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BRIEF REVIEW

Threats to Internal Validity in Exercise Science: A Review of Overlooked Confounding Variables

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Internal validity refers to the degree of control exerted over potential confounding variables to reduce alternative explanations for the effects of various treatments. In exercise and sports-science research and routine testing, internal validity is commonly achieved by controlling variables such as exercise and warm-up protocols, prior training, nutritional intake before testing, ambient temperature, time of testing, hours of sleep, age, and gender. However, a number of other potential confounding variables often do not receive adequate attention in sports physiology and performance research. These confounding variables include instructions on how to perform the test, volume and frequency of verbal encouragement, knowledge of exercise endpoint, number and gender of observers in the room, influence of music played before and during testing, and the effects of mental fatigue on performance. In this review the authors discuss these variables in relation to common testing environments in exercise and sports science and present some recommendations with the goal of reducing possible threats to internal validity.

Keywords*:* experimental design, reliability, scientific process

Internal validity refers to the degree to which observed changes in a dependent variable can be ascribed to changes in the independent variables facilitating trustworthy interpretations about causal relationships.[1–3] By controlling for potentially confounding variables, the risk of an alternative (erroneous) explanation accounting for the treatment effects is reduced.[1–3] Typically, exercise and sports scientists control for threats to internal validity by accounting for technical and biological factors that could potentially influence the results of experiments. Establishing test–retest reliability and careful calibration of equipment are examples of technical factors that are normally controlled by exercise scientists. Furthermore, controlling for age,[4] gender,[5] caffeine and caloric intake,[6,7] time of day,[8] and ambient temperature[9] are examples of common ways to account for biological factors that can affect physical performance. However, a number of other confounding variables have the potential to substantially affect physiological and performance outcomes. For example, while instructing subjects on how to perform a physical test is mandatory, directing their attentional focus to 1 aspect of the test at the expense of another can substantially alter results.[10] Most exercise tests have a known endpoint in regard to their distance, time, or number of repetitions to be performed. Informing subjects about the exercise endpoint either before or during the test can substantially affect performance.[11,12] Providing verbal encouragement is very common in exercise and sports studies; however, rarely is the frequency or type of encouragement controlled despite the likelihood that it might affect the athlete's responses.[13,14] In particular, the volume and pitch of the voice during verbal encouragement appear to affect performance.[15] The number and gender of observers in the room could influence subjects' physiological and psychological responses.[16,17] Mental fatigue during extended submaximal or extended exercise is another consideration.[18,19] Thus, testing subjects after writing an examination or listing to a long and complex lecture could potentially add a confounding variable to the results. Finally, playing background music, either before or during the tests, can skew the results of the study.[20] This is especially the case if fast-tempo music is played, leading to improved performance compared with slower-tempo music.[21] In this review we briefly describe and discuss confounding variables in exercise testing and provide recommendations for reducing the risk of compromising internal validity.

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Attentional Focus

Instructing subjects to focus on 1 aspect of a physical test more than another can lead to meaningful enhancement or deficit in the outcome measures. In particular, guiding subjects to employ an external focus of attention, where an individual focuses on the effects of the movement in relation to the environment, has been repeatedly shown to enhance performance.[10] In contrast, employing an internal focus-of-attention strategy, with a focus on a specific body part, can impair performance.[10] For example, recreationally trained subjects produced 7% greater net joint torque with their elbow flexors when asked to focus on the crank bar of the dynamometer (external focus) rather than their arm muscles (internal focus).[22] The external focus was associated with lower elbow-flexor electromyography (EMG) activity pointing to enhanced movement efficiency. Muscle-endurance performance was also enhanced when employing external attentional focus. Subjects completed more repetitions with 75% of 1-repetition maximum (1RM) in a bench press (11 vs 10 repetitions) and squat (11 vs 10 repetitions) when instructed to focus on moving and exerting force against the barbell (external focus) versus focusing on moving and exerting force with the arms or legs (internal focus).[23] Comparable results were observed when 40 recreationally trained subjects were able to hold a wall-sit task to exhaustion ~8 seconds longer when employing an external focus.[24] In another study university students were able to jump ~1.5 cm higher when asked to focus on the rungs of the vertical-jump measurement device (external focus) than when focusing on the fingers with which the rungs were to be touched (internal focus).[25] Similarly, untrained subjects jumped ~13 cm farther when employing external focus of attention compared with internal focus.[26] It appears that increasing the distance of the external focus of attention relative to the body when performing a long jump tends to increase the covered distance.[27] Moreover, agility performance tested with an "L" run task was 6% faster with external than with internal focus among recreationally trained subjects.[28]

The benefits of external focus are not just limited to strength and power measurements. Balance,[29] accuracy,[30] and sport-specific[31] performance can be improved with external focus of attention. For instance, balance is improved when subjects are asked to minimize movements of the platform or unstable discs (external focus) compared with movement of their feet (internal focus).[29,32] This topic has been previously reviewed in detail by Wulf et al.[10] Collectively, it is clear that directing an athlete's attention externally or internally can substantially skew the results. Specifically, external focus of attention most likely enhances physical performance, whereas heightened internal focus of attention might be a hindrance. Controlling the instructions in exercise testing is important, as alternating between the 2 types of attentional focus might compromise the internal validity of a study or routine testing session.

Knowledge of Exercise Endpoint

Providing athletes with an exercise endpoint in terms of distance, time, or number of repetitions has been shown to affect performance.[11] For example, aerobically trained males completed a 6-km self-selected running trial ~6% faster when receiving accurate feedback of each covered kilometer than when receiving no feedback.[33] Similarly, peak power was reduced by 2% to 8% during a 40-km cycling time trial when elite cyclists were deprived of feedback about the covered distance.[34] Furthermore, Mauger et al[35] had trained cyclists perform 4 consecutive 4-km time trials without knowing the exact distance they cycled. In contrast to the control group, which received distance feedback, the experimental group was considerably slower in the first time trial (–10%), but with each successive trial the difference in time to completion decreased between the groups. However, in a replication of the study with untrained subjects, knowledge of exercise endpoint was not advantageous, suggesting that only trained subjects benefit from exercise-endpoint anchors.[36] The importance of exercise endpoints in relation to performance is also evident by the studies that have examined intermittent activities. Other than the outcome measures, which differed between the studies (elbow-flexor maximum voluntary contractions [MVCs] in Halperin et al[12,37] and lower-body cycling sprints in Billaut et al[38]), all 3 employed a similar design in which before the initiation of the exercise subjects were provided with accurate information about number of repetitions to be performed, no knowledge of exercise endpoint, or inaccurate information about number of repetitions to be performed, in which subjects were told they would perform relatively few repetitions, but once that number was reached they were asked to perform additional work. Despite the fact that subjects completed the same number of repetitions under the 3 conditions and were asked to exert maximal efforts, they tended to underperform when not provided with an endpoint and to overperform when assuming they would complete fewer repetitions. Other studies have reported lower rates of perceived exertion (RPE),[39] heart rate,[33,40] and oxygen consumption[33] ($\dot{V}O_2$) when subjects were required to run or cycle at a given intensity without receiving any distance or time feedback. It appears that the lower physiological demands may be evidence of a subconscious attempt to preserve energy when the exercise endpoint is unknown or unclear (see reviews of Williams et al[11] and Jones et al[41]).

Encouragement and Feedback

Providing verbal encouragement during physical testing is a very common procedure in exercise and sports science. However, rarely is the frequency or the pitch of verbal comments controlled despite evidence of substantial effects on exercise performance. Numerous studies report that providing verbal encouragement can variously enhance maximal force,[42,43] rate of force development,[44,45] muscle endurance,[46] power,[47] and submaximal whole-body endurance tasks.[14] In fact, a positive relationship between the frequency of verbal encouragement and performance has been reported.[14] After an initial $\dot{V}O_{2max}$ test, recreationally trained subjects repeated the test while receiving verbal encouragement every 20 seconds, 60 seconds, 180 seconds, or control conditions. Compared with pretests, relative $\dot{V}O_{2max}$ (13%), time to exhaustion (8%), blood lactate concentration (10%), and RPE (5%) were all substantially higher after frequent encouragement given every 20 seconds. After the 60-second condition, $\dot{V}O_{2max}$, blood lactate, and RPE increased by 9%, 15%, and 5%, respectively. Where encouragement was given every 3 minutes only RPE was higher (5%). A loud auditory stimuli before performing a concentric bench press enhanced rate of force development by 10% to 15%, as well as velocity of the bar by 3% to 4%, compared with silence and a softer auditory stimuli.[45] Similarly, a loud noise before a handgrip MVC enhanced peak (7%) and rate of force development (18%).[44] Finally, greater levels of isometric force were associated with louder than with lower recordings of verbal encouragement (~9% differences).[15] Coaches, trainers, and sports scientists should be alert to the likely effects of verbal encouragement on exercise performance.

However, not all studies report enhanced performance with verbal encouragement.[48,49] For example, peak torque of the knee extensors and flexors remained unaffected with verbal encouragement compared with control conditions.[48,49] Different outcomes between studies are likely explained by observations that certain personality types are affected to a greater extent by verbal encouragement.[50,51] For example, only conscientious subjects enhanced force production with their plantar flexors while receiving encouragement, whereas the nonconscientious subjects did not.[50] Similarly, encouragement was associated with longer time-to-exhaustion cycling tasks accompanied by greater oxygen consumption in persons with type B personalities, whereas performance in subjects with type A personalities was enhanced.[51] Moreover, the type of feedback provided during or between a series of tests can affect results.[13,52] Hutchinson et al[13] provided active physical education students with either false negative or false positive feedback about their performance in a submaximal handgrip endurance test. Performance on the second test improved by ~12% after receiving false positive feedback and decreased by ~13% after receiving false negative feedback. Similarly and somewhat surprisingly, providing false positive feedback about performance enhanced running economy by ~8% to 12% among trained runners compared with no feedback.[52] Thus, controlling the frequency of encouragement during physical tests and the pitch or volume of the voice during encouragement may reduce the risk of potential confounders. It may be of value to test or assess personality types and decide on the use of verbal encouragement for specific individuals. Moreover, positive and negative feedback has meaningful and different effects on performance.

The Number and Gender of Observers

The number and gender of researchers observing exercise testing could have an important effect on performance. In relation to gender, recreationally trained males reported lower RPE values during running and cycling tasks when a female observer was in the room than with a male observer.[53,54] However, recreationally trained females' RPE remained unaffected by the gender of the observer.[53] Furthermore, elite track and field athletes of both genders reported similar RPE values during a cycling task that included light-, medium-, and high-intensity phases regardless if the observer was a male or female.[55] It appears that the effects of gender of observer on RPE might depend on training background of the subjects. In a different study, university students of both genders performed an MVC test with the plantar flexors in front of an opposite- and then a same-sex observer. The male subjects produced greater levels of force when females were observing them.[56] The presence of a female could also elicit different hormonal responses in males.[57] Five minutes of friendly conversation with a female increased testosterone concentration in males in contrast to a similar conversation with males.[57] Male skateboarders demonstrated riskier skateboarding tasks when an attractive female was filming them than when the observer was male. This effect was also accompanied by an increase in concentration of circulating testosterone.[16] Similarly, physical testing of females could be affected by male presence. For example, females reported substantially greater levels of social physique anxiety in response to an all-male and mixed-sex exercise-settings scenario compared with all-female.[58] The females indicated they would shorten their workout in response to all-male compared with mixed-sex and all-female scenarios.[58] In contrast, these settings did not affect males. Such situational gender-related anxiety could affect certain female subjects and should be considered in laboratory- and field-based exercise testing.

In addition to gender, the number of observers could influence the physical outcome. Recreationally trained subjects completed a 1RM bench-press test on 3 occasions: without direct observers, with 15 observers, and without direct observers but in a competitive setting in which the subjects who could lift the most weight relative to body weight won a prize.[59] Subjects lifted greater loads (105 kg) under the 15-observers condition than in either the competitive (103 kg) or without-observers (93 kg) condition. Recreationally trained male and female subjects performed 1RM bench- and leg-press tests under 2 conditions: no observers (included 1 male supervisor) and with observers (included the male supervisor and 2 opposite-sex observers). Both males and females were able to lift heavier loads in the bench press (~3 kg) and leg press (~7 kg) under the observers condition.[17] Thus, the results of the study point to a possible dose-response relationship between number of observers and load lifted. The topic of social facilitation and its effects on various motor tasks has been reviewed in detail.[60]

Mental Fatigue

Mentally fatigued subjects tend to underperform in physical tasks.[18,61,62] Considering that many of the subjects in exercise-related research studies are university or college students, some attention to their mental state before physical testing may be warranted. Even highly trained athletes must attend to employment and education activities, and they might also suffer decrements when training in a state of mental fatigue. A mentally fatiguing task comprising counting backward from 1000 to 0 in 7s while balancing a bubble on a miniature spirit level between the center lines led to decrements in the number of performed push-ups (14%) and sit-ups (25%) in trained athletes.[62] Moreover, recreationally trained subjects cycled for 15% less time during a time-to-exhaustion test after performing an AX Continuous Performance Test (AX-CPT) for 90 minutes.[18] The AX-CPT includes sequences of letters presented on a computer screen to which subjects are instructed to respond by clicking specific keyboard letters. Self-paced activities were affected by mental fatigue. Self-selected power outputs after the AX-CPT were 8% to 13% slower during 2 self-paced cycling trials lasting 10 minutes in recreationally trained subjects.[61] Compared with control conditions, performing a Stroop test for 30 minutes led to a slower 5-km running time trial (23.1 vs 24.4 min) using recreationally trained subjects.[19] During the Stroop test subjects are presented with a list of words representing different colors (eg, "green") in which the ink color and printed name of the color are mismatched. Subjects are then asked to recognize the color of the ink and ignore the printed word representing the color. Notably, even after a very short performance of the Stroop test (3 min and 40 s), untrained subjects performed ~6% less work during a 10-minute cycling task.[63] Furthermore, mentally fatiguing tasks hindered skilled-based task performance.[64] Five minutes of performing a Stroop test decreased accuracy of a dart-throwing task.[64] Finally, it seems that mentally fatiguing tasks affect endurance-based activities more than tasks involving maximal force. Pageaux et al[65] had subjects perform an AX-CPT for 90 minutes before testing for their MVC and muscle activation, as well as a submaximal endurance test with the knee extensors. Whereas the MVC and muscle activation remained unaffected, endurance time was shortened by 13% in recreationally trained subjects. Finally, force production and muscle activation of the knee extensor remained unaffected by a previous Stroop test task lasting 27 minutes.[66] The interested reader is referred to the Hagger et al[67] review paper related to this topic.

Ensuring that subjects are not physically tested after a long day consisting of mentally fatiguing tasks may strengthen the internal validity of a study or routine testing of athletes in a sporting program. This is especially so when the physical tests consist of endurance-based activities, particularly those employing larger muscles, such as running and cycling. In contrast, if the tests comprise MVCs then it seems as if mental fatigue is not as impactful.

Music

Listening to music before or during a physical test can lead to (positive) effects on performance.[20] Due to their nature, some physical tests are conducted in strength and conditioning gyms or testing laboratories that often have background music that might confound the results of the tests. Listening to music can improve muscle strength and endurance performance. Karageorghis et al[68] reported a 4% enhancement of grip force after subjects listened to stimulating music compared with sedative music and/or white noise in sports-science university students. Physically active subjects were able to hold a weight in front of their body at shoulder height for longer while listening to motivational music (211 s) than with rhythm (198 s) or no music (190 s).[69] Shorter anaerobic activities might also be enhanced with music. Peak and mean power outputs were 7% higher in a Wingate anaerobic test when physically active subjects listened to music, and they reported greater levels of task motivation and positive affect.[70] Likewise, completion time of 400-m running sprint improved by 0.2 to 1.2 seconds when trained subjects listened to music compared with control conditions.[71] Music is also associated with a large positive effect on longer endurance activities. A 10-km cycling time trial was covered faster (10–35 s) when trained subjects listened to music than with no-music conditions.[72] Compared with control conditions, untrained subjects covered a greater distance in a submaximal 20-minute cycling task (9.9 vs 8.9 km) and reported higher levels of enjoyments when listening to music.[73]

The tempo of the music also seems to affect both physiological and psychological variables. Waterhouse et al[21] had physically active subjects cycle at self-selected work rates for 25 minutes while listening to music played at either normal tempo, increased by 10%, or decreased by 10%. Subjects covered 6% more distance during the faster tempo than with the slower-tempo condition and reported greater enjoyment of the music. Moreover, in a study in which music was either played at the first half or second half of a 10-km cycling time trial or not played at all, recreationally active subjects cycled faster in the first half of the time trial only when expecting the music to appear in the second half.[74] The fact that subjects cycled faster when expecting music to be introduced at a later stage of the trial points to the effects music can have on self-paced exercise. Music also affected heart and lung function in physically active subjects when measured during a steady-state run under 3 conditions: fast, slow, or no music.[75] Fast music led to substantial increases in $\dot{V}O_2$ (5–8%), cardiac output (12–13%), minute ventilation (5%), and frequency of breaths (5%) compared with the slow and no music.[75] Furthermore, comparing the effects of classical music with those of no music on a 15-minute run at 70% of $\dot{V}O_{2max}$, Szmedra and Bacharach[76] reported lower RPE (10%), heart rate (5%), and blood pressure (4%) under the music conditions. For review please see Karageorghis and Priest.[20]

Recommendations

Systematically reducing the threats to internal validity in regard to the performance and physiological outcomes in athlete testing in research and sporting settings need not be complicated, time consuming, or dependent on specific technologies. Principally, employing the same specific instructions; type, content, and format of feedback; number and gender of observers; the presence or absence of music; and an acceptable mental state of the subjects across testing conditions in a *consistent* manner is very important (see Table 1). Specific details should be documented in operational manuals or protocol documents, included in staff and student training, and detailed in published work, thereby allowing others to replicate the study with similar conditions.

Table 1 Recommendations for Limiting the Effects of Potentially Confounding Variables in Exercise-Related Research Studies and Routine Athlete Testing

	Attentional focus	Knowledge of exercise endpoint	Verbal encouragement and feedback	Number and gender of observers	Mental fatigue	Music
Standardize and report	Instructions of attention focus from known script. (for practical suggestions see Makaruk and Porter[77]).	Frequency and timing of exercise endpoints feedback before and during tests.	Frequency of VE and type of feedback. If possible, control for voice volume and pitch.	Gender and number of observers.	Confirm that testing is not performed in a state of mental fatigue.	Type and volume of music, if present.
What to avoid	Exchanging between internal and external attentional-feedback instructions.	Providing irregular exercise endpoints.	Variations in frequency of VE and type of feedback.	Uneven number of observers. Different gender of observers between tests.	Testing after mentally fatiguing days for some and after "fresh" days for others.	Changing type of music between testing conditions. Radio or random playlists.
Potential effects on performance	↑ External focus of attention. ↓ Internal focus of attention.	↑ Frequent feedback about exercise endpoint. ↑ Deception. ↓ No exercise endpoint.	↑ Loud and repeated VE. ↑ Positive feedback. ↑ Competitive environment. ↓ No VE. ↓ Negative feedback.	↑ Number of observers. ↑ Opposite-sex observers for males. ↑ Same-sex observers for females. ↓ Opposite-sex observers for females.	↓ Mental fatigue.	↑ Music.
Special considerations		Untrained subjects may not be affected by knowledge of exercise endpoints.[36]	Some personality traits may be more susceptible to VE.[50,51]	If relevant, social-physique-anxiety questionnaires may be appropriate.[58]	Mental fatigue does not seem to affect MVC performance.[63,64] Mood and motivation questionnaires may confirm presence or absence of mental fatigue.[78]	

Abbreviations: VE, verbal encouragement; MVC, maximal voluntary contraction.

Conclusions

To maintain a high degree of internal validity in performance and physiological testing, special consideration is warranted to reduce the risk of confounding variables affecting outcome measures. Sports-science researchers and practitioners should typically control for age and gender of the subjects, caffeine and nutrient intake before testing, hour of the day, and ambient temperature. Moreover, the effects of attentional focus, knowledge of exercise endpoint, verbal encouragement, positive and negative feedback, number and gender of observers, music, and mental fatigue should also be considered given their meaningful effects on physical performance. Scientific manuscripts could include, where appropriate, details of specific methodological approaches employed to quantify and/or reduce the risk of confounding factors.

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