Multiple propane gas burns rate procedure to determine accuracy and linearity of indirect calorimetry systems : An experimental assessment of a method

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Objective: Indirect calorimetry (IC) systems measure the fractions of expired carbon dioxide (F_eCO_2), and oxygen (F_eO_2) recorded at the mouth to estimate whole-body energy production. The fundamental principle of IC relates to the catabolism of high-energy substrates such as carbohydrates and lipids to meet the body's energy needs through the oxidative process, which are reflected in the measured oxygen uptake rates ($\dot{V}O_2$) and carbon dioxide production rates ($\dot{V}CO_2$). Accordingly, it is important to know the accuracy and validity of $\dot{V}O_2$ and $\dot{V}CO_2$ measurements when estimating energy production and substrate partitioning for research and clinical purposes. Although several techniques are readily available to assess the accuracy of IC systems at a single point for $\dot{V}CO_2$ and $\dot{V}O_2$, the validity of such procedures is limited when used in testing protocols that incorporate a wide range of energy production (e.g., basal metabolic rate and maximal exercise testing). Accordingly, we built an apparatus that allowed us to manipulate propane burn rate in such a way as to assess the linearity of IC systems. This technical report aimed to assess the accuracy and linearity of three IC systems using our in-house built validation procedure. Approach: A series of trials at different propane burn rates (PBR) (i.e., 200, 300, 400, 500, and 600 mL min^{-1}) were run on three IC systems: Sable, Moxus, and Oxycon Pro. The experimental values for $\dot{V}O_2$ and $\dot{V}CO_2$ measured on the three IC systems were compared to theoretical stoichiometry values. Results: A linear relationship was observed between increasing PBR and measured values for $\dot{V}O_2$ and $\dot{V}CO_2$ (99.6%, 99.2%, 94.8% for the Sable, Moxus, and Jaeger IC systems, respectively). In terms of system error, the

Jaeger system had significantly ($p < 0.001$) greater $\dot{V}O_2$ (mean difference (M) = -0.057, standard error (SE) = 0.004), and $\dot{V}CO_2$ (M = -0.048, SE = 0.002) error compared to either the Sable ($\dot{V}O_2$, M = 0.044, SE = 0.004; $\dot{V}CO_2$, M = 0.024, SE = 0.002) or the Moxus ($\dot{V}O2$, M = 0.046, SE = 0.004; $\dot{V}CO_2$, M = 0.025, SE = 0.002) IC systems. There were no significant differences between the Sable or Moxus IC systems. Conclusion: The multiple PBR approach permitted the assessment of linearity of IC systems in addition to determining the accuracy of fractions of expired gases.

Multiple propane gas burns rate method to determine accuracy and linearity of indirect calorimetry systems: An experimental assessment of a method

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Running head: Validation of indirect calorimetric systems

33 **Abstract**

34 Objective: Indirect calorimetry (IC) systems measure the fractions of expired carbon dioxide

35 (F_eCO_2), and oxygen (F_eO_2) recorded at the mouth to estimate whole-body energy production.

36 The fundamental principle of IC relates to the catabolism of high-energy substrates such as

37 carbohydrates and lipids to meet the body's energy needs through the oxidative process, which

38 are reflected in the measured oxygen uptake rates (VO_2) and carbon dioxide production rates

39 (VCO_2). Accordingly, it is important to know the accuracy and validity of VO_2 and VCO_2

40 measurements when estimating energy production and substrate partitioning for research and

41 clinical purposes. Although several techniques are readily available to assess the accuracy of IC

42 systems at a single point for VCO_2 and VO_2, the validity of such procedures is limited when used

43 in testing protocols that incorporate a wide range of energy production (e.g., basal metabolic rate

44 and maximal exercise testing). Accordingly, we built an apparatus that allowed us to manipulate

45 propane burn rate in such a way as to assess the linearity of IC systems. This technical report

46 aimed to assess the accuracy and linearity of three IC systems using our in-house built validation

47 procedure. Approach: A series of trials at different propane burn rates (PBR) (i.e., 200, 300, 400,

48 500, and 600 mL min^{-1}) were run on three IC systems: Sable, Moxus, and Oxycon Pro. The

49 experimental values for VO_2 and VCO_2 measured on the three IC systems were compared to

50 theoretical stoichiometry values. Results: A linear relationship was observed between increasing

51 PBR and measured values for VO_2 and VCO_2 (99.6%, 99.2%, 94.8% for the Sable, Moxus, and

52 Jaeger IC systems, respectively). In terms of system error, the Jaeger system had significantly (p

53 < 0.001) greater VO_2 (mean difference (M) = -0.057, standard error (SE) = 0.004), and VCO_2 (M

54 = -0.048, SE = 0.002) error compared to either the Sable (VO_2, M = 0.044, SE = 0.004; VCO_2, M

55 = 0.024, SE = 0.002) or the Moxus ($VO2$, M = 0.046, SE = 0.004; VCO_2, M = 0.025, SE = 0.002)

56 IC systems. There were no significant differences between the Sable or Moxus IC systems.

57 Conclusion: The multiple PBR approach permitted the assessment of linearity of IC systems in

58 addition to determining the accuracy of fractions of expired gases.

59

60 **Keywords**: Accuracy, energy production, indirect calorimetry, linearity, propane gas.

61

62

63 **Introduction**

64 Of primary interest to use indirect calorimetry (IC) in the study of human thermoregulation is the

65 measurement of fractions of expired oxygen (F_eO_2) and carbon dioxide (F_eCO_2) for estimation of

66 energy production or substrate turnover under various environmental conditions (e.g., at rest,

67 during exercise, and in cold or hot environments) (Jequier and Felber 1987). Chemical,

68 electronic, and spectroscopic technologies have been implemented to perform these

69 measurements. Indirect calorimetry systems integrate discrete electronic analyzers to record F_eO_2

70 and F_eCO_2 in line with measures of flow rate (e.g., flowthrough respirometry) or ventilation rate

71 (e.g., breath-by-breath measurements) in addition to the temperature, pressure, and humidity of

72 ambient and expired air using computer-controlled analog-to-digital signal processing (Leonard

73 2012). Several instrument configurations range from simple or semi-automated mixing chamber

74 systems to highly sophisticated, fully automated breath-by-breath measurement devices

75 (Matarese 1997). Each instrument incorporated in the IC system contributes errors in measuring

76 whole-body oxygen uptake (VO_2) and carbon dioxide production (VCO_2), affecting estimates of

77 energy production and substrate turnover. The expected accuracy of IC systems can differ

78 between manufacturers, and the user is often left to rely on the devices' technical notes to

79 estimate the accuracy of their measured outcomes. However, technical notes generally report

80 specifications for the individual analyzers and not the IC system as a whole. Accordingly,

81 additional procedures are needed to assess the accuracy of IC measures under conditions that

82 reflect the testing environment, especially when investigating small metabolic differences

83 between the experimental and control conditions (Lighton 2008).

84

85 Researchers and clinicians in the biological/medical sciences routinely implement IC technology

86 to study the effects of behavioral and environmental manipulations on whole-body energy

87 metabolism in humans under resting and exercise conditions (Brooks, Fahey, and Baldwin

88 2005). Several techniques have been developed to assess the accuracy of outcomes recorded

89 through IC; however, few are designed to simulate the energy production rates recorded among

90 humans at rest and during light physical activity. Burning of methanol (Cooper and Storer 2001)

91 or propane (Lighton 2008) and nitrogen dilution (Fedak, Rome, and Seeherman 1981) are the

92 most commonly applied techniques to validate IC measurements (i.e., VO_2 and VCO_2). Yet, the

93 determination of signal linearity through the generation of multiple propane burn rates or levels

94 of nitrogen dilution remains rarely performed. Lack of appropriate validation may cause

95 inaccurate interpretations of VO_2 and VCO_2 measurements and, consequently, lead to large

96 errors in calculating substrate partitioning and energy production (Ferrannini 1988).

97 Advancements in sensor technologies will continue to increase the accuracy and reliability of IC

98 measurements in both laboratory and field settings and render energy production assessment

99 promptly available (Haugen, Chan, and Li 2007). However, the accuracy and linearity of the

100 measurements under various experimental and clinical conditions warrant special considerations

101 for the enhancement of existing methodologies used to validate IC outcomes (Levine, Eberhardt,

102 and Jensen 1999). Therefore, the present manuscript reports a new propane gas validation

103 procedure designed to assess the accuracy and linearity of VO_2 and VCO_2 measurements taken at

104 different propane burn rates (PBR).

105

106 **Materials and methods**

107 The accuracy and linearity of three IC systems available in our exercise physiology laboratory

108 (i.e., Jaeger Oxycon Pro, Jaeger- now CareFusion, Hochberg, Germany; Moxus Modular

109 Metabolic System, AEI technologies, IL, USA; and Sable Classic Line, Sable Systems

110 International, Las Vegas, NV, USA) were assessed with an in-house built device for

111 manipulating PBR and recovering the products of complete propane combustion (i.e., CO_2 and

112 H_2O) into the mixing chambers of the IC systems. All IC systems were set for flow-through

113 respirometry measurements (i.e., excurrent flow measurement or negative pressure system).

114 During Jaeger and Moxus measurements, subsampled air was dried by passing through a twin-

115 tube Nafion sample line or tube filled with magnesium perchlorate, respectively. Water vapor

116 pressure was recorded during Sable measurements, and flow rate (FR), F_eO_2, and F_eCO_2 were

117 corrected (see equations below).

118

119 Technical characteristics of the indirect calorimetric systems

120 The ViaSys Jaeger Oxycon Pro is a quasi-modular IC system consisting of a twin tube (Nafion

121 sample line), a turbine volume transducer (flow range = 0-300 $L•min^{-1}$; accuracy = 2%), a

122 subsample pump (flow rate ranging from 200 to 220 $ml•min^{-1}$), a 6 L mixing chamber, a fuel-cell

123 oxygen sensor (accuracy = 0.05%; resolution = 0.01%; full range = 0-25%; time response = 0.08

124 sec), a dual infrared carbon dioxide sensor (accuracy = 0.05%; resolution = 0.01%; full range =

125 0-15%; time response = 0.08 sec).

126 The Moxus Modular Metabolic System, consists of a turbine for determination of ventilation

127 volume [VMM-400 (flow range = 0-800 L•min-1; accuracy = 1%)], a 4.2 L mixing chamber, a

128 subsample pump (flow rate ranging from 10-500 mL•min-1), a zirconia oxygen sensor [3A/I

129 Oxygen analyzers (accuracy = 0.01%; resolution = 0.01%; full range = 0-100%; time response =

130 0.1 sec)] and a dual infrared carbon dioxide sensor [CD-3A Carbon Dioxide (accuracy = 0.02 %;

131 resolution = 0.01%; full range = 0-15%; time response = 0.025 sec)]. To investigate differences

132 in oxygen sensor technologies (i.e., zirconia vs. paramagnetic), Moxus IC system validity

133 measures were recorded with its gas analyzers (3A/I and CD-3A) integrated with Sable

134 technologies for generating FR and subsample rate (described below).

135

136 The Sable Classic Line is a modular IC system consisting of a subsample pump (sub-sampler,

137 SS4 – linearized mass flow meter ranging from 0-2000 ml•min^{-1}), a water vapor analyzer (RH-

138 300 – resolution = 0.001% and full range = 0-100% RH non-condensing), a dual infrared carbon

139 dioxide sensor [CA-10 Carbon Dioxide (accuracy = 1%; resolution = 0.00001%; full range = 0-

140 10%; time response = 0.5 sec), a paramagnetic oxygen sensor [PA-10 Oxygen analyzers

141 (accuracy = 0.1%; resolution = 0.0001%; full range = 0-100%; time response = 0.2 sec)] and an

142 air mass flow generator and controller (FK-500 – accuracy = 0.05 L•min^{-1}; full range = 50-500

143 L•min^{-1}).

144

145 Calibration of IC Systems

146 Before data collection, oxygen and carbon dioxide analyzers were calibrated with medically

147 certified calibration gases (1% CO_2 and 100% N_2 for Sable and 4% CO_2 and 16% O_2 for Moxus

148 and Jaeger). In terms of FR, the Oxycon Pro's turbine volume transducer was calibrated using

149 the manufacturer's built-in automated calibration procedures. Outcomes of the Sable's air mass

150 flow generator and controller were validated using the nitrogen dilution technique described by

151 Fedak et al. (1981). A series of N_2 gas flows (1000, 500, 250. 150 ml•min^{-1}) were randomly

152 selected at two different ventilation rates (55 and 75 L min^{-1}) and injected into the incurrent air

153 through a canopy. The actual experimental values of N_2 gas were compared to their theoretical

154 value (Fedak, Rome, and Seeherman 1981).

155 Multiple Propane Burn Rate Device

156 The overarching aim of the current methodological study was to develop a procedure to assess

157 the validity of VO_2 and VCO_2 measurements recorded at rest and during light exercise in human

158 subjects. Given that propane gas combustion provides one of the best methods for simulating

159 whole-body energy metabolism (Rising et al. 2017), a device was built to enable the complete

160 combustion of propane at different mass flow rates (described as propane burn rate (PBR) to

161 avoid confusion with the flow rate used during flow-through respirometry) and the recovery of

162 VO_2 and VCO_2. The in-house built propane gas validation system consists of the following

163 sequential connections. First, a tank of chemically pure (99%) propane gas (SPG-PROCHP6 –

164 Air-Liquids Canada) with a two-stage Western Medical gas regulator (model M1-940-PG,

165 Westlake, Ohio) and its gas hose is connected to a one-way Matheson mass-flow transducer

166 (model 8141) that is subsequently connected to a Matheson mass-flow controller, model 8240

167 (East Rutherford, NJ) and to a Bunsen burner – vertical metal tube of 60 mm high and 4 mm

168 inside diameter (ID). The burner is located in a 2.4 L glass canopy that flows into a 0.4 L glass

169 tubing. The entire system is connected to IC systems by a 1.4-inch diameter hose (Figure 1). The

170 pressure in the gas line flowing out of the cylinder is maintained at ten psi. The regulator is fitted

171 with a ¼ inch MNPT brass needle valve and a high-pressure gas hose (4mm ID, 7mm outside

172 diameter, OD) to prevent potential propane leaks. The flow rates were expressed in STP-

173 corrected volumes from the analog output that varied from 0 to 5 volts on a 0% to 100% full

174 scale (Lighton 2008). The data collection is detailed in the next section, and its results are shown

175 in the results section.

176
177 Insert Figure 1 about here
178
179 Data collection and reduction

180 A series of PBRs (200, 300, 400, 500, and 600 mL min^{-1}) were selected and tested at two

181 different FRs to assess each IC system's accuracy and linearity. According to the Jaeger

182 recommendations for resting metabolic rate, the flow rate was set at 20 and 40 L•min^{-1} using the

183 digital volume transducer described above. For the Sable IC system and Moxus gas analyzers,

184 flow rates were set at 55 and 75 L min^{-1}. Three 30-min trials per PBR and FR were randomly

185 performed on each IC system. Also, 15-min of baseline (i.e., room air) was recorded before and

186 after each PBR trial equating to 60-min measurement periods. All trials were conducted at one

187 location and at the same time of the day. In addition, the F_eO_2, F_eCO_2, FR, barometric pressure

188 (BP), water vapor pressure (WVP), chamber temperature (T°C-Ch), and room temperature (T°C-

189 Rm) were recorded.

190 Propane gas stoichiometry equations

191 Pure propane (C_3H_8) is an odorless, colorless, flammable gas. Complete combustion of one mole

192 of 100% C_3H_8 produces three moles of CO_2 and 4 moles of H_2O for every five moles of O_2

193 consumed according to the stoichiometry reaction depicted in Equation 1.

194

$C_3H_8 + 5O_2 => 3CO_2 + 4H_2O$	(Eq.1)

195

196 Therefore, under standard pressure and temperature (STPD), 22.44 L of C_3H_8 would react with

197 112.2 L of O_2 for the reaction to be completed to produce 67.2 L of CO2. At standard conditions,

198 the molecular mass of 100% C_3H_8 is 44 g, and 1 g of propane would then require 2.55 L of O_2

199 (i.e., 112.2 L of O_2 to burn 44 g of propane) to produce 1.53 L CO_2, that is, 67.2 L of CO_2 results

200 from the burning of 44 g of propane. It can then be deduced that optimal C_3H_8 combustion

201 results in RER equal to 0.60 (Lighton 2008).

202

203 The present technical report used a mass flow meter for an accurate PBR. The PBR was

204 calculated using the following formula:

205

Mass flow rate (g min^{-1}) = volume flow rate x propane density	Eq.2

206

207 Flow-through respirometry equations

208 The measured fraction of expired gases (F_eO_2 and F_eCO_2) and FR recorded by the Sable IC

209 system were first corrected for the effect of WVP using Dalton's law of partial pressures.

210

$F'_eO_2 = F_eO_2$ x BP / (BP − WVP)	(Eq.3)

211
212 Where $F'eO_2$ and F_eO_2 represent a fraction of expired air dry and moist oxygen.

213

$F'_eCO_2 = F_eCO_2$ x BP / (BP − WVP)	(Eq.4)

214
215 Where F'_eCO_2 and F_eCO_2 represent a fraction of expired air dry and moist carbon dioxide.

FR' = FR x (BP − WVP) / BP	(Eq.5)

216

217 Where FR' and FR represent dry and moist air FR, respectively.

218

219 To correct for any drift in the fraction of oxygen, the following equation developed by

220 Lieberman et al. (2015) was computed:

221

$F_iO_{2ss} = F_iO_{2f} + [(F_iO_{2f} − F_iO_{2i}) \times (T_{ss} − T_i) / (T_f − T_i)]$	(Eq.6)

222

223 Where F_iO_{2i} is the initial fractional amount of oxygen in the inspired air stream measured at

224 equilibrium before each PBR (baseline pre-); F_iO_{2f} is the final fractional amount of oxygen in the

225 inspired air stream measured at equilibrium after each PBR (baseline post-); T_{ss} is the time into

226 each PBR at steady state; T_f is the time when final inspired oxygen fraction is measured; T_i is the

227 time when initial inspired oxygen fraction is measured.

228

229 The calculation of $\dot{V}O_2$, $\dot{V}CO_2$, and RER was performed using the following equations:

230

$\dot{V}O_2 = FR_e [(F_iO_2 − F_e'O_2) − F_iO_2 (F_e'CO_2 − F_iCO_2)] / (1 − F_iO_2)$	(Eq.7)

231

232 Where FR_e is expired flow rate; F_iO_2 stands for the fraction of inspired unscrubbed oxygen;

233 $F_e'O_2$ stands for expired dry oxygen; $F_e'CO_2$ for expired dry carbon dioxide; and F_iCO_2 stands

234 for the fraction of inspired unscrubbed carbon dioxide.

235

$\dot{V}CO_2 = FR_e [(F_e'CO_2 − F_iCO_2) + F_iCO_2 (FiO_2 − F_e'O_2)] / (1 + F_iCO_2)$	(Eq.8)

236

237 where acronyms stand as in Eq.7

238

$RER = \dot{V}CO_2 / \dot{V}O_2$	(Eq.9)

239

240 Where RER is the quotient of $\dot{V}CO_2$ over $\dot{V}O_2$.

241

242 The metabolic data ($\dot{V}O_2$, $\dot{V}CO_2$, and RER) were then truncated by 10 min (5 min at the

243 beginning and end of each data collection period). An average value was reported for the

244 remaining 20 min. To determine the accuracy and linearity of the three different IC systems,

245 respirometry data were compared to the stoichiometry theoretical $\dot{V}O_2$, and $\dot{V}CO_2$ values under

246 standard conditions for the five PBRs studied. Given that the same propane burn rates were used

247 to validate the three IC systems, data are expressed as the mean difference between

248 stoichiometry theoretical value and mean experimental values (i.e., $M\Delta$=STV-MEV).

249

250 Statistical analysis

251 Statistical analyses were performed using SPSS, version 23 (SPSS Inc., Chicago, IL, USA).

252 Unless otherwise specified, all values are reported as mean ± standard deviation, and an alpha

253 level of 0.05 was used to indicate statistical significance. Tests for statistical assumptions were

254 performed; homogeneity of variance was tested using Levene's test, and normality was tested

255 using the Kolmogorov-Smirnov test. First, descriptive statistics were conducted. Second, a series

256 of one-way ANOVA was used to assess the effect of ventilation rates (55 L min-1, 75 L min^{-1} for

257 Sable and Moxus, 20 L min^{-1}, 40 L min^{-1} for Jaeger) on the fraction of gases. Third, a linear

258 regression analysis was performed to examine the linearity between the volumes of $\dot{V}O_2$, and

259 $\dot{V}CO_2$, with PBR for the three systems. Regression analysis was also performed to test the

260 linearity between N_2 flow rates with $\dot{V}O_2$ during validation of the Sable mass flow generator and

261 controller. Fourth, Bland-Altman plots followed by linear regressions were created to evaluate

262 the mean difference (error) between systems outputs and the stoichiometry theoretical $\dot{V}O_2$,

263 $\dot{V}CO_2$, and RER values for all systems at all PBRs. Lastly, two-factor ANOVAs were conducted

264 to evaluate the effects of the three IC systems and the five PBRs for $\dot{V}O_2$, $\dot{V}CO_2$, and RER error.

265 A mixed models design was used with systems being a fixed effect and PBR a random effect. A

266 corrected F-test was calculated (Neter et al. 1996) for the random factor as mixed models in

267 SPSS incorrectly uses MS *from the interaction* as the error term (denominator). The correction is

268 to use MS *error* as the denominator. SPSS correctly uses MS *error* in the F-test for the

269 interaction, which is also considered random. SPSS correctly uses the MS *from the interaction* as

270 the error term (denominator) for the fixed factor's F-test. Effect sizes were calculated for F-test:

271 Omega-squared (Ω^2) was used for the fixed effect of the system, and rho (ρ) was used for the

272 random effect of flow rate as was the interaction effect. In case of significant interactions, Tukey

273 and Bonferroni post-hoc tests were applied.

274

275 **Results**

276 Exploratory and Descriptive Statistics

277 Descriptive statistics were performed on VO_2, VCO_2, and RER (Table 1). Levene's test for

278 testing homogeneity of variance (VO_2, VCO_2) for PBRs was significant. Homogeneity of

279 variance between levels of PBR was violated for all outcome variables for all systems. However,

280 F-statistics are robust when there are no equal variances (Field 2015), especially in a balanced

281 design. A test of normality (Kolmogorov-Smirnov) was performed within PBRs. The assumption

282 of normality was not met for all data. Within overall PBRs (regardless of system), normality was

283 only met for the 200 ml min^{-1} condition for both VO_2 and VCO_2. When normality was examined

284 by system, assumptions of normality were met for both VO_2 and VCO_2 for every PBR.

285

286 Validation of Sable mass flow generator

287 Simple linear regression analyses were conducted to assess the linear relationship between N_2

288 flow rates and actual experimental VO_2 (mL min^1) for the Sable system. Results showed a strong

289 linear relationship between the actual experimental VO_2 and the N_2 flow rates (R_{adj}^2 = 0.996; β =

290 0.998; 95% CI (0.174-0.183); $p < 0.001$).

291

292 Effect of flow rate on respirometry outcomes

293 A series of 15 one-way ANOVAs [2 FR (55 L min^{-1}, 75 L min^{-1} for Sable and Moxus, 20 L min^{-1}

294 , 40 L min^{-1}, for Jaeger) X 5 PBRs (200, 300, 400, 500, 600 mL min^{-1})] revealed no significant

295 effect of FR on VO_2 and VCO_2 outcomes for the Sable, Moxus, and Jaeger IC systems.

296 Accordingly, data was pooled for each PBR (i.e., FR as a variable was ignored). The average of

297 the six experimental trials per PBR is shown in Table 1.

298
299   Insert table 1 about here
300
301 Linear relationship of VO_2 and VCO_2 by PBR per system

302 A linear regression analysis was run to assess the linearity between the VO_2, VCO_2, and the PBR

303 through the determination of linear regression equations ($y = b_0 + b_x + \varepsilon$) for each system. Table

304 2 shows that for the Sable system, PBR explains 99.6 % of the variability in VO_2 and VCO_2

305 (R^2=0.996), while it explains 99.2% and 99.4% of the variability in VO_2 and VCO_2, respectively,

306 for the Moxus. However, the Jaeger system had the worst linear scores compared to the other

307   two systems (94.8%, and 94.2 %, for VO_2 and VCO_2, respectively). Furthermore, the mean

308   values of VO_2 and VCO_2 were lowest for the 200 ml min^{-1} condition and increased as PBR

309   increased for the three systems (Figure 2).

310
311   Insert table 2 and Figure 2 about here
312
313   Assessing error of VO_2, VCO_2, and RER for each system

314   To assess the relationship between the error in the system (VO_2, VCO_2, and RER) and the PBR,

315   Bland-Altman plots were created and were followed by linear regression analysis for each

316   system. As shown in Table 3, there were weak, moderate, and strong linear relationships between

317   the VO_2, VCO_2 errors, and the PBR for the Sable, Moxus, and Jaeger systems, respectively.

318   Also, VO_2 and VCO_2 errors of the Sable system had a non-significant *p*-value, which indicates

319   that the Sable system had the lowest error in both volumes compared to the other two systems.

320   However, although *p*-values were significant for both Moxus and Jaeger systems, the VO_2 and

321   VCO_2 errors of the Moxus system had a weaker relationship with PBR and, therefore, lower error

322   than the Jaeger system. The Jaeger system had the highest error among the three systems.

323
324   Insert Table 3 about here
325
326   A two-factor [3 (IC) × 5 (PBR's)] ANOVA was conducted to evaluate the effects of the three

327   systems and the five PBR's on VO_2, VCO_2, and RER error. For VO_2 the results indicate a

328   significant interaction between system and propane burn rate ($F_{(8,75)} = 3.328$, $p = 0.0026$).

329   Approximately 26.6% ($\rho = 0.266$) of the variance in VO_2 error is accounted for by the interaction

330   factor. The results show a non-significant main effect of propane burn rate on VO_2 error ($F_{(4,75)} =$

331   1.0714, $p = 0.3767$), and it accounts for only 0.2% ($\rho = 0.00286$) of the variability in VO_2 error.

332   The main fixed effect of system was found to be significant ($F_{(2,8)} = 67.028$, $p < 0.001$). All else

333   held constant, the fixed main effect of system accounts for approximately 83% ($\Omega^2 = 0.8293$) of

334   the variability in VO_2. The Jaeger system had significantly ($p < 0.001$) greater VO_2 error ($M = -$

335   0.057, $SE = 0.004$) compared to either the Sable ($M = 0.044$, $SE = 0.004$) or Moxus ($M = 0.046$,

336    $SE = 0.004$) systems. There were no significant differences between Sable or Moxus systems on

337   the above-mentioned variables (Figure 3).

338
339   Insert Figure 3 about here

340   The results for VCO_2 indicate a significant interaction between systems and PBR ($F_{(8,75)} =$

341   10.722, $p < 0.001$). Approximately 54% ($\rho = 0.539$) of the variance in VCO_2 is accounted for by

342   the interaction factor. The results show a significant main effect of PBR on VCO_2 ($F_{(4,75)} = 9.375$,

343    $p < 0.001$) and it accounts for 14.8% ($\rho = 0.148$) of the variability in VCO_2. The main fixed

344   effect of system is also significant ($F_{(2,8)} = 34.966$, $p < 0.001$). All else held constant, the fixed

345   main effect of system accounts for approximately 89% ($\Omega^2 = 0.8947$) of the variability in VCO_2.

346   The Jaeger system had significantly ($p < 0.001$) greater VCO_2 error ($M = -0.048$, $SE = 0.002$)

347   compared to either the Sable ($M = 0.024$, $SE = 0.002$) or Moxus ($M = 0.025$, $SE = 0.002$)

348   systems. There were no significant differences between Sable and Moxus systems on the above-

349   mentioned variable (Figure 3).

350

351   The results for RER indicate a significant interaction between system and PBR ($F_{(8,75)} = 3.332$, p

352    $= 0.003$). Approximately 20% ($\rho = 0.2044$) of the variance in RER is accounted for by the

353   interaction factor. The results show a significant main effect of PBR on RER ($F_{(4,75)} = 7.8125$, p

354   < 0.001) and it accounts for 21.8% ($\rho = 0.2184$) of the variability in RER. The main fixed effect

355   of system is also significant ($F_{(2,8)} = 4.993$, $p = 0.039$). All else held constant, the fixed main

356   effect of system accounts for approximately 23.8% ($\Omega^2 = 0.2381$) of the variability in RER. The

357   Jaeger system had significantly ($p < 0.001$) greater RER error ($M = -0.028$, $SE = 0.003$)

358   compared to either the Sable ($M = -0.005$, $SE = 0.003$) or Moxus ($M = -0.005$, $SE = 0.003$)

359   systems. There were no significant differences between Sable and Moxus systems on the above-

360   mentioned variable (Figure 3).

361

362   **Discussion**

363   Laboratories using IC routinely implement propane gas validation techniques (Melanson et al.

364   2010, White et al. 1996). However, procedures are rarely performed to assess both the accuracy

365   and the linearity of individual systems. We, therefore, built a device that enabled the

366   manipulation of PBR during flow-through respirometry measurements. The custom-built device

367   was used to evaluate the accuracy and linearity of IC systems readily available in our exercise

368   physiology laboratory. Also, the multiple PBR procedure was implemented at two flow rates

369   with each IC system (55 L min^{-1}, 75 L min^{-1} for Sable and Moxus, 20 L min^{-1}, 40 L min^{-1}, for

370   Jaeger) to investigate the effect of flow rate on VO_2 and VCO_2 measurements. The primary
371 outcome of the current study revealed that the multiple PBR procedure agreed with the

372 stoichiometric theoretical values (Table 2), and strong linear responses were observed,

373 suggesting that the technique could be used to evaluate the accuracy and linearity of IC systems.

374 Further, burning propane gas at rates within the range of whole-body energy metabolism in

375 human subjects at rest and during light physical activity revealed differences in accuracy and

376 linearity between IC systems.

377

378 Accuracy and linearity of the indirect calorimetry systems

379 The accuracy of the IC system typically refers to the closeness between the measured value and

380 the "true" value (theoretical stoichiometry value). In contrast, linearity refers to systematic and

381 random errors (Cooper and Storer 2001). Regression analyses and ANOVA were performed to

382 determine the accuracy and linearity of the three IC systems on VO_2, VCO_2, and RER. The

383 ventilation rates were pooled because statistical outcomes showed that the two ventilation rates

384 at the studied PBRs did not alter the VO_2 and VCO_2. As shown in Table 1, the mean difference

385 between experimental and theoretical values of the Sable and Moxus systems overestimated

386 VO_2, and the mean differences were stable across the 5 PBRs. On the contrary, the Jaeger system

387 underestimated VO_2, and the mean differences increased with progressing PBR. The multiple

388 PBR procedure demonstrated a high consistency for RER values for both Sable and Moxus

389 systems. Our technical report showed that both Sable and Moxus systems responded similarly to

390 propane gas combustion. However, it is important to note that the Moxus O_2 and CO_2 analyzers

391 were incorporated into the Sable Classic Line technologies. Accordingly, further studies are

392 needed using the complete Moxus Modular Metabolic System before making comparisons

393 between the two IC systems. Special consideration should be given to the technologies used to

394 measure FR during flow-through respirometry. The Sable system incorporates a mass flow

395 meter, which is not affected by fluctuations in temperature and pressure (Lighton 2008).

396 However, systems that integrate volumetric flow meters need to correct for changes in

397 temperature and pressure (Lighton 2008). The increased discrepancy between experimental and

398 theoretical values observed with the Jaeger system at increasing PBR may reflect a lack of

399 appropriate correction for temperature changes.

400

401 The regression analysis of PBR vs. experimental values revealed a robust linear relationship for
402 the three systems, meaning that both VO_2 and VCO_2 increased with propane gas combustion
403 (Table 2). However, when linear regression was performed on the VO_2 and VCO_2 errors
404 (difference), the Sable system provided the most accurate measurements evidenced by the least
405 error values (i.e., R_{adj}^2 was the least with a non-significant p values ($p= 0.37$, $p= 0.13$) for VO_2
406 and VCO_2, respectively).
407
408 The Sable and Moxus systems showed no change in mean difference for VO_2, VCO_2, and RER
409 throughout progressive PBRs (Figure 3), indicating more stable and reliable metabolic outcomes
410 than the Jaeger system. The observed mean differences between analyzers could stem from the
411 type of O_2 analyzer (sensor) implemented in these systems. As time elapses, the fuel-cell oxygen
412 sensor becomes noisy, unstable, and unreliable, an issue not always addressed by the
413 manufacturer's conventional medical gas calibration procedures. This is particularly true for fuel
414 cells with faster response times but shorter lifespans. Conversely, paramagnetic (Sable System)
415 and zirconia (Moxus) oxygen analyzers do not degrade over time if well maintained. Therefore,
416 one cannot discard the temperature effect to account for the difference in O_2 response. The
417 paramagnetic oxygen analyzer has a temperature compensation circuitry that efficiently reduces
418 the impact of temperature fluctuations. In contrast, the zirconia-cell oxygen analyzer does not
419 fluctuate with temperature change owing to its high temperature operating capability. However, a
420 sudden shift in temperature or a high operating temperature may induce significant drift in the
421 fuel-cell O_2 analyzer (Lighton 2008), which the thermal compensation array may not correct. On
422 the other hand, the CO_2 analyzers offer far more stable readings with little to no drift.
423 Accordingly, the observed discrepancy between experimental and theoretical values for both
424 VO_2 and VCO_2 with increasing PBR (Figure 3-B) suggests that the effects of temperature on
425 flow rate may explain the poor performance of the Jaeger system compared to the other two IC
426 systems.
427
428 Reliability of the indirect calorimetry systems
429 Reliability refers to the reproducibility or repeatability of the readings of the gas analyzer under
430 identical conditions (Cooper and Storer 2001). The reliability of each IC system was determined
431 by calculating the coefficient of variation (CV) of VO_2 and VCO_2. Our results showed that CV

432   values of VO_2 and VCO_2 ranged from 1% to 5% and from 1% to 4% for the Sable and Moxus

433   systems, respectively. On the other hand, the Jaeger system had CV ranging from 7% to 10% for

434   VO_2 and VCO_2, respectively. Our data indicate that the Sable and Moxus systems provided more

435   reliable measures of VO_2 and VCO_2 compared to the Jaeger system.

436

437   Propane gas technique compared to other techniques

438   The present study was conducted to validate the outcomes of IC systems using commercially

439   available gas mass flow devices, and as such, it represents a novel approach in comparison to

440   techniques/procedures routinely performed in other human physiology laboratories. For example,

441   Melanson et al. (2010) conducted propane gas combustion tests to validate the outcomes of their

442   whole room IC system, which integrated similar Sable System technologies (the O_2 analyzer

443   incorporated a fuel cell rather than paramagnetic). The Sable system provided more than 98% of

444   the expected recovery of VO_2 and VCO_2 during the propane combustion trials. In addition to

445   validation through propane combustion tests, the authors also investigated the system's ability to

446   detect known changes in energy metabolism in humans transitioning from rest to light exercise,

447   and then to more moderate intensity exercise. Again, the whole room IC system performed as

448   anticipated. However, in the latter experiment, the expected accuracy of the measurements taken

449   at different metabolic rates is dependent on the systems linearity. Accordingly, performing the

450   propane combustion test at multiple PBRs, as outlined in the current study, would strengthen the

451   interpretation of these outcomes.

452

453   In a recent study, Rising et al. (2015) used propane gas combustion to determine the accuracy of

454   another whole room Sable metabolic system. The IC system was subjected to multiple (n=10)

455   propane (99.5% purity) combustion tests to simulate 24-hour metabolic measurements. The burn

456   rate (0.15 ± 0.025 g/min) was determined by obtaining the weight before and after completion of

457   each combustion test using a calibrated analytical balance. Although within acceptable and non-

458   statistically different limits, the Sable system was observed to under report mean VO_2 values

459   when comparing the outcomes of combustion to that of propane stoichiometry (Rising et al.

460   2015). In the current study, mean experimental values for VO_2 were higher than the

461   stoichiometry theoretical value at the five PBRs tested. Slight discrepancies between studies

462   could be explained by differences in oxygen sensor technology (i.e., fuel cell vs. paramagnetic),

463   indirect calorimetry method (i.e., ventilated hood vs. whole room) and validation procedure. The

464   current multiple burn rate procedure adds to previously implemented propane combustion

465   validation techniques by enabling the assessment of system accuracy and linearity within a single

466   session. Furthermore, assessment of the accuracy and linearity of metabolic outcomes could

467   allow for the correction of systematic error, which will be the focus of future work.

468

469   Other commonly used techniques to calibrate IC systems include alcohol combustion and butane

470   gas burning. The two methods bear on stoichiometry calculations similar to that for the

471   combustion of propane gas (i.e., O_2 is consumed, and CO_2 and water are produced) (Toien 2013).

472   For instance, Marks et al. (1987) calculated $\dot{V}O_2$ and $\dot{V}CO_2$ after burning known masses of

473   ethanol and methanol gases (pure alcohol) in an open-circuit IC system. The authors reported

474   that gas volumes differed by less than 5% from their theoretical values, confirming alcohol

475   combustion as a valid technique for calibrating IC systems in neonates. Furthermore, Miodownik

476   et al. (1998) described a methanol-burning lung model, which reported less than 0.005% reading

477   error attributed to carbon monoxide gas production. Finally, Nunn, Makita, and Royston (1989)

478   showed that a known amount of butane gas burned in a closed-circuit IC system yielded, for one

479   mole of butane, 6.5 moles of O_2, and an RER of 0.61, providing a simple and robust technique to

480   test IC system performance. However, although all these procedures produced good outcomes,

481   they are not routinely implemented in a manner to evaluate the linearity of IC systems. quickly

482   and efficiently. Therefore, the use of gas mass flow meter technology to monitor PBR represents

483   a more comprehensive procedure that will enhance the validation IC outcomes under a variety

484   experimental condition. In fact, Perez-Suarez et al. (2018) published a quality control study in

485   which they implemented the proposed PBR method based on our approach (Ismail 2017).

486

487 Conclusion

488   This work aimed to refine the propane gas validation technique. We had no intent to compare

489   indirect calorimetry systems to promote one over the others. Each system was built for specific

490   applications and, as such, differed from each other. The current technical report describes a

491   complimentary propane gas validation procedure to assess the accuracy and linearity of indirect

492   calorimetry. The IC systems output must yield an accurate fraction of gases for the computation

493   of $\dot{V}O_2$ and $\dot{V}CO_2$ and energy production in medicine, nutrition, and exercise sciences.

494 Generally, companies manufacturing indirect calorimetry systems provide customers with

495 calibration procedures to address accuracy issues. However, to ascertain the quality of data

496 acquisition, IC's accuracy and linear response should be validated regularly. We recommend

497 using the described multiple propane burn rate validation procedure to assess the accuracy and

498 linearity of IC systems.

499

500

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504 10. Declaration of interest

505 The authors report no declarations of interest.

506

507

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572

Figure 1

Table 1

Descriptive statistics of the Sable, Moxus, and Jaeger metabolic carts including the SD, SE,

CV of mean $\dot{V}O_2$ (ml min^{-1}), $\dot{V}CO_2$ (ml min^{-1}), and RER.

System	PBR	$\dot{V}O_2$ (ml•min^{-1})					$\dot{V}CO_2$ (ml•min^{-1})					RER				
		Mean	SD	SE	CV	Mean difference	Mean	SD	SE	CV	Mean difference	Mean	SD	SE	CV	Mean difference
Sable	200	263	14.0	5.7	0.05	-48	158	7.9	3.2	0.05	-29	0.60	0.01	0.00	0.02	0.00
	300	363	6.9	2.8	0.02	-40	215	4.5	1.8	0.02	-21	0.59	0.01	0.00	0.02	0.01
	400	478	5.8	2.4	0.01	-48	284	5.0	2.0	0.02	-26	0.59	0.01	0.00	0.02	0.01
	500	585	12.6	5.1	0.02	-47	346	6.4	2.6	0.02	-23	0.59	0.01	0.00	0.02	0.01
	600	685	9.0	3.7	0.01	-39	409	4.9	2.0	0.01	-22	0.59	0.01	0.00	0.02	0.01
Moxus	200	245	5.8	2.4	0.02	-30	148	3.7	1.5	0.03	-19	0.60	0.02	0.01	0.03	0.00
	300	368	4.2	1.7	0.01	-45	217	3.8	1.6	0.02	-23	0.59	0.01	0.00	0.02	0.01
	400	486	14.6	6.0	0.03	-56	288	8.8	3.6	0.03	-30	0.59	0.01	0.01	0.02	0.01
	500	583	21.8	8.9	0.04	-45	346	11.5	4.7	0.03	-23	0.59	0.02	0.01	0.03	0.01
	600	700	12.6	5.1	0.02	-54	418	6.0	2.5	0.01	-31	0.59	0.01	0.00	0.02	0.00
Jaeger	200	187	19.4	8.0	0.1	28	114	8.2	3.3	0.07	15	0.61	0.05	0.02	0.08	-0.01
	300	281	26.5	10.8	0.1	43	163	11.9	4.9	0.07	31	0.58	0.03	0.01	0.05	0.02
	400	368	40.6	16.6	0.1	62	208	20.1	8.2	0.1	50	0.56	0.01	0.01	0.02	0.03
	500	474	31.9	13.0	0.07	64	262	17.7	7.2	0.07	61	0.55	0.01	0.00	0.02	0.04
	600	560	42.2	17.2	0.08	86	307	26.8	11.0	0.09	80	0.57	0.01	0.01	0.02	0.05

PBR: propane gas burn rate, $\dot{V}O_2$: volume of oxygen, $\dot{V}CO_2$: volume of carbon dioxide, SD: standard deviation, SE: standard error. CV: coefficient of variance, RER: respiratory exchange ratio

Figure 2

Table 2

Linearity analysis of the Sable, Moxus, and Jaeger metabolic carts including Beta, R^2,

Confidence Interval (CI), and p-value of $\dot{V}O_2$ (ml min^{-1}) and $\dot{V}CO_2$ (ml min^{-1}) errors.

System	$\dot{V}O_2$ (ml•min^{-1}) Error				$\dot{V}CO_2$ (ml•min^{-1}) Error			
	β	R^2	95 % CI	p-value	β	R^2	95 % CI	p-value
Sable	0.998	0.996	(0.001, 0.001)	0.001	0.998	0.996	(0.001,0.001)	0.001
Moxus	0.996	0.992	(0.001, 0.001)	0.001	0.997	0.994	(0.001,0.001)	0.001
Jaeger	0.974	0.948	(0.001, 0.001)	0.001	0.944	0.942	(0.00, 0.001)	0.001

$\dot{V}O_2$: volume of oxygen per minute, $\dot{V}CO_2$: volume of carbon dioxide per minute

Figure 3

Table 3

Regression analysis of $\dot{V}O_2$ (ml min^{-1}), $\dot{V}CO_2$ (ml min^{-1}), and RER errors by propane flow levels which followed the Bland-Altman plots for each metabolic cart (Sable, Moxus, Jaeger).

System	$\dot{V}O_2$ (ml•min^{-1}) Error				$\dot{V}CO_2$ (ml•min^{-1}) Error				RER Error			
	β	SE	R_{adj}^2	t-value (p-value)	β	SE	R_{adj}^2	t-value (p-value)	β	SE	R_{adj}^2	t-value (p-value)
Sable	-0.168	0.000	-0.006	-0.903 (0.37)	-0.281	0.000	0.046	-1.552 (0.13)	-0.379	0.000	0.134	-3.848 (0.001)
Moxus	0.438	0.000	0.163	2.575 (0.02)	0.425	0.000	0.151	2.484 (0.02)	-0.162	0.000	-0.009	0.869 (0.4)
Jaeger	-0.536	0.000	0.262	-3.363 (0.002)	-0.807	0.000	0.639	-7.227 (0.001)	-0.679	0.000	0.441	-4.891 (0.001)

$\dot{V}O_2$: volume of oxygen per minute, $\dot{V}CO_2$: volume of carbon dioxide per minute, SE: standard error, RER: respiratory exchange ratio

Figure 4

Figure 1

Schematic representation of the in-house built propane gas device utilized to assess the accuracy and linearity of indirect calorimetry systems. The propane gas device encompasses a tank of chemically pure propane gas with a two-stage Western Medical gas regulator and its gas hose connected to a one-way gas mass-flow transducer subsequently connected to a gas mass-flow controller, and to a Bunsen burner. The burner is located into a 2.4 L glass canopy that flows into a 0.4 L glass tubing. From there, the entire system is connected to IC by a 1.4-inch diameter hose.
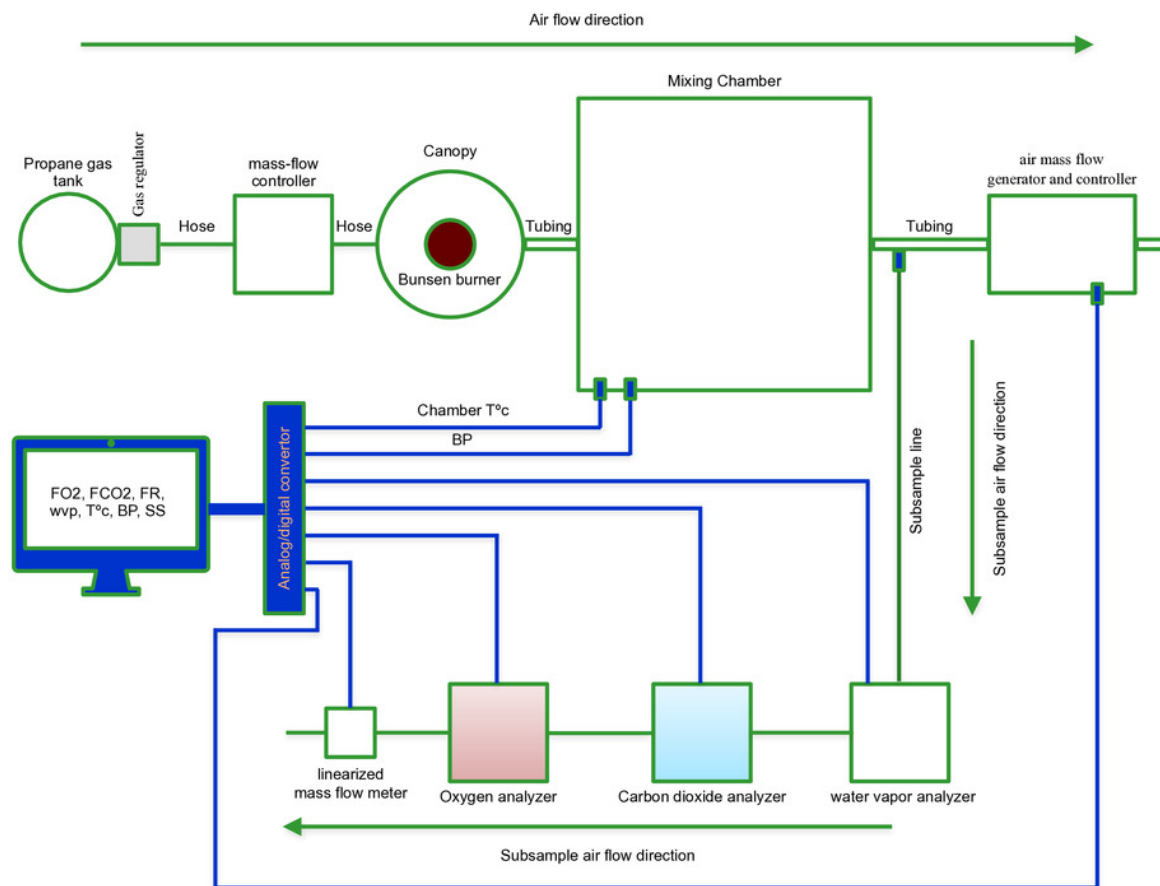
Figure 5

Figure 2

Mean rates of oxygen uptake ($\dot{V}O_2$, L min^{-1}; A), carbon dioxide production ($\dot{V}CO_2$, L min^{-1}; B), and respiratory exchange ratio (RER; C) measured during propane gas combustion tests with three metabolic carts: Sable system (dashed line), Moxus system (solid line), and Jaeger system (dotted line). Values are means SD.
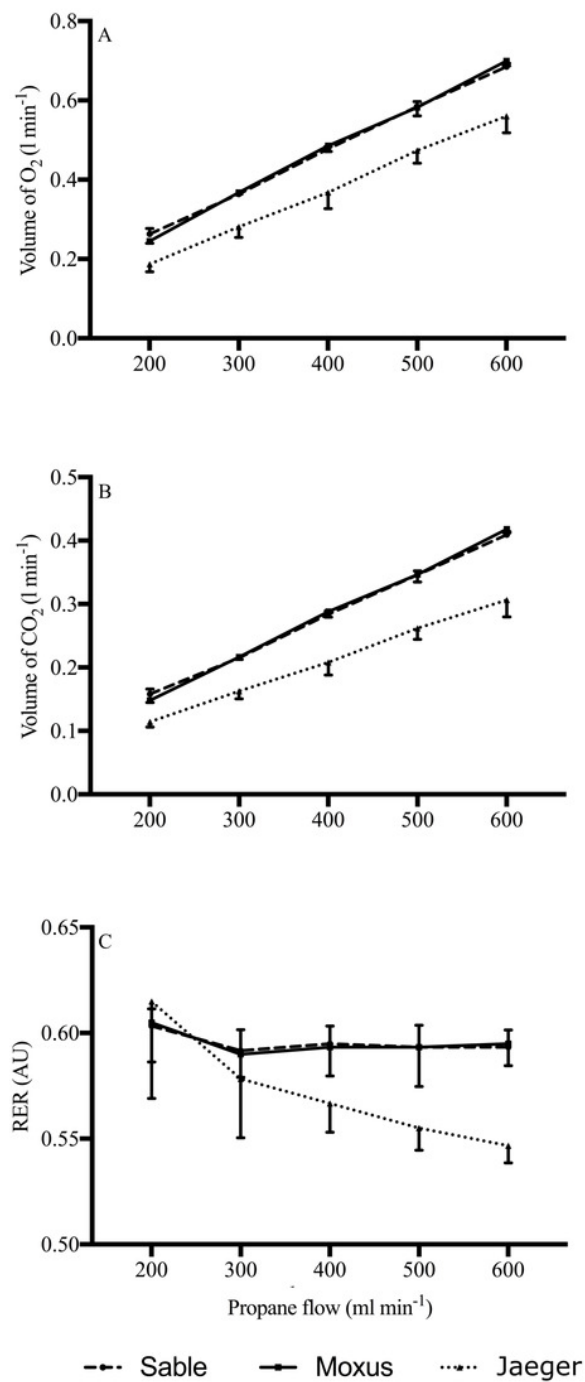
Figure 6

Figure 3

Mean difference (delta) between the stoichiometric theoretical values and the actual experimental values of oxygen uptake ($\dot{V}O_2$, L min^{-1}; A), carbon dioxide production ($\dot{V}CO_2$, L min^{-1}; B) and respiratory exchange ratio (RER; C) measured during propane gas combustion tests with three indirect calorimetric systems: Sable system (dashed line), Moxus system (solid line), and Jaeger system (dotted line). Values are means