter of debate and several criteria have been proposed to confirm whether
74 the oxygen uptake ($\dot{V}O_2$) obtained at the end of a maximal incremental effort is representative of
75 the individual's maximum capacity to consume oxygen. Among them, the most known and
76 possibly most used criterion is the presence of a $\dot{V}O_2$ plateau (6). Although considered the gold
77 standard for $\dot{V}O_{2\max}$ determination, a plateau in $\dot{V}O_2$ is not always observed, ranging from 0% to
78 100%, and is influenced by other factors such as the exercise mode and test protocol employed (3,
79 7), as well as data analysis and interpretation methods (1). Consequently, reliance solely on the
80 plateau criterion has been criticized (3, 8–11).

81

82 In the absence of a plateau, secondary criteria have been used as an indication that a maximal effort
83 was attained during graded exercise testing (GXT) (12). These include attaining age-estimated
84 maximal heart rate (HR), respiratory exchange ratio (RER) > 1.1 or 1.15, and Borg's rating of
85 perceived exertion (RPE) values (8, 9). Although previous researchers have stated that the presence
86 of at least two of these criteria is a strong indicator that $\dot{V}O_{2\max}$ was attained (8, 12), others have
87 criticized its capacity to discriminate a "true" $\dot{V}O_{2\max}$ (1, 10, 13), with some authors even
88 suggesting a complete rejection of secondary criteria (14, 15). As a consequence, alternative
89 strategies have been proposed, such as the so-called verification phase (VER) (9, 10), which is

90 characterized as an additional effort performed above critical power, or ideally above the work
91 rate that yielded $\dot{V}O_{2\max}$, after GXT itself (16). Lack of a difference between the $\dot{V}O_2$ at the end of
92 GXT and VER is perhaps an ideal method for determining true $\dot{V}O_{2\max}$ was reached during GXT
93 (14–17).

94

95 In clinical settings, GXT has been typically confirmed using traditional criteria (i.e. $\dot{V}O_2$ plateau
96 and secondary criteria) in a symptom-limited manner. However, this practice has also been
97 recently criticized (17) and, based on the low incidence of plateaus and the possibility that
98 secondary criteria underestimate $\dot{V}O_{2\max}$ it makes the search for alternatives necessary and
99 relevant. Within this context, VER has been previously tested and found valid for use in physically
100 active elderly men and women (18), but its applicability and benefit for clinical populations
101 deserve further investigation.

102

103 Systemic arterial hypertension is a worldwide highly prevalent condition, with global estimates
104 suggesting about 31% of the adult population is hypertensive (19). High cardiorespiratory fitness
105 is an important factor associated with a lower risk of death from cardiovascular diseases, including
106 hypertension (5). As such, adequate $\dot{V}O_{2\max}$ assessment is essential to both clinical and laboratory
107 practice to support proper diagnosis and prediction of cardiovascular risk, develop individual
108 recommendations about physical activity and exercise, as well as to track and follow-up training-
109 related outcomes (20–22). To date, however, it is unknown whether VER is both feasible and
110 provides additional information to confirm $\dot{V}O_{2\max}$ in clinical populations, such as hypertensive
111 individuals. Considering the prevalence of hypertension in older adults, which may exceed 70%
112 in those aged 60 and over (23), hypertensive patients were used in the present investigation as a

113 model for individuals and/or conditions that are typically associated to lower exercise tolerance or
114 fitness levels as compared to healthy or physically active older adults, that presents. Therefore, the
115 aim of this study was to investigate whether VER was able to confirm the $\dot{V}O_{2max}$ results of a GXT
116 in older adults with hypertension. Furthermore, we compared VER with other criteria previously
117 employed to validate a maximum GXT (e.g. $\dot{V}O_2$ plateau, cutoff point in RER, estimated
118 maximum HR and near-maximum RPE). Our hypothesis was that VER would improve the
119 determination of $\dot{V}O_{2max}$ in the studied population compared to other criteria.

120

121 **METHODS**

122 This investigation is a cross-sectional parallel study based on the *Hypertension Approaches in the*
123 *Elderly: a Lifestyle Study* (HAEL) trial, a multicenter randomized clinical trial that compared the
124 effects of pragmatic combined exercise training vs. a health education program on ambulatory
125 blood pressure and cardiorespiratory fitness in older adults with hypertension (ClinicalTrials.gov
126 NCT03264443). The current manuscript focuses on the Pelotas center participants' maximal GXT
127 and VER tests and $\dot{V}O_2$ data at baseline, which were carried out from February 2018 to June 2019
128 and includes all but the first recruitment wave of the study. For further details of the Hael Study
129 please refer to the protocol manuscript (24).

130

131 **Participants**

132 Out of the forty-two individuals included in the Hael Study during the recruitment period of the
133 current investigation, two did not perform GXT, six refused to participate in the VER study, and
134 data from one participant could not be retrieved due to equipment malfunctioning during VER.
135 Thus, thirty-three participants (9 men and 24 women) ≥ 60 years old previously diagnosed with

136 hypertension agreed to participate and were included in the present analysis (Table 1). Briefly,
137 exclusion criteria followed those of the HAEL Study and included a history of myocardial
138 infarction, myocardial revascularization, deep vein thrombosis, stroke or pulmonary embolism in
139 the last 12 months; severe cardiovascular disease (e.g. class III or IV chronic heart failure or
140 unstable arrhythmia); chronic lung disease requiring O₂ or corticosteroids; kidney disease
141 requiring dialysis; neurological disorders; cancer requiring treatment in the last two years or
142 medical contraindication to exercising. Prior to any procedure, all participants read and signed an
143 informed consent form and the research project was previously approved by the Federal University
144 of Pelotas Institutional Review Board (CAAE: 62427616.0.2001.5313) in accordance with the
145 Declaration of Helsinki.

146

147 ****Insert Table 1 near here****

148

149 **Experimental procedures**

150 The present study was conducted during the baseline maximal GXT session of the HAEL Study
151 (24). Specifically, participants performed a GXT to determine their $\dot{V}O_{2max}$ followed by VER
152 (explained in detail below) after a brief recovery period. For safety reasons, all tests were
153 supervised by a physician and participants were familiarized with the equipment and safety
154 procedures prior to the experimental session.

155

156 **Protocols**

157 *Maximal incremental test.* Participants attended the laboratory and, after a 5-min resting interval,
158 baseline resting blood pressure measurement was taken and the participants were presented to the

159 physician along with a brief case summary of the patient. After clearance, participants were
160 submitted to a maximal GXT to exhaustion on a motorized treadmill. Participants warmed-up at 3
161 $\text{km}\cdot\text{h}^{-1}$ for 3 min, and the test started with $0.5 \text{ km}\cdot\text{h}^{-1}$ and 1% grade increments per minute until
162 volitional failure or at the physician's request. For safety, after every two minutes, and at least 30
163 seconds after the workload was increased, measurements of blood pressure and RPE (Borg 6-20)
164 were obtained, and the participant's HR was recorded. All participants were instructed to have
165 their last meal at least 3 hours before the test, and to abstain from intense physical exercises and
166 ingestion of caffeine and alcohol 24 hours before the experimental session.

167
168 *Verification Phase.* After a 10-min passive recovery interval, each participant underwent a VER
169 to determine whether the $\dot{V}\text{O}_{2\text{max}}$ value obtained during GXT was a true maximum. Specifically,
170 a new multistage effort bout was performed, which consisted of 2 min at 50% of the maximal
171 speed and grade reached during GXT, followed by 1 min at 70% of the maximal speed and grade
172 at GXT, and then participants exercised to volitional failure at one stage above the last completed
173 stage in their individual GXT (16, 25). We chose a multistage VER rather than a single square-
174 wave protocol to minimize the chance of patients reaching fatigue before $\dot{V}\text{O}_{2\text{max}}$ was attained due
175 to the possibility of slow $\dot{V}\text{O}_2$ kinetics (26).

176
177 During both GXT and VER, gas exchange data were obtained using a portable metabolic system
178 (VO2000, MedGraphics®, Ann Arbor, MI, USA), which was calibrated prior to each session
179 according to manufacturer's specifications and recalibrated between GXT and VER to minimize
180 drift. Participant's HR was measured telemetrically using a HR monitor (Polar FT1, Polar Electro,
181 Finland), and the exercise test would be terminated if adverse symptoms were observed, such as

182 blood pressure above 250/115 mm Hg, a decline in systolic blood pressure with increasing work
183 rate or at the physician's discretion (20), although no test had to be interrupted because of these
184 reasons.

185

186 **Data analysis**

187 $\dot{V}O_2$ values were time-averaged every 20 s and the average from the last two 20-s intervals in both
188 GXT and VER were considered as phase-specific $\dot{V}O_{2\max}$ (13, 27). The $\dot{V}O_{2\max}$ during GXT was
189 confirmed when the difference between VER and GXT $\dot{V}O_{2\max}$ was less than 3% (25, 28). In
190 addition, to determine the number of valid tests, GXT results were also confirmed based on the
191 presence of a $\dot{V}O_2$ plateau ($\dot{V}O_2$ change ≤ 150 ml·min⁻¹ with an increase in workload); or when at
192 least two secondary criteria were met (i.e. ± 10 bpm of age-predicted HR (≥ 220 -age), RER > 1.10
193 or RPE ≥ 18) (6, 20). To gain insight into GXT capacity to confirm $\dot{V}O_{2\max}$ in relation to VER, a
194 novel individualized plateau criterion proposed by Midgley et al. (25) was also used in which a
195 least squares linear regression line was fitted to the linear portion of GXT $\dot{V}O_2$ -workrate data
196 excluding the final 2 min. The regression line was then extrapolated to the end of the test and a
197 plateau was confirmed when a difference between GXT modeled and measured $\dot{V}O_{2\max}$ was
198 greater than 50% of the regression slope for the linear portion (25).

199

200 **Statistical Analysis**

201 Data are reported as mean \pm standard deviation (SD) or confidence intervals (CI) unless stated
202 otherwise. Initially, comparisons between the GXT and VER mean outcomes were performed
203 using paired sample t-tests. Considering that the comparison between mean values may conceal
204 between-trials variability, comparisons between GXT and VER $\dot{V}O_{2\max}$ results were also

205 conducted individually based on the $\dot{V}O_{2max}$ difference observed between the exercise bouts.
206 Accordingly, GXT was found to be valid when VER $\dot{V}O_{2max}$ was less than 3% higher than observed
207 during GXT (25, 28), following previous recommendations (16, 17). Finally, the agreement
208 between GXT and VER $\dot{V}O_{2max}$ measures was assessed using Bland-Altman plots and reliability
209 was measured using the coefficient of variation. The level of significance was set at $\alpha = 0.05$ and
210 all analyses were performed using SPSS software (v. 25.0., IBM, USA).

211

212 **RESULTS**

213 *Mean comparisons*

214 Testing was well tolerated by all participants, and no adverse event was observed. Mean data from
215 both GXT and VER are presented in Table 2. Both absolute and relative $\dot{V}O_{2max}$ values were higher
216 in VER than in GXT ($p = 0.011$ and 0.014 , respectively). RER and RPE were lower in VER ($p <$
217 0.001 and $p = 0.002$, respectively), whereas HR_{max} was not different between the two trials ($p =$
218 0.286). Coefficient of variation for $\dot{V}O_{2max}$ was 8.1% (CI95%: 6.1 to 10.1%) between GXT and
219 VER. Bland-Altman plots are presented in Figure 1. Accordingly, no agreement was observed for
220 both absolute ($p = 0.011$) and relative ($p = 0.014$) $\dot{V}O_{2max}$ results between trials, and there was no
221 evidence of proportional bias ($p = 0.300$, $p = 0.189$, respectively).

222

223 ****Insert Table 2 near here****

224

225 ****Insert Figure 1 near here****

226

227

228 *Individual comparisons*

229 $\dot{V}O_{2max}$ comparison between GXT and VER based on individual tests revealed that 54.6% of the
230 participants (18/33) achieved a $\dot{V}O_{2max}$ value that was $\geq 3\%$ higher during VER (range: from 3%
231 to 22.1%). A total of 24.2% (8/33) had VER $\dot{V}O_{2max}$ within $\pm 2.9\%$ of their GXT result (range:
232 from -2.9 to 1.5%), whereas 21.2% (7/33) displayed VER results that were $\geq 3\%$ lower than GXT
233 (range: from -5.1 to -29.4%). A waterfall plot of the individual differences between VER and
234 GXT is shown in Figure 2.

235

236 ****Insert Figure 2 near here****

237

238 *Classic criteria*

239 When GXTs were analyzed based on the classic established criteria (i.e. $\dot{V}O_2$ plateau and/or
240 secondary criteria), results diverged from those observed in the individual comparisons between
241 VER and GXT (Table 3). Specifically, if only one criterion was used to validate GXT, 97% (32/33)
242 of the participants would have had their tests confirmed as valid. If at least two criteria were used,
243 as proposed by Howley et al. (12), 87.9% (29/33) of the present sample would have had their GXT
244 validated. When only those tests that were deemed to be underestimated during GXT were
245 analyzed separately, 94.4% (17/18) of them would have been confirmed as valid based on the
246 occurrence of a $\dot{V}O_2$ plateau or the presence of at least two secondary criteria.

247

248 ****Insert Table 3 near here****

249

250 As an additional analysis, GXTs that would have been validated by classic criteria were further
251 separated according to the specific criteria that were reached and then compared to VER results
252 (see Table, Supplemental Digital Content, Comparison between GXT and VER $\dot{V}O_{2\max}$ results
253 stratified according to the primary and secondary criteria achieved during GXT). The analyses
254 revealed that both absolute and relative $\dot{V}O_{2\max}$ were lower in GXT for those who achieved (i) a
255 plateau ($\leq 150 \text{ ml}\cdot\text{min}^{-1}$; $p < 0.001$); (ii) HR_{\max} and RER ($p \leq 0.022$); (iii) HR_{\max} and RPE ($p \leq$
256 0.049); (iv) as well as RER and RPE ($p \leq 0.004$) criteria. No difference was observed between
257 GXT and VER $\dot{V}O_{2\max}$ for those who presented a plateau as proposed by Midgley et al. ($p = 0.063$)
258 or who reached all three secondary criteria ($p = 0.075$ and 0.059). In addition, RER and RPE were
259 lower during VER ($p \leq 0.034$) and no difference was found in HR_{\max} between VER and GXT ($p >$
260 0.05).

261

262 **DISCUSSION**

263 The main finding of the present study was that in hypertensive older individuals GXT
264 underestimated $\dot{V}O_{2\max}$ in over half of the participants, as demonstrated by VER, even though close
265 to ninety percent of the tests would have been validated as a maximal effort if the classic criteria
266 were employed. Moreover, VER testing appeared to be safe for the study population with no
267 adverse event registered. Hence, our data demonstrate that VER can be a practical and sensitive
268 method to verify GXT $\dot{V}O_{2\max}$ and can be performed during the same session as GXT in
269 hypertensive individuals.

270

271 This is the first study to our knowledge that investigated VER in older adults with hypertension.
272 Most of the previous studies were performed on healthy young men and women and showed that

273 VER is indeed able to confirm whether $\dot{V}O_{2max}$ was attained (25, 28–32). Our results, however,
274 suggest otherwise in the present population, in disagreement with previous data on physically
275 active middle-aged and older adults (18). Using a constant-load VER at 105% of the peak power
276 output, Dalleck et al. (18) found no difference between GXT and VER mean $\dot{V}O_{2max}$ results,
277 whereas only 11% of their older participants reached $\dot{V}O_2$ values during VER that were greater
278 than GXT by more than 3%. This contrasts with our current findings, with 55% of participants
279 displaying greater $\dot{V}O_2$ during VER compared to GXT. In fact, when only the underestimated tests
280 were considered, $\dot{V}O_{2max}$ was found to be, on average, 13.5% ($\sim 3.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) higher in VER
281 (Cohen's $d = 0.62$). Such marked differences between studies may be related to several factors,
282 including participant's motivation to achieve maximal effort and the protocols used during the
283 VER phase.

284

285 An important aspect that might have influenced the aforementioned results is the difference in
286 fitness between our participants and that of Dalleck et al.'s. The latter was composed mainly of
287 older individuals who were actively engaged in an exercise program. Supporting this notion, it was
288 previously demonstrated that while no differences were observed between GXT and VER among
289 young individuals with moderate to high fitness levels ($\dot{V}O_{2max} > \text{than } 40 \text{ and } 50 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$,
290 respectively), those considered as low fitness ($\dot{V}O_{2max} > 30 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) achieved higher $\dot{V}O_{2max}$
291 during VER (33). This is also in line with studies on cancer (34) and chronic heart failure patients
292 (35), which are conditions typically related to lower exercise tolerance and fitness level. Indeed,
293 most of the literature investigating VER ability to confirm $\dot{V}O_{2max}$ on sedentary individuals was
294 performed on inactive but otherwise healthy men and women, with relatively low cardiovascular
295 risk (1, 36, 37). Thus, those with chronic conditions, such as hypertension, have been overlooked.

296

297 Because mean value comparisons might mask a considerable between-trials variability in some
298 participants, previous authors recommended that comparisons between GXT and VER $\dot{V}O_{2max}$
299 should be performed on an individual case-by-case basis (15, 16, 38–40). Our results demonstrated
300 not only a substantial $\dot{V}O_{2max}$ underestimation when individual data were compared, but also an
301 underestimation when mean values were used for analysis, which might relate to the multistage
302 VER protocol approach employed in this study.

303

304 Square-wave tests are the most common VER protocols employed in previous investigations (16).
305 In such studies, the entire VER is performed at a constant load that typically can be sustained for
306 ~2 min (e.g., 18, 30, 33, 35, 37, 41). When a multistage protocol is performed, however,
307 participants are allowed a certain time for $\dot{V}O_2$ to adjust before the final workload is delivered. In
308 the case of the present study, this approach resulted in VER duration of about 5 min on average.
309 Even though the duration of exercise at supra GXT intensity might be similar or slightly lower
310 than those of square-wave protocols, it is possible that the first submaximal stages allowed
311 participants to reach the final stage at a higher $\dot{V}O_2$ and, in turn, less time was possibly required at
312 this supramaximal intensity to reach or surpass the $\dot{V}O_2$ observed during GXT.

313

314 It has been reported that a previous heavy intensity exercise bout may speed $\dot{V}O_2$ kinetics while
315 attenuating the $\dot{V}CO_2$ response during a subsequent bout of heavy or maximal exercise, leading to
316 reduced RER values, as observed in VER compared to GXT in the present study (42, 43).
317 Considering that less fit individuals normally possess a lower anaerobic capacity and slower $\dot{V}O_2$
318 kinetics, multistage approaches may also reduce the anaerobic burden and assist these participants

319 to sustain efforts for longer (1, 26). Although not yet tested, this hypothesis is supported by the
320 lower RPE and RER reported after VER compared to GXT.

321
322 In accordance with our initial hypothesis, 85% of the participants would have had their maximal
323 tests considered valid based on the achievement of at least two of the secondary criteria
324 investigated (Table 3), even though VER demonstrated a considerable $\dot{V}O_{2\max}$ underestimation
325 during GXT. As such, the use of these secondary criteria has been questioned (14, 17, 25, 44), with
326 some authors reporting that the same secondary criteria employed in the current study (e.g. HR_{\max}
327 and RER) may be reached as early as 80% $\dot{V}O_{2\max}$. These criteria are also affected by the rate at
328 which GXT workload is increased. For example, final RER has been reported to be higher when
329 the workload is increased at a faster rate (35). Accordingly, these criteria have been strongly
330 criticized for their validity and sensitivity to verify $\dot{V}O_{2\max}$ (14, 15). This is further reinforced by
331 the differences between GXT and VER that were maintained even when data were stratified
332 between primary and secondary criteria (see Table, Supplemental Digital Content, Comparison
333 between GXT and VER $\dot{V}O_{2\max}$ results stratified according to the primary and secondary criteria
334 achieved during GXT).

335
336 This additional exploratory analysis indicated that $\dot{V}O_{2\max}$ was not different when GXT and VER
337 were compared only in those who achieved either a $\dot{V}O_2$ plateau as proposed by Midgley or all
338 three secondary criteria (see Table, Supplemental Digital Content, Comparison between GXT and
339 VER $\dot{V}O_{2\max}$ results stratified according to the primary and secondary criteria achieved during
340 GXT). Such results would indicate that a plateau determined based on the individual $\dot{V}O_2$ rate of
341 increase, rather than a fixed or average increase (e.g. $< 150 \text{ ml}\cdot\text{min}^{-1}$), would likely be more suitable

342 to validate and confirm $\dot{V}O_{2\max}$ attainment (25), whereas participants reaching both HR_{\max} , RPE
343 and RER criteria would have provided a greater effort during the incremental test compared to
344 those who have reached only two. In this regard, however, it is important to note that likely due to
345 their more stringent nature these criteria were the ones fulfilled by the least number of participants
346 ($n = 11$ and 12 , respectively). It is possible, therefore, that these exploratory analyses lacked the
347 power to detect such differences. Irrespective of this, the aforementioned results suggest that, if a
348 criterion is to be found to validate GXT on its own, an individualized approach is more likely to
349 succeed (25).

350

351 **LIMITATIONS**

352 The present study is not exempt from limitations. First, the 3% cutoff threshold adopted here to
353 validate a difference between GXT and VER was not based on data from our laboratory and,
354 therefore, it is possible that the GXT test-retest reliability in our laboratory might be slightly
355 different than the one used. Nevertheless, this value has been suggested previously (28) and
356 employed with some degree of success by others in young (11, 30, 32, 45), middle-aged, and older
357 adults (18). In fact, even if a 9% cutoff value was adopted, as previously reported in cystic fibrosis
358 patients, more than 40% of the current tests would still result in higher $\dot{V}O_{2\max}$ during VER. In
359 addition, it should be noted that the lack of a non-hypertensive control group is a limitation to
360 make inferences about the influence of hypertension *per se* on our findings and additional research
361 is necessary to elucidate this. Finally, the absence of a sample size estimation may also be
362 suggested as a limitation, however, our study seemed enough powered (all ≥ 0.82) to detect
363 differences in all outcomes that were expected to differ between GXT and VER), as shown in
364 Table 2.

365

366 **CONCLUSION**

367 In conclusion, our results demonstrate the relevance that verifying the $\dot{V}O_{2\max}$ obtained during
368 GXT with a relatively simple strategy can have in clinical populations such as the one studied in
369 the present investigation. The use of VER might be an alternative approach to consider, as it is not
370 very time-consuming and enables direct $\dot{V}O_2$ comparisons between test phases. Thus, even though
371 it is not possible to assume that VER represents the participant's "true" $\dot{V}O_{2\max}$ when it is higher
372 than GXT, by implementing a VER researchers are provided with the highest $\dot{V}O_2$ from the two
373 phases. This would not be possible through other criteria based solely on a single incremental test
374 (e.g., HR_{\max} or RER). This is especially important when the performance of another GXT is not
375 feasible because of logistics, such as during large clinical trials.

376

377

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387

388 **Data availability**

389 Data from the present study will be made available upon request to the corresponding author.

390

391 **Conflicts of Interest**

392 All authors declare that they have no competing financial interests related to this publication. All authors
393 declare that the results of the study are presented clearly, honestly, and without fabrication, falsification,
394 or inappropriate data manipulation. The results of the present study do not constitute an endorsement by
395 the American College of Sports Medicine.

396

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508

509 **Figure legends**

510

511 **Figure 1.** Bland-Altman plots for the absolute (A) and relative (B) maximal oxygen uptake
512 ($\dot{V}O_{2max}$) responses between the graded exercise test and verification phase. The solid line
513 represents the mean bias and dashed lines the limits of agreement (mean $\pm 1.96*SD$).

514

515 **Figure 2.** Mean (bars) and individual (lines) maximal oxygen uptake ($\dot{V}O_{2max}$) responses between
516 the graded exercise test (GXT) and the verification phase (VER) (A). Waterfall plot (B) of the
517 individual differences in $\dot{V}O_{2max}$ ($\Delta\dot{V}O_{2max}$) between VER and GXT. In (B), values within the
518 dotted lines (gray bars) represent participants with a lower than 3% difference between VER and
519 GXT $\dot{V}O_{2max}$ results, whereas those above (black bars) and below (white bars) correspond to
520 differences greater and lower than 3%, respectively (as proposed by Midgley et al. 2009). * =
521 significantly different from GXT ($p = 0.014$).

522

523

524 **Supplemental Digital Content**

525 **Supplementary Table 1.** Comparison between GXT and VER $\dot{V}O_{2max}$ results stratified according to the
526 primary and secondary criteria achieved during GXT

Table 1. Participants' characteristics (n = 33).

	Mean \pm SD
Age (years)	67.1 \pm 5.1
Sex	24 female / 9 male
Race	24 white / 9 black
Height (m)	1.55 \pm 0.09
Body mass (kg)	77.3 \pm 13.8
Waist circumference (cm)	103.2 \pm 10.6
SBP (mmHg)	128 \pm 10.8
DBP (mmHg)	72 \pm 7.4
Antihypertensive medications*	
Diuretics (n)	19
ARB (n)	23
CCB (n)	5
Beta-blockers (n)	11
ACE inhibitors (n)	8

SBP 24-h ambulatory systolic blood pressure; *DBP* 24-h ambulatory diastolic blood pressure. *ARB* angiotensin receptor blockers; *CCB* calcium channel blockers *ACE* angiotensinogen converting enzyme.

*Number of medications per drug class is presented as frequency.

Table 2. GXT and VER outcomes in older adults with hypertension (mean \pm SD).

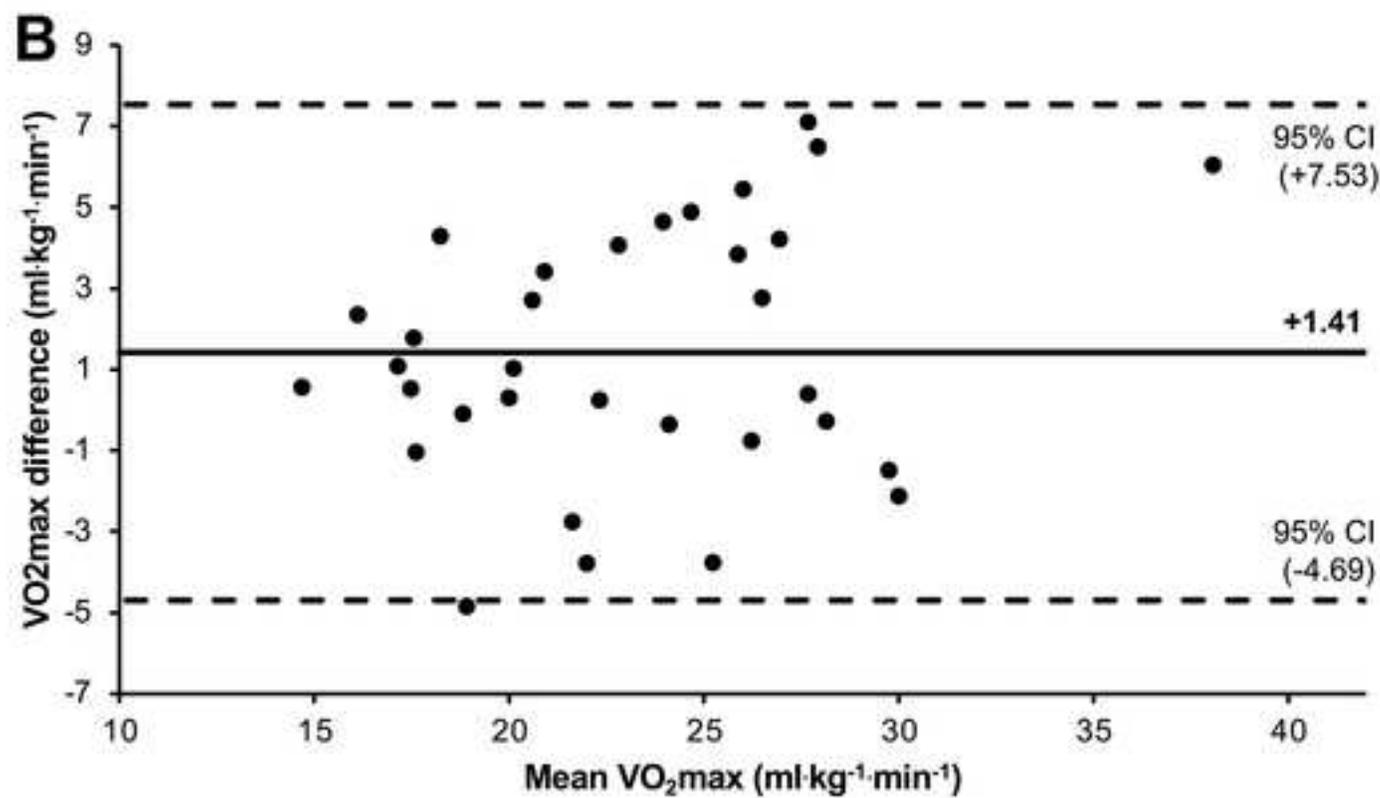
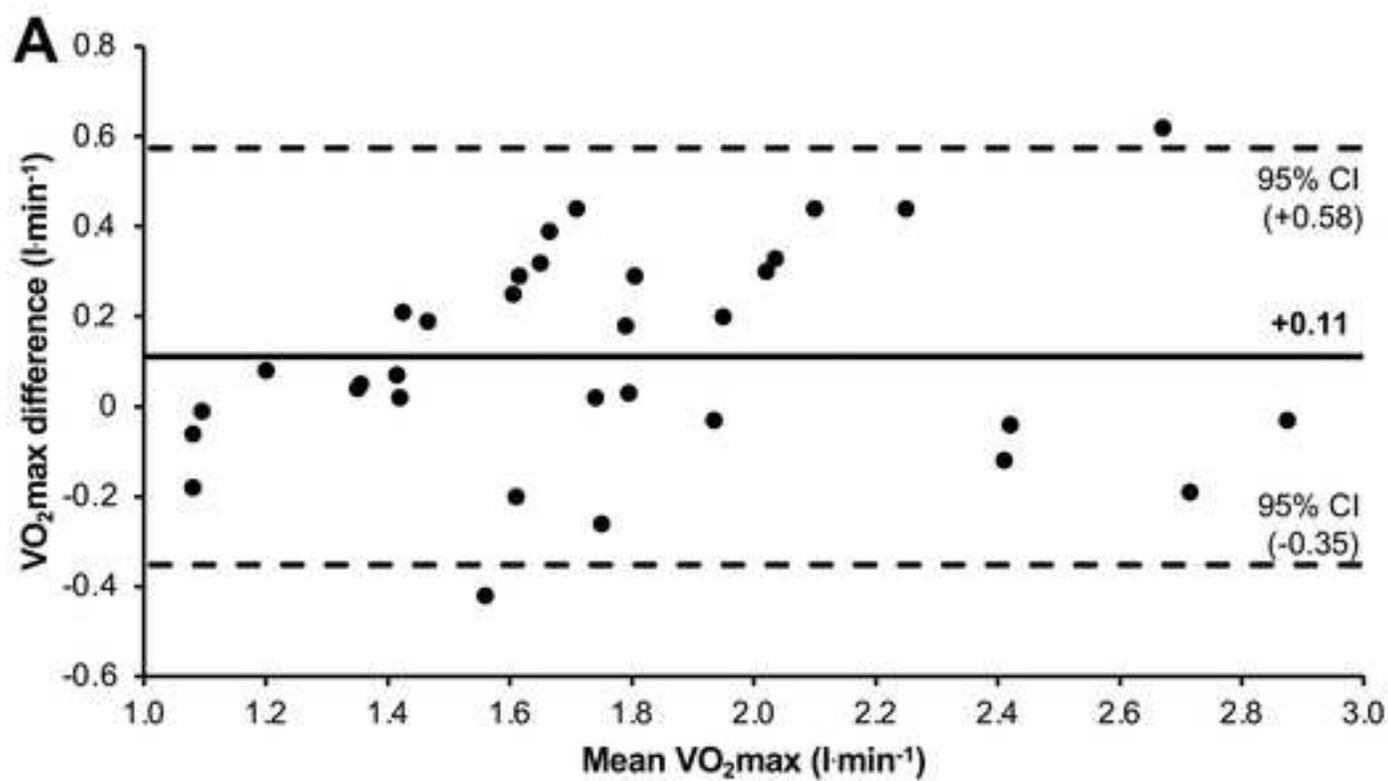
	Incremental phase	Verification phase	p
$\dot{V}O_{2max}$ (l·min ⁻¹)	1.72 \pm 0.46	1.83 \pm 0.50	0.011
$\dot{V}O_{2max}$ (ml·kg ⁻¹ ·min ⁻¹)	22.5 \pm 4.9	23.9 \pm 5.6	0.014
HR _{max} (beats·min ⁻¹)	150.4 \pm 16.4	151.8 \pm 15.7	0.286
RER	1.15 \pm 0.18	0.97 \pm 0.08	<0.001
RPE	17.7 \pm 1.9	16.7 \pm 2.3	0.002
Duration (s)	714.8 \pm 126.8	286.5 \pm 26.5	<0.001

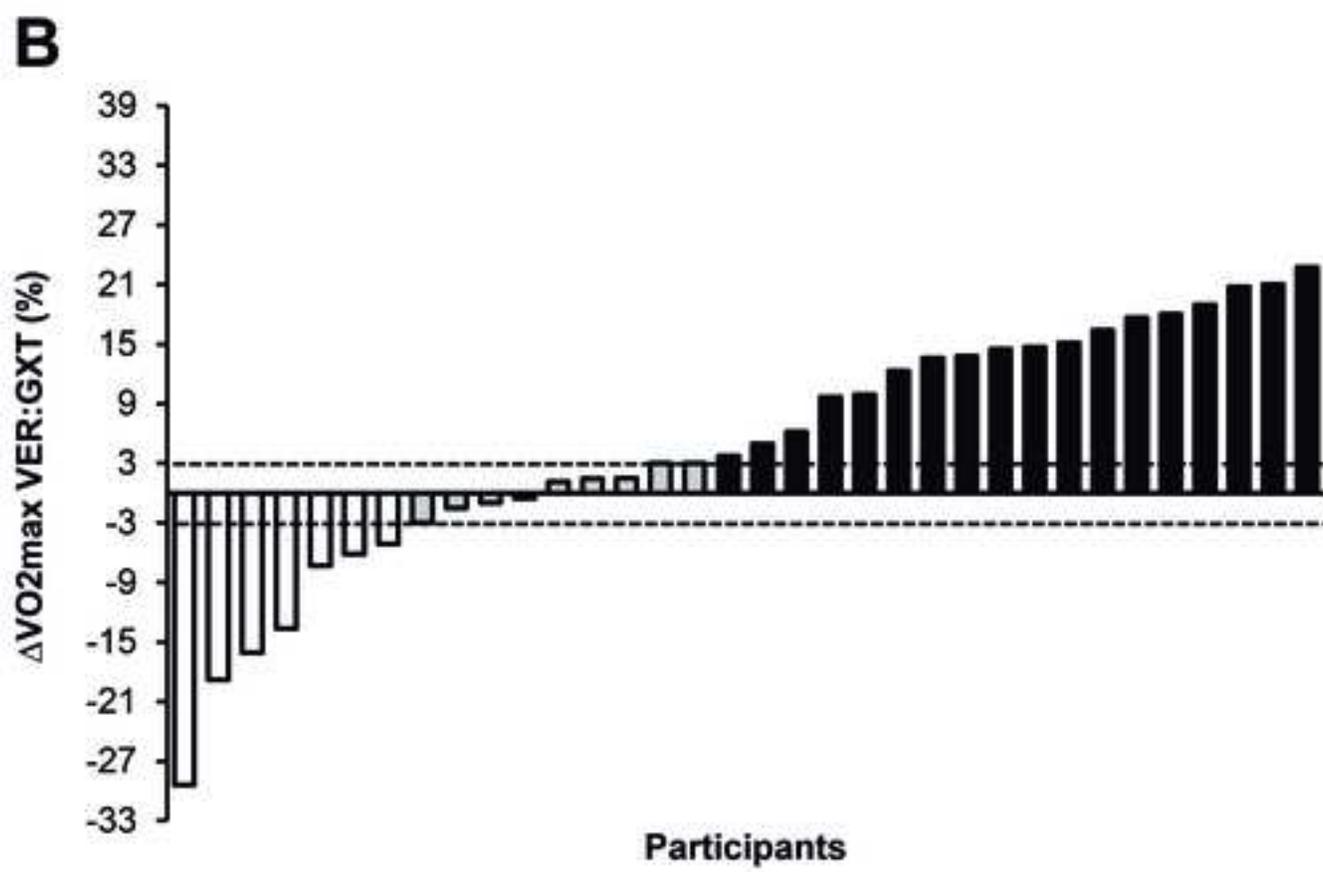
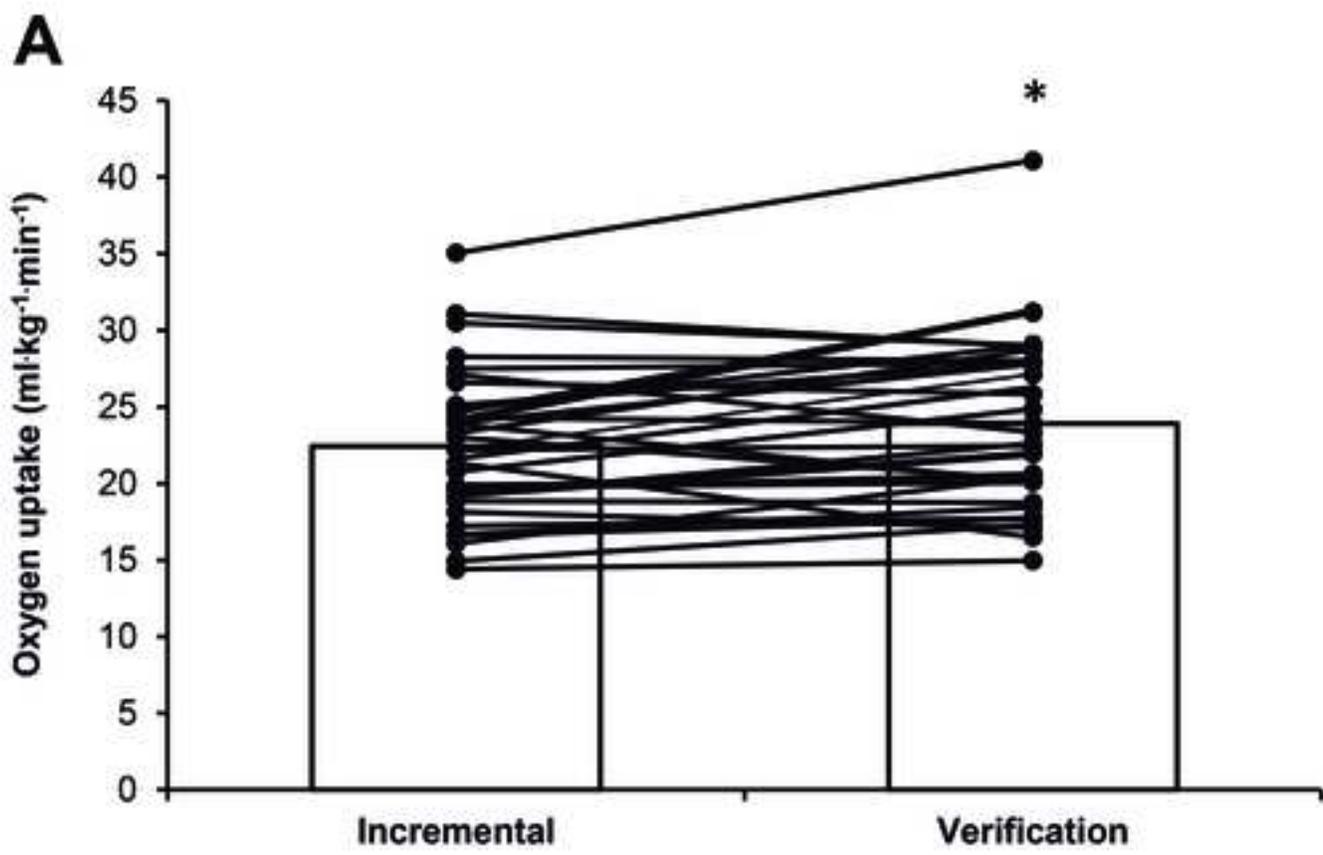
$\dot{V}O_{2max}$ maximal oxygen uptake; HR_{max} maximal heart rate; *RER* respiratory exchange ratio; *RPE* rating of perceived exertion.

Table 3. Frequency of primary and secondary criteria attainment during the incremental test and number of tests deemed valid (n = 33).

	Frequency (%)	
	No	Yes
$\dot{V}O_2$ plateau (Midgley et al.)	23 (69.7)	10 (30.3)
$\dot{V}O_2$ plateau (Taylor et al.)	18 (54.5)	15 (45.5)
HR_{max}	9 (27.3)	24 (72.7)
RER	12 (36.4)	21 (63.6)
RPE	9 (27.3)	24 (72.7)
<i>Validated tests*</i>		
One criterion	1 (2.9)	32 (96.7)
Two criteria	5 (15.1)	28 (84.9)

$\dot{V}O_{2max}$ maximal oxygen uptake; HR_{max} maximal heart rate; *RER* respiratory exchange ratio; RPE rating of perceived exertion. *results are similar using both plateau criterion.







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Supplemental Data File (.doc, .tif, pdf, etc.)

9. Supplemental table 1_MSSE_final.docx

