The effect of test modality on dynamic exercise biomarkers in children, adolescents, and young adults

Ronen Bar-Yoseph1, Janos Porszasz2, Shlomit Radom-Aizik1, Annamarie Stehli1, Pearl Law1 & Dan M. Cooper1,3

1 Pediatric Exercise and Genomics Research Center (PERC), Department of Pediatrics, University of California Irvine, Irvine, California
2 Rehabilitation Clinical Trials Center, Division of Respiratory and Critical Care Physiology and Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California
3 University of California Irvine Institute for Clinical and Translational Science, Irvine, California

Keywords
Cycle ergometry, peak oxygen uptake (V̇O₂), puberty, submaximal biomarkers of fitness, treadmill.

Abstract
Cardiopulmonary exercise testing (CPET) modalities, treadmill (TM), and cycle ergometer (CE), influence maximal gas exchange and heart rate (HR) responses. Little is known regarding CPET modality effect on submaximal biomarkers during childhood and adolescence. Ninety-four healthy participants (7–34 y.o., 53% female) performed TM and CE CPET to address two major gaps: (1) the effect of modality on submaximal CPET biomarkers, and (2) estimation of work rate in TM CPET. Breath-by-breath gas exchange enabled calculation of linear regression slopes such as V̇O₂/ΔHR and ΔV̇E/ΔV̇CO₂. Lean body mass (LBM) was measured with dual X-ray absorptiometry. We tested a novel TM CPET estimate of work rate based on TM velocity, incline, and body mass (VIM). Like the linear relationship between V̇O₂ and work rate in CE CPET, V̇O₂ increased linearly with TM VIM. TM ΔV̇O₂/ΔHR was highly correlated with CE (r = 0.92), and each increased substantially with LBM (P < 0.0001 for TM and CE). ΔV̇O₂/ΔHR was to a small (~8.7%) but significant extent larger in TM (1.6 mL/min/beat, P = 0.04). In contrast, TM and CE ΔV̇E/ΔV̇CO₂ decreased significantly with LBM, supporting earlier observations from CE CPET. For both CE and TM, males had significantly higher ΔV̇O₂/ΔHR but lower ΔV̇E/ΔV̇CO₂ than females. Novel TM CPET biomarkers such as ΔVIM/ΔHR and ΔV̇O₂/ΔV̇IM paralleled effects of LBM observed in CE CPET. TM and CE CPET submaximal biomarkers are not interchangeable, but similarly reflect maturation during critical periods. CPET analysis that utilizes data actually measured (rather than estimated) may improve the clinical value of TM and CE CPET.

Introduction
The goal of this study was to test hypotheses focused on the effect of the two most common exercise testing modalities, cycle ergometry (CE), and treadmill (TM), on cardiopulmonary exercise testing (CPET) results in children, adolescents, and young adults. We addressed two key challenges in comparing TM and CE CPET among children, adolescents, and young adults: (1) the difficulty in quantifying the work performed in TM CPET, and (2) useful approaches to scaling CPET results when body size and physiologic function change so dramatically over the course of growth and development (Cooper et al., 1987; Cooper et al., 2014). CPET biomarkers are used to assess disease severity, progress, and response to therapy (including exercise prescriptions) across an expanding range...
of childhood diseases and conditions and across the lifespan (Ploeger et al., 2009; Pahkala et al., 2013; Liem et al., 2015; Sule and Fontaine, 2016; Cordingley et al., 2016; Gualano et al., 2017; Li et al., 2017). Despite these factors, CPET has failed to fulfil its promise in child health research and clinical practice (Ashish et al., 2015). A major barrier to more accurate and effective clinical use of CPET in children and adults has been a lack of harmonization of protocol types and exercise modalities (Ashish et al., 2015), factors that influence CPET results (Fredriksen et al., 1998; Beltrami et al., 2012; Bires et al., 2013; May et al., 2014; Cunha et al., 2015). For example, in clinical trials involving CPET in children over the past five years, a PubMed search revealed 40 published studies that used CE and 113 that used TM.

We concentrated on dynamic submaximal physiologic output variables (Fig. 1) that are less effort-dependent than the traditional VO2 max and, arguably, more acceptable in children and adolescents, particularly those with chronic diseases or conditions (Stein et al., 2003; Cooper et al., 2014). Dynamic relationships among CPET variables (such as HR, VE, VCO2, and VO2) reveal novel insights into cardiorespiratory function in health and disease (Cooper et al., 1984; Troutman et al., 1998; Moser et al., 2000; Chen et al., 2014; Elbehairy et al., 2015; Hestnes et al., 2017), and can be obtained in both CE and TM modalities without necessarily measuring work rate. The effect of exercise modality on submaximal physiologic output variables has not been adequately studied in children and adolescents.

In CE, the work rate is usually measured directly from the known resistance on the ergometer’s flywheel. VO2 is linearly related to work rate (Whipp et al., 1981) and, as a consequence, CE protocols can be easily designed to produce a linear relationship between protocol duration (i.e., exercise time) and VO2, which simplifies the ultimate analysis of CPET data. In contrast, it is difficult to estimate the relationship between work rate and VO2 in TM because of the complexity of both the physics and human mechanical efficiency of treadmill walking and running (Workman and Armstrong, 1963; Kyröläinen et al., 1995; Porszasz et al., 2003; Keir et al., 2012; Azuma, 2014). Treadmill work is determined by kinetic energy [functions of the velocity (V) of the TM and the body mass (M) of the participant] and work against gravity imposed by the TM grade or incline (Workman and Armstrong, 1963; Ruckstuhl et al., 2010). While body weight is often used to scale CPET values in an effort to compare results among individuals of different size, recent data suggest that LBM is significantly better correlated to size-dependent CPET biomarkers (Cooper et al., 2014). Lean body mass (LBM) is a more direct measure of skeletal muscle, the predominant metabolizing tissue in exercise, than body weight.

Consequently, we measured LBM using dual X-ray absorptiometry DXA (Bridge et al., 2011), and used LBM in our comparison of the two CPET modalities.

Although the magnitude of peak or maximal VO2 is similar in CE and TM CPET, one observation made consistently in both children and adults is that peak VO2 tends to be somewhat greater in TM CPET (Turley and Wilmore, 1997). We hypothesized that CPET slope variables would reflect exercise modality differences as well. We further hypothesized that the metabolic response to TM is proportional to the kinetic energy exerted on the center of gravity of the body, therefore an estimate of TM work rate (ΔVO2/ΔWR and ΔHR/ΔWR) available only for the CE.

Methods

Participants

The study was approved by the UC Irvine Institutional Review Board. Inclusion criteria included healthy 7–35 y.o. participants (53% female) without any known
respiratory, cardiac, or metabolic disease, and not taking any chronic prescribed medication. BMI of each participant was less than the 95th percentile for children (http://www.cdc.gov/healthyweight/assessing/bmi/chi) and BMI less than 30 for adults. Each volunteer visited the laboratory on two occasions. During the first visit, informed consent was obtained (parental consent + child assent for participants <18 y/o), demographic and anthropometric data were recorded, Tanner stage (by questionnaire) was assessed, and DXA was performed. A maximal progressive exercise protocol on a CE was also done on the first visit. At least 48 h later, each participant performed TM CPET with a protocol designed to mimic the rate of power increase found in the CE CPET.

**Anthropometric measurement and body composition**

Standard calibrated scales and stadiometers were used to determine weight and height. Body composition, including LBM, fat mass, and percent body fat were determined by DXA using a Hologic QDR 4500 densitometer. Participants were scanned while lying supine and wearing light clothing. On the day of each test, the DXA instrument was calibrated using the procedures provided by the manufacturer, and DXA scans were performed and analyzed using pediatric software where appropriate.

**CE and TM protocols**

The CE protocol consisted of a ramp-type progressive cycle ergometry used previously in this and other laboratories to measure peak VO₂ in children and adults (Cooper et al., 2016). After a 2-min period of unloaded cycling (0 W), power output was increased by 8–30 W/min. The increase in the ramp W/min was individualized for each participant empirically from the following formula:

\[
\text{work rate increment} = \frac{\text{(bodyweight + 3) / 10}}{\text{body weight in kg. This formula was derived empirically from}}
\]

where work rate increment is in watts per minute and body weight in kg. This formula was derived empirically from the thousands of tests we have performed and is fairly reliable in producing CPET duration of 8–15 min, an interval previously determined to optimize evaluation of breath-by-breath data (Buchfuhrer et al., 1983; Myers and Froelicher, 1993). Participants cycled at a constant pedaling rate between 60 and 70 revolutions per minute (rpm) throughout the test on an electronically braked, servo-controlled cycle ergometer. The increasing work rate was discontinued when the participants indicated that they had reached the limit of their tolerance and/or a drop occurred in pedaling rate below 60 rpm despite strong verbal encouragement. At this point, the work rate was lowered to 0 W and the participants continued to pedal for at least 5 more minutes while lowering the pedaling rate to below 40 rpm in order to prevent an excessively sudden drop in blood pressure (Kenney and Seals, 1993).

Since our goal was to compare the two modalities using tests in which VO₂ increased linearly over a duration of about 8–15 min, we used the results of the CE CPET (in which work rate was precisely known) to guide the velocity and incline configuration for the TM CPET performed on a separate data. Our coauthor (Dr. Porszasz) and coworkers (Porszasz et al., 2003) previously developed a TM CPET protocol that linearized the VO₂ increase over a 10–15 min exercise duration. They found that a protocol combining an initial slow walking speed that progressively increased in concert with a dynamically changing incline met the demands of an initially low exercise metabolic rate and optimum test duration.

In designing the protocol, we first determined the desired work rate for each of the 1-min steps assuming a linear increase both in work rate and speed. The speed-range we used was between 0.5 and 10.5 miles per hour (0.8 km/h and 16.8 km/h, respectively); baseline speed was set to 0.5 mph and every minute was increased by 0.5 mph up to 10.5 mph max. Each step had the same work rate and speed increments; having formulated these, the inclination was determined by the following formula:

\[
\text{TM inclination angle} = \text{arctan} \left( \frac{\text{work rate}}{(\text{mass} \times g \times v^2)} \right)
\]

where inclination angle is in radians; work rate in watts, mass in kg, g as 9.81m/sec², and v in m/sec. The inclination as percentage was then determined and set for each step in the protocol. This resulted in a decreasing incline profile (set individually, with 30% as the highest incline in the cohort), which approached 1% toward the end of the test. The general formula [Equation (2)] was used to calculate the desired change in TM incline to produce a given work rate at a particular velocity and work rate (Porszasz et al., 2003). We assumed that the work rate in TM CPET would be related to the kinetic energy equation, work = \( \frac{1}{2} m v^2 \) and the following equation was used to estimate TM work rate:

\[
\text{WR(TM)} = k \cdot \text{mass} \cdot v^2 \cdot (I + 1)
\]

where mass is body weight (kg), \( v \) is treadmill speed (m/sec), \( I \) is incline (%), and \( k \) is a conversion factor constant. We arbitrarily used the expression \( I + 1 \) because at 0% incline, work rate would not be possible to calculate due to multiplication by zero. For convenience, we refer to \( k \text{mass} v^2 (I + 1) \) as VIM. Using these equations and protocol, we achieved a largely linear relationship between...
VO₂ and exercise work intensity up to a reasonable level and the test duration was between 8 and 15 min.

Gas exchange measurement
Gas exchange was measured breath-by-breath using the SensorMedics metabolic system (Vmax Encore 229, Yorba Linda, CA). The breath-by-breath gas exchange data were interpolated to 1-sec and 10-sec bin averages were formed and used for all later analyses. Physiologically abnormal data for HR and gas exchange (e.g., HR < 50 beat/min or >230 beat/min, or VO₂ = 0 L/min or >5 L/min) and outliers, based on each subject, are occasionally observed in breath-by-breath CPET data obtained in children. These data were identified and excluded for slope or peak calculation.

Calculation of submaximal CPET slopes and peak values
Submaximal slopes (ΔVO₂/ΔHR, ΔVE/ΔVCO₂, etc.) were calculated using standard linear regression as described previously (Cooper et al., 2014) omitting the first minute and the last 30 sec of the exercise. The peak values were taken as the highest values in 20-sec bin averages over the last 2 min of exercise. There is currently no validated, universally accepted approach for the determination of peak VO₂ in children. We used a criterion implemented in a large study by Rowland et al. (2008) defined by inability to maintain the pedaling cadence in association with subjective evidence of fatigue (sweating, hyperpnea) and HR >185 bpm (children) or >170 bpm (adults) and/or respiratory exchange ratio (RER, VCO₂/VO₂) >1.00 (children) or >1.10 (adults).

Comparing CE and TM work rate input
We assumed that the linear relationship between work rate and VO₂ (Whipp et al., 1981) in both exercise modalities have the same slope and intercept, we used the linear regression parameters for the measured VO₂ on the CE (in which work rate was known) to determine the work rate on the TM (in which the velocity, incline, and participant’s weight were known) for each participant. The equivalent work rate on TM exercise was marked as WR’.

\[
\dot{V}O_2(CE) = a \cdot WR(CE) + b
\]

\[
WR' = \left[\dot{V}O_2(TM) - b\right]/a
\]

We used standard linear regression techniques to estimate the parameters ‘a’ and ‘b’ for each participant and calculated the WR’ for TM CPET.

Comparison of fitness variables obtained from CE CPET and TM CPET
We compared the CPET variables described above obtained from the two modalities. To eliminate any confounding effect introduced by the estimation of WR’ (due to its dependence on the VO₂-WR relationship derived from CPET-CE), we also compared CPET values using VIM itself. In addition, we tested the degree to which CE and TM CPET variables scaled to body mass and composition, factors essential for understanding CPET in the growing child. For example, while the numerical value of ΔWR/ΔHR and ΔVIM/ΔHR will be quite different, we expected that their relationship and correlation to key variables such as age, body weight, lean body mass (LBM), and sex would be quite similar when comparing the two modalities.

Statistical analysis
For each peak VO₂, ΔVO₂/ΔHR slope, ΔVE/ΔVCO₂ slope, and other slopes, statistical comparisons of CE versus TM were performed using mixed models (via SAS PROC MIXED) to account for subject level intercorrelation between the paired modality measurements (TM and CE). Each model also included puberty group (children tanner 1–2, adolescents 4-5, adults >18 years), sex, puberty × sex, puberty group × modality interaction, sex × modality interaction, and puberty group × sex × modality interaction. Post hoc comparisons of model-generated, least-square (LS) means were evaluated where significant fixed effects were found. This was done according to the hierarchy principle such that if an interaction was present, only the appropriate conditional means were compared and interpreted. Significance for the post hoc comparisons was determined by Tukey-adjusted P-values of the LS mean differences. We performed a standard Bland-Altman (BA) analysis to compare to peak VO₂ and submaximal slopes between the two modalities.

Results
Participants characteristics
Representative examples of CE and TM CPET are shown in Figure 2. A total of 111 healthy children and young adults (7–34 y.o.) participated in this study. We excluded 17 of them from the final analysis: five due to technical problems, three due to incompletion of the study protocol, two due to a submaximal effort on the TM, six due to inability to assess the Tanner score, and one due to exercise-induced bronchoconstriction following CE ramp test. Ninety-four participants were
included in the analysis, and demographic and anthropometric data are presented in Table 1. For analysis of peak VO\(_2\), data from 88 participants were analyzed (six participants did not meet the criteria for a maximal test as noted above). Submaximal slopes, and peak CPET values are shown in Tables 2, 3, 4. Detailed summary of statistical analyses are shown in Tables 5 and 6.

**Linearity of VO\(_2\) with work rate estimate and exercise duration in the two modalities**

Corroborating the previous work by Porszasz et al., (2003, we achieved success in linearizing the relationship between VO\(_2\) and exercise duration (time). This was evidenced both by visual inspection of the exercise tests (e.g., Fig. 2) as well as by the remarkably high correlation between VO\(_2\) and time for the TM CPET (mean \(r = 0.978\)). We compared the correlation coefficients of two linear regressions: \(\dot{V}O_2\) versus \(v \times (I + 1) \times \text{mass}\) and \(\dot{V}O_2 \times (I + 1) \times \text{mass}\). For the former, the average \(R^2\) was 0.7889 and for the latter \(R^2\) was 0.9255. A paired \(t\)-test for the mean difference was statistically significant \([\Delta \dot{V}O_2; R^2 = 0.1367, P < 0.00001]\), suggesting stronger prediction of VO\(_2\) by \(\dot{V}^2I\text{M}\). The duration of exercise for TM and CE modality in each group of participants is shown in Table 4. For the adolescents and young adults, TM duration was significantly longer than CE. In general, exercise duration for both modalities was longer in males than in females.

**Submaximal CPET variables**

\(\Delta\dot{V}O_2/\DeltaHR\)

\(\Delta\dot{V}O_2/\DeltaHR\) results are shown in Figure 3 and Table 2. The CE and TM values were highly correlated \((P < 0.0001, \text{Fig. 3A})\). A small but significantly higher mean \(\Delta\dot{V}O_2/\DeltaHR\) difference \((1.7 \pm 0.81 \text{ mL/beat, about } 10\%)\) was found in TM versus CE. For the group as a whole, BA analysis revealed higher \(\Delta\dot{V}O_2/\DeltaHR\) for TM CPET (bias of 1.74, 95% CI of 1.06 to 2.42). Statistically significant maturation-dependent differences were observed in both males and females (Table 6). Consistent with our previous study of cycle ergometer exercise in children and adolescents (Cooper et al., 2014), \(\Delta\dot{V}O_2/\DeltaHR\) increased with LBM \((r = 0.88, P < 0.0001)\). For both CE and TM (Fig. 3B), peak VO\(_2\) was highly correlated to \(\Delta\dot{V}O_2/\DeltaHR\). We used the linear regression equations relating \(\Delta\dot{V}O_2/\DeltaHR\) and LBM to calculate a predicted value for each participant, then compared the percent predicted from the CE and TM CPET to determine how interchangeable the two modalities were. A moderate correlation was found (correlation coefficient \(r = 0.66, P < 0.0001, \text{Fig. 3C})\).
The Effect of Test Modality on Dynamic Exercise Biomarkers
R. Bar-Yoseph et al.

Δ\(\text{VE}/\Delta\text{VCO}_2\)

Δ\(\text{VE}/\Delta\text{VCO}_2\) results are shown in Figure 4 and Table 2. The CE and TM values were significantly correlated (\(P < 0.0001\), Fig. 4A). A small but significantly higher mean Δ\(\text{VE}/\Delta\text{VCO}_2\) difference (1.151 ± 0.527, about 3.7%) was found in CE vs. TM. For the group as a whole, BA analysis revealed lower Δ\(\text{VE}/\Delta\text{VCO}_2\) in TM CPET (bias of –1.13, 95%CI of –1.79 to –0.47). In the males only, the values were significantly greater (\(P < 0.0001\)) in the children compared to the adolescents and the adults. For the participant population as a whole, Δ\(\text{VE}/\Delta\text{VCO}_2\) was inversely correlated with LBM (\(P < 0.0001\), Fig. 4B).

Table 1. Participant characteristics.

| Group | Children | Children | Adolescent | Adolescent | Adult | Adult |
|-------|----------|----------|------------|------------|-------|-------|
| Sex   | M        | F        | M          | F          | M     | F     |
| Tanner | 1–2      | 1–2      | 4–5        | 4–5        | NA    | NA    |
| N     | 18       | 18       | 18         | 23         | 8     | 11    |
| Age (year) | 10.8 ± 1.7 | 8.8 ± 1.1 | 16.9 ± 1.4 | 15.5 ± 1.8 | 29.0 ± 2.3 | 26.3 ± 4.6 |
| Height (cm) | 144.0 ± 11.8 | 132.1 ± 9.4 | 172.4 ± 7.0 | 161.4 ± 6.0 | 178.8 ± 9.0 | 161.8 ± 4.8 |
| Total body mass (kg) | 37.4 ± 12.0 | 29.7 ± 8.0 | 62.4 ± 9.4 | 54.8 ± 8.4 | 79.5 ± 8.3 | 58.1 ± 7.6 |
| Lean body mass (kg) | 25.2 ± 6.5 | 19.0 ± 3.8 | 47.3 ± 6.5 | 35.4 ± 4.6 | 59.5 ± 7.0 | 38.4 ± 5.0 |
| % Body fat | 29.0 ± 5.9 | 32.6 ± 5.6 | 21.3 ± 5.0 | 32.6 ± 5.2 | 22.5 ± 5.2 | 31.2 ± 4.3 |
| BMI (kg/m\(^2\)) | 17.5 ± 3.0 | 16.7 ± 2.4 | 17.2 ± 3.3 | 20.9 ± 2.2 | 24.9 ± 1.9 | 22.1 ± 2.3 |
| BMI percentile | 47.3 ± 30.7 | 49.4 ± 29.0 | 49.9 ± 29.4 | 43.1 ± 23.5 | N/A | N/A |
| Ethnicity (Hispanic or Latino) | 0 | 1 | 1 | 2 | 0 | 0 |
| Race (White/Asian/African-American) | 16/1/1 | 13/2/1 | 8/10/0 | 16/7/0 | 5/3/0 | 7/3/1 |

M, Male; F, Female; BMI, body mass index.
Data are presented as mean ± SD.

Table 2. Submaximal slopes of CPET variables obtainable from both CE and TM modalities.

| Group | Children | Children | Adolescent | Adolescent | Adult | Adult |
|-------|----------|----------|------------|------------|-------|-------|
| Sex   | M        | F        | M          | F          | M     | F     |
| CE Δ\(\text{VE}/\Delta\text{VCO}_2\) | 31.4 ± 3.5 | 31.2 ± 2.7 | 27.9 ± 4.0 | 30.7 ± 4.0 | 27.2 ± 3.9 | 29.4 ± 5.4 |
| TM Δ\(\text{VE}/\Delta\text{VCO}_2\) | 30.6 ± 2.2 | 29.8 ± 2.7 | 27.9 ± 2.2 | 28.7 ± 2.5 | 25.2 ± 3.7 | 28.9 ± 3.7 |
| CE Δ\(\text{VO}_2/\Delta\text{HR}\) (mL/beat) | 13.7 ± 4.1 | 9.4 ± 1.9 | 26.0 ± 6.4 | 15.8 ± 4.8 | 29.2 ± 8.0 | 21.2 ± 5.9 |
| TM Δ\(\text{VO}_2/\Delta\text{HR}\) (mL/beat) | 14.9 ± 4.6 | 10.8 ± 2.6 | 27.2 ± 7.1 | 18.8 ± 4.27 | 31.7 ± 7.6 | 22.0 ± 5.0 |

Data are presented as mean ± SD.

Table 3. Similar maturation and sex patterns in heart rate response to work rate in CE CPET (work rate actually measured) and TM CPET (work rate estimated by WR’ and VIM).

| Group | Children | Children | Adolescent | Adolescent | Adult | Adult |
|-------|----------|----------|------------|------------|-------|-------|
| Sex   | M        | F        | M          | F          | M     | F     |
| CE Δ\(\text{WR}/\Delta\text{HR}\) (watts/beat/min) | 1.17 ± 0.41 | 0.77 ± 0.19 | 2.33 ± 0.49 | 1.58 ± 0.34 | 2.61 ± 0.62 | 2.09 ± 0.61 |
| TM Δ\(\text{WR}/\Delta\text{HR}\) (watts/beat/min) | 1.29 ± 0.48 | 0.90 ± 0.27 | 2.46 ± 0.55 | 1.92 ± 0.43 | 2.85 ± 0.61 | 2.20 ± 0.64 |
| TM Δ\(\text{VIM}/\Delta\text{HR}\) | 123.9 ± 43.4 | 85.9 ± 22.7 | 334.2 ± 130.2 | 198.1 ± 75.5 | 368.9 ± 110.6 | 245.1 ± 78.9 |

Data are presented as mean ± SD.

For CE WR-HR and TM WR’-HR, the sex difference is significant for all three puberty groups. For VIM-HR the sex difference is significant for adolescents and adults, but not children. In males, all three measures, demonstrate a puberty effect with adolescents and adults having larger slopes than children. Adolescents and adult males are not significantly different from each other. In females all three measures also show adolescents and adults having larger slopes than children. In addition, CE WR-HR and TM VIM-HR show adult females having significantly larger slopes than adolescent females, but this was not the case for TM WR’-HR.
Sex effects on ΔVO₂/ΔHR and ΔVE/ΔVCO₂

Both CE and TM CPET revealed significant sex effects. ΔVO₂/ΔHR was greater in males, and ΔVE/ΔVCO₂ was greater in females. The sex effect was not influenced by CPET modality.

Relationships between HR, WR, WR', and VIM

As shown in Figure 5, we found strong correlations between LBM and either ΔWR/ΔHR from CE CPET or ΔWR'/ΔHR from TM CPET (Fig. 5A). ΔVIM/ΔHR from TM CPET was highly correlated to LBM and to ΔWR/ΔHR from CE CPET (Fig. 5B and 5, respectively). As shown in Table 3, the WR, WR’, and VIM relationships with HR all reflected comparable patterns within the subpopulations of the participants.

Peak VO₂ comparison: CE vs. TM and relationship to submaximal CPET variables

Figure 6 and Table 4 shows the correlations between peak VO₂ for the whole participant population from the two modalities. Peak VO₂ was highly correlated between CE and TM (Fig. 6A), and both CE and TM CPET peak VO₂ demonstrated high correlation with LBM (correlations with weight were high, but not as high as with LBM, Fig. 6B). Overall, a small (5.9 ± 1.3%) but significantly higher mean peak VO₂ difference was found in TM. For the group as a whole, BA analysis revealed higher peak VO₂ for TM CPET (bias of 122 mL/min, 95% CI of 62–184 mL/min). However, within the puberty subgroups, there was no significant difference between CE and TM. Males had higher peak VO₂ than females at all puberty levels. In males, there were no differences among puberty groups. In females, adolescents had the lowest mean values, statistically significant only younger ages.

We used the linear regression equations relating peak VO₂ and LBM to calculate a predicted value for peak VO₂, then compared the percent predicted from the CE and TM CPET to determine how interchangeable the two modalities were. A moderate correlation was found (Fig. 6C).

Discussion

For TM CPET, we were able to design a protocol that linearized the relationship of VO₂ to both exercise duration
and an estimate of work rate using the participant’s body weight and data easily obtained from TM CPET, namely TM speed and incline. The dynamic relationship between the novel VIM estimate of work rate and CPET variables like HR and VO₂ paralleled the relationships we found using CE CPET in which work rate is measured directly. This can provide investigators with new tools to gauge fitness in children and adolescents using TM CPET. Dynamic submaximal CPET variables (such as ΔVO₂/ΔHR and ΔVE/ΔVCO₂) were highly correlated between the new linear TM and CE CPET protocols: this is the first attempt to analyze these submaximal CPET parameters in a cohort of children, adolescents, and young adults. Furthermore, we found that the relationship of these CPET results to critical exercise-response determinants such as body size were similar in both exercise modalities. Although the HR and gas exchange results of TM and CE exercise were comparable, our data corroborated previous work establishing that CPET TM peak VO₂ is somewhat and significantly greater than CE CPET. We extended this finding to a submaximal CPET variable, ΔVO₂/ΔHR. The dynamic submaximal relationship between VE and VCO₂ (ΔVE/ΔVCO₂) was to a small but significant degree higher in CE CPET.

The mechanisms responsible for the larger ΔVO₂/ΔHR in TM CPET are not clearly evident. The Fick equation [VO₂ = HR×SV × (a − v)O₂, where HR is heart rate, SV is stroke volume, and (a − v)O₂ is arteriovenous oxygen content difference] indicates that a greater increase in VO₂ per given change in HR can occur only as a result of a greater change in SV or widening of the arteriovenous O₂ concentration difference. A possible mechanism influencing stroke volume could be higher venous return and increased muscle mass involved in exercise during TM versus CE. We reanalyzed the data cited earlier from Turley et al. (1997) who measured (a − v)O₂ and SV indirectly and noninvasively in 24 children and 24 adults during both TM and CE progressive exercise. Interestingly, while we could find no systematic differences in SV between cycle and treadmill exercise, we did find that the average (a − v)O₂ during exercise was significantly (P < 0.01) higher in TM exercise (10.7/100 mL) compared with CE exercise (9.6/100 mL). Further studies will be needed to examine the matching of blood flow distribution in the exercising muscle to determine possible mechanisms leading to greater O₂ extraction during TM exercise, leading to the small but significant differences in the ΔVO₂/ΔHR.

ΔVE/ΔVCO₂ values obtained from TM and CE CPET were correlated, but not as strongly as the ΔVO₂/ΔHR CPET variable (Fig. 3A, 4A). The relationship of VE to VCO₂ during exercise reveals useful clinical information regarding respiratory dead space and the systemic set point of CO₂ concentration that ultimately modulates respiratory control centers in the brainstem and carotid bodies (Armon et al., 1991; Rausch et al., 1991). Clinical

Table 6. Paired comparisons of mixed model least square means.

| CPET variable  | ΔVO₂/ΔHR (mL/beat) | ΔVE/ΔVCO₂ | Peak VO₂ per weight | Peak VO₂ per LBM |
|----------------|------------------|-----------|---------------------|------------------|
|                | Mean diff       | P         | Mean diff           | P                |
| Pubertal status (child = C; adolescent = ADO; adult = ADU) | | | | |
| C vs. ADI      | −9.725          | <0.0001   | 2.110               | 0.0003           |
| C vs. ADU      | −13.824         | <0.0001   | 3.105               | <0.0001          |
| ADO vs. ADU    | −4.099          | 0.0002    | 0.995               | 0.1436           |
| Sex (M,F)      |                |           |                     |                  |
| M vs. F        | 7.448           | <0.0001   | −1.506              | 0.0053           |
| Modality       | −1.679          | 0.0402    | 1.151               | 0.0317           |
| Pubertal status × sex | | | | |
| C: M vs. F     | 4.219           | 0.0009    | 0.539               | 0.5153           |
| ADO: M vs. F   | 9.263           | <0.0001   | −2.130              | 0.0060           |
| ADU: M vs. F   | 8.861           | <0.0001   | −2.926              | 0.0103           |
| M: C vs ADO    | −12.247         | <0.0001   | 3.444               | <0.0001          |
| M: C vs ADU    | −15.144         | <0.0001   | 4.837               | <0.0001          |
| M: ADU vs ADO  | −3.898          | 0.0149    | 1.393               | 0.1759           |
| F: C vs ADO    | −7.203          | <0.0001   | 0.776               | 0.3240           |
| F: C vs ADU    | −11.503         | <0.0001   | 1.372               | 0.1483           |
| F: ADU vs ADU  | −4.299          | 0.0023    | 0.597               | 0.5000           |

*No significant interactions.
insights using CPET-derived $\Delta VE/\Delta VCO_2$ have been gained in children and adults from both TM and CE CPET in diseases ranging from cystic fibrosis to heart failure (Moser et al., 2000; Ingle et al., 2012). We did observe small but statistically significant differences in $\Delta VE/\Delta VCO_2$, for example, a 3.7% larger value overall for CE exercise. Our study was not configured to determine the mechanism of this difference (e.g., greater ventilatory dead space or a lower CO2 set point in CE compared with TM CPET). Nonetheless, while the differences were small in this cohort of children and adults with no history of lung disease, one might speculate that variables like $\Delta VE/\Delta VCO_2$ might become more useful in participants with chronic lung disease.

One reason for the somewhat smaller correlation for the $\Delta VE/\Delta VCO_2$ variable between the two modalities may be that the range of $\Delta VE/\Delta VCO_2$ values in our cohort

Figure 3. Interoperability of $\Delta VO_2/\Delta HR$ derived from CPET-CE and CPET–TM. (A) The slope of the linear regression equation was highly significant, 0.923 ± 0.0419, $P < 0.0001$; the y-intercept, 3.11 ± 0.82 mL O2/beat, was significant at $P = 0.0003$, and $r = 0.92$. (B) Relationship of CE and TM $\Delta VO_2/\Delta HR$ to peak VO2. Both modalities revealed very high correlations. Linear regression parameters for CPET CE (solid line) were: peak VO2 (mL/min) = 113.3 × $\Delta VO_2/\Delta HR$ (mL/beat) + 361.7, $r = 0.94$; and for CPET TM (dotted line) peak VO2 (mL/min) = 115.2 × $\Delta VO_2/\Delta HR$ (mL/beat) + 247.0, $r = 0.94$. (C) We calculated the percent predicted peak VO2 based on the LBM linear regression (see text) and plotted TM percent predicted vs. CE percent predicted peak VO2. The correlation coefficient was $r = 0.66$, $P < 0.0001$. 

Figure 4. Interoperability of $\Delta VE/\Delta VCO_2$ derived from CPET-CE and CPET–TM. (A) The slopes were significantly correlated $\Delta VE/\Delta VCO_2$ (TM) = 0.48 × $\Delta VE/\Delta VCO_2$ (CE) + 14.4, $r = 0.63$, $- P < 0.0001$. (B) The slopes from both modalities were inversely correlated with LBM (CE: $\Delta VE/\Delta VCO_2 = -0.12 \times LBM$ (kg) + 34.3, $r = -0.40, P < 0.0001$; TM: $\Delta VE/\Delta VCO_2 = -0.11 \times LBM$ (kg) + 32.3, $r = -0.48, P < 0.0001$. These data corroborate previous studies showing generally higher $\Delta VE/\Delta VCO_2$ in younger (smaller) children compared with adolescents and young adults.
Figure 5. Interoperability of work rate–heart rate slopes derived from CPET-CE and CPET-TM. (A) The slopes for \( \Delta \text{WR}/\Delta \text{HR} \) and \( \Delta \text{VIM}/\Delta \text{HR} \) as a function of LBM were similar: \( \Delta \text{WR}/\Delta \text{HR} \) (CE) = 0.048 \( \times \) LBM – 0.070, \( r = 0.89, P < 0.0001 \); \( \Delta \text{VIM}/\Delta \text{HR} \) (TM) = 0.050 \( \times \) LBM + 0.041, \( r = 0.89, P < 0.0001 \). (B) Using data derived solely from CPET TM showed a very similar relationship, \( \Delta \text{VIM}/\Delta \text{HR} \) (TM) = 7.24 \( \times \) LBM – 50.12, \( r = 0.86, P < 0.0001 \). (C) Dynamic WR-HR data obtained from the two modalities were highly correlated. \( \Delta \text{VIM}/\Delta \text{HR} \) (TM) = 140.4 \( \times \) \( \Delta \text{WR}/\Delta \text{HR} \) (CE) – 24.5, \( r = 0.90, P < 0.0001 \).

Figure 6. Interoperability of peak VO2 derived from CPET-CE and CPET–TM. (A) The slope of the linear regression equation was highly significant, \( 0.975 \pm 0.0316, P < 0.0001 \); the y-intercept, 182.81 \pm 81.87 mL O2/min, was significant at \( P = 0.028 \), and \( r = 0.95 \). (B) Relationship of CE and TM peak VO2 to lean body mass. Both modalities revealed very high correlations. Linear regression parameters for CPET CE (solid line) were: peak VO2 CE \( \text{(mL/min)} = 67.3 \times \text{LBM (kg)} + 21.8, r = 0.91 \); and for CPET TM (dotted line) peak VO2 TM \( \text{(mL/min)} = 71.3 \times \text{LBM (kg)} + 4.39, r = 0.94 \). The correlation coefficients for LBM were higher than for weight in both modalities: CE, peak VO2 = 26.4 \( \times \) Weight + 46.4, \( r = 0.82 \); TM, peak VO2 = 49.6 \( \times \) Weight – 13.0, \( r = 0.86 \). (C) We calculated the percent predicted peak VO2 based on the LBM linear regression and plotted TM percent predicted versus CE percent predicted peak VO2. The correlation coefficient was \( r = 0.66 \).
was substantially smaller than for other CPET variables. For example, ΔVO₂/ΔHR ranged from about 7–45 mL O₂/beat while ΔVE/ΔVCO₂ ranged only from about 25–35 (unitless). Within a participant group of healthy individuals with no history of lung or heart disease, a major determinant of many CPET variables is body size, particularly muscle mass (Cooper et al., 2014). Previous studies using CE CPET demonstrated high correlations between body size and ΔVO₂/ΔHR and weak, but significant, inverse correlations with ΔVE/ΔVCO₂.

We found that the relationships between ΔVO₂/ΔHR and body mass in TM CPET paralleled the relationships we found previously using CE CPET. The correlation between TM CPET-derived ΔVO₂/ΔHR and body weight was strong, but even stronger when correlated with LBM. These results emphasize the need to scale ΔVO₂/ΔHR to some metric of body size in order to interpret the results correctly. The TM CPET-derived ΔVE/ΔVCO₂ was to a small but significant degree inversely correlated to body size, similar to the earlier studies using CE CPET (Cooper et al., 1984; Nagano et al., 1998). These similar results from the two different exercise modalities bolster the idea that physiologic mechanisms, such as the CO₂ set point or the relationship between dead space and tidal volume, systematically change over childhood and adolescence.

Both modalities revealed similar and significant sex effects in ΔVO₂/ΔHR and ΔVE/ΔVCO₂. The higher oxygen extraction per beat found in male participants reflects, as noted above, the influence of stroke volume and (a – v)O₂. In adults, left ventricular size is smaller in females compared to males (Gebhard et al., 2013). Similar observations have been made in children (Vinet et al., 2003). These results might explain the sexual dimorphism of the ΔVO₂/ΔHR. Although not as well studied as heart size, one study in young and middle-aged adults also showed generally higher VE/VECO₂ based parameters in females compared with males (Sun et al., 2002), an observation not seen in one exercise study in younger volunteers (Guerrero et al., 2008). Sexual dimorphism in respiratory control in adults is a known phenomenon, but the impact of sex on respiratory control during exercise in children is not well understood. Whether the generally higher ΔVE/ΔVCO₂ that we found in females indicate greater deadspace ventilation or, alternatively, a lower CO₂ set point, has yet to be determined.

**CPET typically consists of an ergometer programmed to increase the participant’s work rate coupled with a set of devices capable of measuring physiologic responses such as gas exchange or HR. These physiologic outputs are useful only insofar as they can be scaled. For example, an isolated HR measured during exercise is uninterpretable unless it is dynamically scaled to a CPET input such as the work rate. We used several approaches to address the challenging problem of estimating work performed during TM CPET. There are very compelling reasons to do this; one of the most potentially impactful would be in reanalyzing fitness data from many studies in children in which TM CPET in some form is used to estimate, rather than measure, peak VO₂ [e.g., NHANES (Astrand and Ryhming, 1954; Jackson et al., 1990)]. Subsequent calculated estimates of VO₂max derived from the submaximal CPET may include variables or constants reflecting levels of habitual physical activity or normative values obtained from studies in adults. Such approaches can contribute to the increasingly recognized problems that confound data interpretation due to mis-specification, collinearity, and mathematical coupling (Tu et al., 2004; Aggarwal and Ranganathan, 2016).

An analysis of TM CPET that relies predominantly on actually measured data would advance our ability to accurately gauge fitness from CPET. In the current study, we were able to calculate the WR’ TM exercise based on the VO₂-WR relationship measured during CE CPET. As shown in Figure 5A, ΔWR/ΔHR from CE CPET and ΔWR/ΔHR from TM CPET had virtually identical relationships with LBM (and body weight, data not shown). Additional parallel effects of sex are shown in Table 4.

Using the VIM estimate of TM work rate led to a linear relationship with VO₂ (Fig. 2), mimicking the well-established relationship between VO₂ and work rate observed consistently in CE CPET. The potential value of this approach to TM CPET, which uses only the actually measured data, that is, body mass, HR, and treadmill speed and incline, is highlighted in Figure 5C, showing the very high correlation between the submaximal ΔVIM/ΔHR of TM CPET and ΔWR/ΔHR measured in CE CPET. As shown in Table 3, maturation- and sex-related changes in TM CPET-derived ΔVIM/ΔHR paralleled, as expected, the changes in CE ΔWR/ΔHR. Similarly, for exercise biomarkers expected to be relatively size independent, we found, as expected, little or no differences across our subgroups for CE CPET ΔVO₂/ΔWR and the parallel TM CPET ΔVO₂/ΔVIM.

We found strong correlations between peak VO₂ obtained from TM and CE CPET (Fig. 6A). Our data are consistent with previous studies demonstrating generally lower values for CE peak VO₂. For both TM and CE CPET, there was a strong correlation between ΔVO₂/ΔHR and peak VO₂ (Fig. 3B). This observation may be particularly useful in instances when a participant or patient does not meet standard criteria for peak VO₂, not an infrequent occurrence (Paridon et al., 2008). In these cases, investigators or clinicians might consider using ΔVO₂/ΔHR (a value not dependent on maximal effort) as a surrogate index for fitness.
Myers et al. (2017) and Kaminsky et al. (2017) recently published normative values for CPET in separate populations of adults using TM and CE. Although the investigators found generally lower peak VO2 values in CE CPET, they were unable to identify a unique conversion factor that could eliminate differences between the two ergometer types across the age groups of their study. A number of investigators have compared TM and CE CPET in which participants performed both modalities (Jacobs and Sjödin, 1985; Turley and Wilmore, 1997; Basset and Boulay, 2000; Mitchell et al., 2010; Gordon et al., 2012; Itô et al., 2013) and consistently higher TM CPET peak VO2 has been observed. As noted, Turley and Wilmore (1997) studied both children and adults, and found that CE peak VO2 in all groups was lower than TM to a small but consistent degree. There are a number of possible explanations for the higher peak VO2 values in TM exercise, including the energy cost of maintaining an upright posture (Miles-Chan et al., 2013; Júdice et al., 2016) and/or factors related to work efficiency, skeletal muscle mass, and activation that occur in TM but not CE CPET. Muscle mass clearly plays a role; for example, peak VO2 is, as expected, smaller in upper body ergometry compared with TM or CE CPET (Drescher et al., 2015). It is noteworthy that we could not find significant changes in any of our submaximal slopes in the transition from walking to running on the treadmill, suggesting that the predominant component of energy costs of TM exercise is related to velocity, mass, and incline.

Using the strong relationships of LBM to both TM and CE peak VO2 and ΔVO2/ΔHR, we addressed the question of whether the two modalities reflected similar hierarchies in fitness among the participants. To do this, we used the linear regression relationship between LBM and both peak VO2 and ΔVO2/ΔHR to determine a predicted value for each participant based on LBM. We then correlated the TM and CE percent predicted value for each participant. As shown in Figures 3C and 6C, we found modest but significant correlations in fitness hierarchy for both submaximal ΔVO2/ΔHR and peak VO2. In summary, a participant in our study with a relatively high or low peak VO2 would likely have respectively high or low ΔVO2/ΔHR on both TM and CE modalities. However, the variability in our data also cautions that relative fitness ascertained by CPET biomarkers is not fully interchangeable between the two modalities.

Limitations: Due to the same order of exercise test modalities a sequence effect may influence the second test session. In this study supramaximal test was not performed. In children VO2peak is more commonly used than in adults and supramaximal tests are equivocal. This study focused on submaximal values and novel TM protocol; thus, measuring peak or max values were presented as secondary end point.

In conclusion, our data reveal the effect of the two predominant modalities of laboratory exercise testing in children, adolescents, and young adults on submaximal and peak CPET results. Both modalities similarly reflected effects of body size on ΔVO2/ΔHR, ΔVE/ΔVCO2, and peak VO2. Results from the two modalities, however, are not interchangeable and may reflect the complexities of how external work, particularly on the treadmill, is transduced to physiologic responses such as VO2 (Pandolf et al., 1977; Epstein et al., 1987; Hall et al., 2004) The reasons for using TM or CE exercise for assessment of exercise biomarkers in the clinic or in research ultimately depend on a variety of factors, including the skill and experience of the laboratory, available equipment, and perceived capabilities of the targeted participants or patients. We provide a novel approach for analyzing TM CPET data relying on actually measured HR, body mass, and the velocity and incline. This approach might prove useful in reanalyzing existing datasets where such measurements are available and in the future establishment of normative values for CPET testing in children and adolescents, where reliable datasets in large numbers of healthy participants are currently lacking.

Acknowledgments

We thank Dr. Kim Lu for reviewing the manuscript, Hoang Pham for assistance in performing the exercise testing and the children and their families who participated in this study.

Conflict of Interest

The authors declare no conflicts of interest. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

References

Aggarwal, R., and P. Ranganathan. 2016. Common pitfalls in statistical analysis: The use of correlation techniques. Perspect Clin Res 7:187–190.

Armon, Y., D. M. Cooper, and S. Zanconato. 1991. Maturation of ventilatory responses to 1-minute exercise. Pediatr. Res. 29:362–368.

Ashish, N., M. M. Bamman, F. J. Cerny, D. M. Cooper, P. D’Hemecourt, J. C. Eisenmann, et al. 2015. The clinical translation gap in child health exercise research: a call for disruptive innovation. Clin. Transl. Sci. 8:67–76.

Astrand, P. O., and I. Ryhming. 1954. A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during sub-maximal work. J. Appl. Physiol. 7:218–221.

Azuma, A. 2014. Effects of a vigorous gait on mechanical work and oxygen uptake during treadmill walking. Percept. Mot Skills 119:6–19.
Basset, F. A., and M. R. Boulay. 2000. Specificity of treadmill and cycle ergometer tests in triathletes, runners and cyclists. Eur. J. Appl. Physiol. 81:214–221.

Beltrami, F. G., C. Froyd, A. R. Mauger, A. J. Metcalfe, F. Marino, and T. D. Noakes. 2012. Conventional testing methods produce submaximal values of maximum oxygen consumption. Br. J. Sports Med. 46:23–29.

Bires, A. M., D. Lawson, T. E. Wasser, and D. Raber-Baer. 2013. Comparison of Bruce treadmill exercise test protocols: is ramped Bruce equal or superior to standard Bruce in producing clinically valid studies for patients presenting for evaluation of cardiac ischemia or Arrhythmia with body mass index equal to or greater than 30?. J. Nucl. Med. Technol. 41:274–278. https://doi.org/10.1097/JMT.0b013e318279b3e7.

Bridge, P., N. A. Pocock, T. Nguyen, C. Munns, C. T. Cowell, N. Forwood, et al. 2011. Validation of longitudinal DXA changes in body composition from pre- to mid-adolescence using MRI as reference. J. Clin. Densitom. 14:340–347.

Buchfuhrer, M. J., J. E. Hansen, T. E. Robinson, D. Y. Sue, K. Cooper, D. M., M. R. Kaplan, L. Baumgarten, D. Weiler-Ravell, B. J. Whipp, and K. Wasserman. 1983. Optimizing the exercise protocol for cardiopulmonary assessment. J. Appl. Physiol. 55:1558–1564.

Chen, C. A., S. Y. Chen, H. H. Chiu, J. K. Wang, C. I. Chang, I. S. Chiu, et al. 2014. Prognostic value of submaximal exercise data for cardiac morbidity in Fontan patients. Med. Sci. SportsExerc. 46:10–15.

Cooper, D. M., D. Weiler-Ravell, B. J. Whipp, and K. Wasserman. 1984. Aerobic parameters of exercise as a function of body size during growth in children. J. Appl. Physiol. 56:628–634.

Cooper, D. M., M. R. Kaplan, L. Baumgarten, D. Weiler-Ravell, B. J. Whipp, and K. Wasserman. 1987. Coupling of ventilation and CO2 production during exercise in children. Pediatr. Res. 21:568–572.

Cooper, D. M., S. Y. Leu, P. Galassetti, and S. Radom-Aizik. 2014. Dynamic interactions of gas exchange, body mass, and progressive exercise in children. Med. Sci. Sports Exerc. 46:877–886.

Cooper, D. M., S.-Y. Leu, C. Taylor-Lucas, K. Lu, P. Galassetti, and S. Radom-Aizik. 2016. Cardiopulmonary exercise testing in children and adolescents with high body mass index. Pediatr. Exerc. Sci. 28:98–108.

Cordingley, D., R. Girardin, K. Reimer, L. Ritchie, J. Leiter, K. Russell, et al. 2016. Graded aerobic treadmill testing in pediatric sports-related concussion: safety, clinical use, and patient outcomes. J. Neurol. Neurosurg. Pediatr. 18:693–702.

Cunha, F. A., A. W. Midgley, T. Gonçalves, P. P. Soares, and P. Farinatti. 2015. Parasympathetic reactivation after maximal CPET depends on exercise modality and resting vagal activity in healthy men. Springerplus 4:100. https://doi.org/10.1186/s40064-015-0882-1.

Drescher, U., J. Koschate, and U. Hoffmann. 2015. Oxygen uptake and heart rate kinetics during dynamic upper and lower body exercise: an investigation by time-series analysis. Eur. J. Appl. Physiol. 115:1665–1672.

Elbeheiry, A. F., C. E. Ciavaglia, K. A. Webb, J. A. Guenette, D. Jensen, S. M. Mourad, et al. 2015. Canadian respiratory research network. pulmonary gas exchange abnormalities in mild chronic obstructive pulmonary disease. Implications for dyspnea and exercise intolerance. Am. J. Respir. Crit. Care Med. 191:1384–1394.

Epstein, Y., L. A. Stroschein, and K. B. Pandolf. 1987. Predicting metabolic cost of running with and without backpack loads. Eur. J. Appl. Physiol. Occup. Physiol. 56:495–500.

Fredriksen, P. M., F. Ingier, W. Nystad, and E. Thaulow. 1998. Aerobic endurance testing of children and adolescents–a comparison of two treadmill-protocols. Scand. J. Med. Sci. Sports 8:203–207.

Gebhard, C., B. E. Stähli, C. E. Gebhard, H. Tasnady, D. Zöhler, M. B. Wischnewsky, et al. 2013. Age- and gender-dependent left ventricular remodeling. Echocardiography 30:1143–1150.

Gordon, D., M. Mehter, M. Gernigon, O. Caddy, D. Keiller, and L. Clark. 2012. Abnormalities of the ventilatory equivalent with prediction equations. Med. Sci. Sports Exerc. 46:215–221.

Guerrero, L., J. Naranjo, and M. D. Carranza. 2008. Influence of gender on ventilatory efficiency during exercise in young children. J. Sports Sci. 26:1455–1457.

Hall, C., A. Figueroa, B. Fernhall, and J. A. Kanaley. 2004. Energy expenditure of walking and running: comparison with prediction equations. Med. Sci. Sports Exerc. 36:2128–2134.

Hestnes, J., H. Hoel, O. J. Risa, H. O. Romstøl, O. Røksund, B. Frisk, et al. 2017. Ventilatory efficiency in children and adolescents born extremely preterm. Front. Physiol. 8:499.

Ingle, L., R. Sloan, S. Carroll, K. Goode, J. G. Cleland, and A. L. Clark. 2012. Abnormalities of the ventilatory equivalent for carbon dioxide in patients with chronic heart failure. Pulm. Med. 2012:1–6.

Itoh, H., R. Ajiyaka, A. Koike, S. Makita, K. Omiya, Y. Kato, et al. 2013. Heart rate and blood pressure response to ramp exercise and exercise capacity in relation to age, gender, and mode of exercise in a healthy population. J. Cardiol. 61:71–78.

Jackson, A. S., S. N. Blair, M. T. Mahar, L. T. Wier, R. M. Ross, and J. E. Stuteville. 2012. The effects of exercise modality on the incidence of plateau at VO2max. Clin. Physiol. Funct. Imaging 32:394–399.

Gualano, B., E. Bonfá, R. M. R. Pereira, and C. A. Silva. 2017. Physical activity for paediatric rheumatic diseases: standing up against old paradigms. Nat. Rev. Rheumatol. 13:368–379.

Guerrero, L., J. Naranjo, and M. D. Carranza. 2008. Influence of gender on ventilatory efficiency during exercise in young children. J. Sports Sci. 26:1455–1457.

Hall, C., A. Figueroa, B. Fernhall, and J. A. Kanaley. 2004. Energy expenditure of walking and running: comparison with prediction equations. Med. Sci. Sports Exerc. 36:2128–2134.

Hestnes, J., H. Hoel, O. J. Risa, H. O. Romstøl, O. Røksund, B. Frisk, et al. 2017. Ventilatory efficiency in children and adolescents born extremely preterm. Front. Physiol. 8:499.

Ingel, L., R. Sloan, S. Carroll, K. Goode, J. G. Cleland, and A. L. Clark. 2012. Abnormalities of the ventilatory equivalent for carbon dioxide in patients with chronic heart failure. Pulm. Med. 2012:1–6.

Itoh, H., R. Ajiyaka, A. Koike, S. Makita, K. Omiya, Y. Kato, et al. 2013. Heart rate and blood pressure response to ramp exercise and exercise capacity in relation to age, gender, and mode of exercise in a healthy population. J. Cardiol. 61:71–78.

Jackson, A. S., S. N. Blair, M. T. Mahar, L. T. Wier, R. M. Ross, and J. E. Stuteville. 1990. Prediction of functional aerobic capacity without exercise testing. Med. Sci. Sport Exerc. 22:863.

Jacobs, L., and B. Sjödin. 1985. Relationship of ergometer-specific VO2 max and muscle enzymes to blood lactate during submaximal exercise. Br. J Sports Med. 19:77–80.

Jüdic, P. B., M. T. Hamilton, L. B. Sardinha, T. W. Zderic, and A. M. Silva. 2016. What is the metabolic and energy
cost of sitting, standing and sit/stand transitions? Eur. J. Appl. Physiol. 116:263–273.
Kaminsky, L. A., M. T. Imboden, R. Arena, and J. Myers. 2017. Reference standards for cardiorespiratory fitness measured with cardiopulmonary exercise testing using cycle ergometry: data from the fitness registry and the importance of exercise National Database (FRIEND) Registry. Mayo Clin. Proc. C 92:228–233.
Keir, D. A., R. Zory, C. Boudreau-Lariviére, and O. Serresse. 2012. Mechanical efficiency of treadmill running exercise: Effect of anaerobic-energy contribution at various speeds. Int. J. Sports Physiol. Perform. 7:382–389.
Kennedy, M. J., and D. R. Seals. 1993. Postexercise hypotension. Key features, mechanisms, and clinical significance. Hypertens 22:653–664.
Kyröläinen, H., P. V. Komi, and A. Belli. 1995. Mechanical efficiency in athletes during running. Scand. J. Med. Sci. Sports 5:200–208.
Li, C., F. Feng, X. Xiong, R. Li, and N. Chen. 2017. Exercise coupled with dietary restriction reduces oxidative stress in male adolescents with obesity. J. Sports Sci. 35:663–668.
Liem, R. I., M. Reddy, S. A. Pelligra, A. P. Savant, B. Fernhall, M. Rodeghier, et al. 2015. Reduced fitness and abnormal cardiopulmonary responses to maximal exercise testing in children and young adults with sickle cell anemia. Physiol. Rep. 3:1–8.
May, L. J., R. Punn, I. Olson, J. A. Kazmucha, M. Y. Liu, and C. Chin. 2014. Supine cycling in pediatric exercise testing: disparity in performance measures. Pediatr. Cardiol. 35:705–710.
Miles-Chan, J. L., D. Sarafian, J. P. Montani, Y. Schutz, and A. Dulloo. 2013. Heterogeneity in the energy cost of posture maintenance during standing relative to sitting: phenotyping according to magnitude and time-course. PLoS ONE 8: e65827.
Mitchell, J., W. B. Kist, K. Mears, J. Nalls, and K. Ritter. 2010. Does standing on a cycle-ergometer, towards the conclusion of a graded exercise test, yield cardiorespiratory values equivalent to treadmill testing? Int. J. Exerc. Sci. 3:117–125.
Moser, C., P. Tirakitoontorn, E. Nussbaum, R. Newcomb, and D. M. Cooper. 2000. Muscle size and cardiorespiratory response to exercise in Cystic Fibrosis. Am. J. Respir. Crit. Care Med. 162:1823–1827.
Myers, J., and V. F. Froelicher. 1993. Exercise testing. Procedures and implementation. Cardiol. Clin. 11:199–213.
Myers, J., L. A. Kaminsky, R. Lima, J. W. Christie, E. Ashley, and R. Arena. 2017. A Reference equation for normal standards for VO2MAX: analysis from the Fitness Registry and the Importance of Exercise National Database (FRIEND Registry). Prog. Cardiovasc. Dis. 60:21–29.
Nagano, Y., R. Baba, K. Kuraishi, T. Yasuda, M. Ikoma, K. Nishibata, et al. 1998. Ventilatory control during exercise in normal children. Pediatr. Res. 43:704–707.
Vinet, A., S. Mandigout, S. Nottin, L. Nguyen, A.-M. Lecoq, D. Courteix, et al. 2003. Influence of body composition, hemoglobin concentration, and cardiac size and function of gender differences in maximal oxygen uptake in prepubertal children. Chest 124:1494–1499.

Whipp, B. J., J. A. Davis, F. Torres, and K. Wasserman. 1981. A test to determine parameters of aerobic function during exercise. J. Appl. Physiol. 50:217–221.

Workman, J. M., and B. W. Armstrong. 1963. Oxygen cost of treadmill walking. J. Appl. Physiol. 18:798–803.