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 range of exercise intensities:
 the equation matters**

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**Calculating metabolic energy expenditure across a wide range of exercise intensities:
the equation matters**

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Abstract: 75 words or less for brief communication and tech notes

We compared ten published equations for calculating energy expenditure from oxygen consumption and carbon dioxide production using data for 10 high-caliber male distance runners over a wide range of running velocities. We found up to a 5.2% difference in calculated metabolic rate between two widely used equations. We urge our fellow researchers abandon out-of-date equations with published acknowledgments of errors or inappropriate biochemical/physical assumptions.

Keywords: Indirect calorimetry, expired gas analysis, metabolic rate, running economy, respiratory exchange ratio

Introduction:

Running economy (RE) is a primary physiological determinant of endurance running (Joyner, 1991). Traditionally, RE is defined as the mass specific rate of oxygen uptake required to run at a given submaximal velocity, where the oxygen required serves as a proxy for the metabolic energy expended. However, because different velocities/exercise intensities influence the ratio of carbohydrates (CHO) and fats metabolized, and therefore the amount of energy liberated per liter of oxygen, more recent studies express RE as metabolic rate (kcal/min/kg or W/kg). By dividing oxygen uptake or metabolic rate by running velocity, one can alternatively calculate RE as the cost of transport (COT)- the amount oxygen or energy needed to transport a kilogram of body mass a given distance (mlO₂/kg/km, kcal/kg/km or J/kg/m).

Fletcher et al. (2009) found that oxygen COT remained nearly constant across a range of running velocities, whereas when calculated in energy units, COT significantly increased with velocity. They concluded that expressing COT in units of energy was more sensitive to changes in intensity because it takes into account the different ratio of substrates metabolized at different submaximal velocities. Shaw et al. (2013) corroborated those findings, showing that COT in units of energy has greater validity than COT expressed in units of oxygen.

Indirect calorimetry is the most widely used technique for determining energy expenditure during exercise via the measurement of oxygen uptake (VO₂) and carbon dioxide production (VCO₂). Assuming that VO₂ and VCO₂ collected at the mouth accurately reflect metabolism at the tissues (i.e. respiratory exchange ratio (RER) = respiratory quotient (RQ)), it is possible to calculate the mixtures of fuels being oxidized for energy. However, this is true only when the intensity of the exercise involves a metabolic steady state.

Multiple published equations exist for calculating the energetic cost of exercise (Table 1). These equations calculate the rate of energy production ($-\Delta H$) from measured VO_2 and VCO_2 in L/s, and excreted nitrogen (N). Coefficients (c_1 , c_2 , c_3), for VO_2 , VCO_2 and N are based upon the heat of combustion of various mixtures of CHO, lipids and proteins oxidized (Equation 1).

$$-\Delta H(\text{kJ/s}) = c_1(\text{kJ/l}) \cdot \text{VO}_2 + c_2(\text{kJ/l}) \cdot \text{VCO}_2 - c_3(\text{kJ/g}) \cdot \text{N (g)} \quad (\text{Equation 1})$$

The coefficients (c_1 , c_2 , c_3) in these equations differ in part due to small differences in the stoichiometry of the dietary species used. For example, humans consume a diet with a ratio of saccharides which differ slightly in their caloric values (supplementary table S1). Based on the chosen ratio of these different saccharides, a unique heat of combustion can be calculated for CHO. Additionally, the stoichiometry of fat metabolism also differs depending on the length of the fatty acids chosen.

Once proteins are broken down into amino acids and deaminated, the remaining keto-acids can be oxidized. Thus, the RER for expired gases reflects the metabolism of a mixture of CHO, fats and proteins. However, because the contribution of protein as an energy source is so small, it has generally been considered negligible and ignored when estimating total energy expenditure during exercise (Brooks, 1997). Calculating protein oxidation requires measuring the excreted nitrogen in sweat and urine, or using radioactive isotopes. However, virtually all studies that use established equations for exercise energetics studies ignore the small nitrogen component.

Generally, it has been concluded that as long as the heats of combustion from dietary CHO and body lipids are correct, errors in estimating heat production based on the specific

dietary species likely to lead to errors in estimating heat production less than 1% (Blaxter 1962). However, a few studies have suggested that large errors are introduced depending upon whether CHO oxidation primarily involves glucose or glycogen (Ferrannini, 1988; Jeukendrup and Wallis, 2005). Further, Jeukendrup and Wallis (2005) suggested that because the intensity of exercise plays a role in the relative contributions of glucose and glycogen oxidized, assuming 100% glucose metabolized could overestimate energy expenditure by 8%.

Our primary purpose was to compare multiple, published equations for calculating energy expenditure from expired gas analysis across a wide range of exercise intensities (i.e. running velocities) while assuming the protein contribution is nil. We hypothesized that there would be less than 1% difference in the calculated energy expenditure between the different equations.

Methods:

Participants

Ten high-caliber, male runners participated (27.4 ± 2.7 years, 64.6 ± 4.6 kg, 178.8 ± 5.8 cm). All had recently run a sub-31 minute 10km race at sea level, or an equivalent performance in a different distance running event. Participants gave written informed consent that followed the guidelines of the University of Colorado Boulder IRB. We collected these data as part of a larger study (Kipp, 2017).

Experimental set-up and protocol

To habituate subjects to the treadmill and gas collection setup, they performed a series of running trials on a level motorized treadmill (Treadmetrix, Park City, UT). On Day 1, we verified that they could run submaximally during 5-minute trials at 14, 16 and 18 km/hr. We

measured VO_2 and VCO_2 with an open-circuit expired-gas analysis system (Parvomedics TrueOne 2400, Sandy, UT). We monitored $[\text{La}]$ to assure a primary reliance on oxidative metabolism. To do so, we obtained 50 μL of blood from the subject's fingers to determine lactate concentration ($[\text{La}]$) at rest and at the completion of each 5-minute stage and analyzed the blood with a YSI 2300 lactate analyzer (YSI, Yellow Springs, OH). Subjects who could run with a $[\text{La}]$ below 4 mmol/l (Heck, 1985) and with an $\text{RER} < 1.0$ were considered capable of running at all velocities submaximally. We acknowledge that with these criteria it is possible that some subjects still could be producing some non-metabolic CO_2 . However, the comparison of equations will not be influenced by small changes in non-metabolically produced CO_2 in the small subset of subjects tested.

On Day 2, subjects arrived to the laboratory 2 hours postprandial to help control for potential effects of diet on metabolic rate. They ran six 5-minute trials (with 5-minute breaks) at velocities of 8, 10, 12, 14, 16 and 18 km/hr in randomized order, while we measured VO_2 and VCO_2 . We calculated mean VO_2 and VCO_2 for the last 2-minutes of each trial and calculated metabolic rate using ten different equations (Table 1) assuming zero protein metabolism. When needed, we converted units, so that all equations expressed metabolic power in kJ/s. We maintained the assumptions for energy release and substrate utilization of each paper.

Statistics

We calculated mean metabolic rates and standard deviations (SD) for each equation. To determine whether any differences existed between the equation used and velocity, we used RStudio (version 0.99.892, Boston, MA, USA) to perform a two-way repeated measures ANOVA followed by multiple pairwise comparisons using Tukey's honestly significant difference tests. Criterion of statistical significance was $P < 0.05$.

Results:

The Péronnet and Massicotte (1991) equation resulted in the highest calculated metabolic power, while the Weir (1949) equation resulted in the lowest calculated metabolic power. There was a significant 5.0 - 5.2% difference in calculated metabolic power between the Weir (1949) equation and Péronnet and Massicotte (1991) equation from 8 to 18km/hr ($P < 0.001$).

Additionally, there was a significant 3.8 - 3.7% difference in calculated metabolic power between the Ferrannini (1988) equation assuming 100% glucose and the Péronnet and Massicotte (1991) equation ($P = 0.03$). Similarly there was as 3.8 - 3.6% difference between calculated metabolic power between the Garby and Astrup equation and the Péronnet and Massicotte (1991) equation ($P = 0.04$). The differences between equations were nearly constant across velocity, i.e. no interaction effect (Table 2).

There were numerical differences, but no statistical significant differences between the other equations. We found a $3.6\% \pm 0.03$ mean numerical difference in calculated metabolic power between Brockway (1987) and Péronnet and Massicotte (1991) ($P = 0.055$), and a $3.5\% \pm 0.03$ mean difference in calculated metabolic power between the Ferrannini glycogen equation (1988) and Péronnet and Massicotte (1991) ($P = 0.069$).

Discussion:

The equation used to calculate metabolic power can result in difference of up to 5.2%. Thus, we reject our hypothesis that there would be less than a 1% difference in calculated energy expenditure regardless of the chosen equation. However, the relative differences in metabolic power calculated with different equations did not change across the velocity range we tested.

The original Weir (1949) equation resulted in metabolic power values were 1.7% to 5.2% lower than all of the other equations we compared. Using Lusk's (1928) table of caloric values for CHO, Weir used Cathcart and Cuthbertson's (1931) updated analysis of human fats to propose an elegant and simple formula format for heat output that was adopted by many others that followed after him. However, Brockway (1987) pointed out Weir (1949) used what was most likely a typographic error in the published source data for fat from Cathcart and Cuthbertson (1931). Unfortunately, Weir's original equation is still being used by numerous studies. In the 2016 alone, Weir's original paper was cited 227 times. The Weir (1949) equation is presented in textbooks, programmed into indirect calorimetry software for resting energy expenditure (Parvo Medics TrueOne 2400), and used to create popular energy prediction equations (Pandolf et al., 1977). We feel that Weir still should be given credit for his contribution to the fields of physiology and metabolism but his original equation should be abandoned.

When considering which equation to use, Jeukendrup and Wallis (2005) suggested that to obtain more accurate results, exercise researchers should recognize the increased contribution of muscle and liver glycogen to overall CHO oxidation at greater exercise intensities. The molecular weight of glycogen (162.143g, one glycosyl unit) is less than the molecular weight of glucose (180.158g), and thus oxidized glycogen releases more energy on a per gram basis (glucose-15.64 kJ/g vs. glycogen-17.50 kJ/g). However, our data showed that when we utilized the Jeukendrup and Wallis equations over our wide range of intensities, there was no significant difference from more traditional equations that assume 100% glucose. It has been reported that at moderate to high exercise intensities ($>75\%$ VO_2max) muscle glycogen can account for 75-85% of the CHO oxidation (Romijn et al., 1993, van Loon et al., 2001). However, during lower

intensity exercise, the ratio of glucose vs. glycogen utilized are less clear. Empirical studies have reported at intensities below 50% $\text{VO}_{2\text{max}}$, the contribution of glycogen to overall CHO oxidation to be as high as 77% (van Loon et al., 2001) or as low as 0% (Romijn et al., 1993). Theoretically, at higher intensities, Jeukendrup and Wallis' (2005) equations could be more accurate. While there is some controversy over the contribution blood glucose plays during exercise, our results do not support an equation effect for varying the carbohydrate energy sources.

The stoichiometry of fat metabolism varies depending on the length of the fatty acid chain. Lusk (1923) used published data on pork lard to determine fat oxidation. Equations to follow used palmitic acid or similar fatty acids because they are abundant in the human diet (Ferrannini, 1988; Frayn, 1983), where 1g palmitic acid ($\text{C}_{16}\text{H}_{32}\text{O}_2$) yields 39.75 kJ. Péronnet and Massicotte (1991) took a more meticulous approach, reporting the respective average of 13 fatty acids that represent more than 99% of the fatty acids found in human triacylglycerols, where 1g of the averaged fatty acids ($\text{C}_{17.2702}\text{H}_{32.7142}\text{O}_2$) yields 40.79 kJ.

In addition to slight differences in the dietary species used to create the equation, Péronnet and Massicotte (1991), note that oxygen and carbon dioxide are not ideal gasses as previous equations have treated them. One mole of oxygen actually occupies 22.3858 liters STPD and one mole of carbon dioxide actually occupies 22.2966 liters STPD (Weast, 1972). For example, consider the oxidation of glucose where 2817 kJ of heat are released.

Since 6 moles of oxygen are required for oxidation, one mole of glucose requires 6×22.41 L. The ratio of heat produced to oxygen consumed then becomes $2817 / (6 \times 22.41)$ or 20.95 kJ/L. However, if 22.3858 L/mol is used, 20.97 kJ/L is actually released. Using the common 22.41

L/mol for an ideal gas leads to slight underestimations of the energy equivalent per liter of gas. Accounting for oxygen and carbon dioxide not being ideal gases and the higher energy fat species utilized partly explains why the Péronnet and Massicotte's (1991) equation results in the greatest metabolic power values.

For most within study designs, the equation used might seem irrelevant, but when comparing across studies, it is important to consider which equations were used to calculate metabolic rate. We encourage researchers to report both VO_2 and VCO_2 , or VO_2 and RER to allow readers to calculate energy expenditure using a common equation. Additionally, we urge investigators to abandon out-of-date equations with published acknowledgments of errors or inappropriate biochemical/physical assumptions.

Conflict of interest statement

The authors declare no conflict of interest.

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Table 1. Published equations for calculating the energetic expenditure, where VO_2 and VCO_2 is in L/s, and N is in g.

	Equation
Lusk, 1923 ^a	$16.00 \text{ VO}_2 + 5.15 \text{ VCO}_2 - 7.80\text{N}$
Weir, 1949	$16.50 \text{ VO}_2 + 4.62 \text{ VCO}_2 - 9.06\text{N}$
Weir ^b (corrected)	$16.62 \text{ VO}_2 + 4.51 \text{ VCO}_2 - 9.22\text{N}$
Garby and Astrup, 1987	$16.04 \text{ VO}_2 + 4.94 \text{ VCO}_2$
Ferrannini, 1988 (glucose)	$16.15 \text{ VO}_2 + 4.82 \text{ VCO}_2 - 4.79\text{N}$
Ferrannini, 1988 (glycogen)	$16.38 \text{ VO}_2 + 4.64 \text{ VCO}_2 - 4.51\text{N}$
Brockway, 1987	$16.58 \text{ VO}_2 + 4.51 \text{ VCO}_2 - 5.90\text{N}$
Péronnet and Massicotte, 1991	$16.89 \text{ VO}_2 + 4.84 \text{ VCO}_2$
Jeukendrup and Wallis, 2005 (Intensities between 40-50% $\text{VO}_{2\text{max}}$)	$18.56 \text{ VO}_2 + 2.40 \text{ VCO}_2 - 4.14\text{N}$
Jeukendrup and Wallis, 2005 (Intensities between 50-75% $\text{VO}_{2\text{max}}$)	$18.71 \text{ VO}_2 + 2.30 \text{ VCO}_2 - 4.14\text{N}$

^a as calculated by Abramson et al., 1942.

^b Weir's equation as corrected in Brockway, 1987.

Table 2. Metabolic rate calculated using each equation. * indicates $P < 0.05$ when compared to Peronnet & Massicotte for the same corresponding velocity. Values presented are means $\pm$ SD.

Velocity (km/hr)	Weir, 1949 (W/kg)	Ferrannin, 1988 glucose (W/kg)	Garby and Astrup, 1987 (W/kg)	Brockway, 1987 (W/kg)	Ferrannini, 1988 glycogen (W/kg)	Lusk, 1923 (W/kg)	Weir corrected (W/kg)	Jeukendrup and Wallis, 2005 (W/kg)	Péronnet and Massicotte, 1991 (W/kg)
8	8.79* ± 0.49	8.91* ± 0.52	8.93* ± 0.52	8.94 ± 0.49	8.95 ± 0.52	8.97 ± 0.52	9.01 ± 0.53	9.10 ± 0.56	9.26 ± 0.54
10	10.40* ± 0.56	10.55* ± 0.61	10.55* ± 0.61	10.58 ± 0.57	10.58 ± 0.61	10.62 ± 0.61	10.66 ± 0.62	10.75 ± 0.62	10.96 ± 0.63
12	12.17* ± 0.76	12.35* ± 0.84	12.35* ± 0.85	12.38 ± 0.77	12.39 ± 0.84	12.43 ± 0.85	12.48 ± 0.85	12.57 ± 0.86	12.83 ± 0.87
14	14.49* ± 0.64	14.70* ± 0.75	14.71* ± 0.75	14.74 ± 0.65	14.75 ± 0.75	14.81 ± 0.75	14.86 ± 0.76	15.00 ± 0.78	15.27 ± 0.78
16	17.31* ± 0.92	17.58* ± 1.01	17.59* ± 1.01	17.61 ± 0.94	17.63 ± 1.01	17.71 ± 1.02	17.76 ± 1.01	17.86 ± 0.99	18.25 ± 1.04
18	20.38* ± 0.91	20.72* ± 0.96	20.73* ± 0.97	20.75 ± 0.92	20.78 ± 0.97	20.89 ± 0.97	20.92 ± 0.97	20.92 ± 0.99	21.51 ± 1.00