Combined arm-leg ergometry in persons with a lower limb amputation

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Inter-observer and intra-observer reliability of determining aerobic threshold and respiratory compensation point using the combined arm-leg (Cruiser) ergometer in able bodied subjects and subjects with a lower limb amputation

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Abstract

Purpose: To analyze inter-observer and intra-observer reliability of determining first (VT1) and second (VT2) ventilatory thresholds in subjects with a lower limb amputation (LLA) and able bodied (AB) subjects during a peak exercise test on the arm-leg (Cruiser) ergometer.

Materials and Methods: Previously published data of exercise tests on the Cruiser ergometer of subjects with a LLA (n=17) and AB subjects (n=30) were analyzed twice by two observers. The VT1 and VT2 were determined based on ventilation plots . Differences in determining the VT1 and VT2 between the observers for the first and second analysis were analyzed. To quantify variation in measurement a variance component analysis was performed. Bland and Altmann plots were made and limits of agreement were calculated.

Results: The number of observations in which thresholds could not be determined differed significantly between observers and analysis. Variation in VT1 between and within observers was small (0-1.6%) compared to the total variation, for both the subjects with a LLA and AB subjects. The reliability coefficient for VT1 was > 0.75 and limits of agreement were good.

Conclusions: Intra- and inter-observer reliability of determining the VT1 on the Cruiser ergometer is good. Determination of VT2 was less reliable.

Keywords: ventilatory thresholds, ergometer, exercise test, exercise training, lower limb amputation.

Introduction

Most persons with a lower limb amputation (LLA) are elderly with a high prevalence of cardiovascular disease and low physical fitness [1-3]. Low physical fitness results in an undesirable decrease of activities and participation[4,5]. Therefore, it is important for persons with a LLA to start exercising prior to or as soon as possible after amputation in a safe, comfortable, and efficient manner to improve physical fitness. Before starting exercise training, a valid, reliable and safe physical exercise test especially with regard to cardiovascular risks, is required, to design exercise training programs. Additionally, outcomes of exercise tests should help to predict successful ambulation with a prosthesis[6-8]. To stimulate physical fitness as soon as possible after surgery, persons with a LLA have to be able to perform large muscle exercises even in the absence of a prosthesis . Testing and exercising on a bicycle ergometer is limited as persons with a LLA cannot make a complete cycling movement with one leg without help and a limited muscle mass is used because of the amputation[8].
The Cruiser ergometer, a combined arm-leg ergometer, is appropriate for persons with a unilateral LLA to test physical fitness[9-13]. Advantages of the Cruiser ergometer are that persons with a LLA can sit on it with adequate back support and support for the residual limb[13]; they can safely exercise with one leg, both arms and trunk without help of a test assistant and they use relatively large muscle mass.

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A protocol for peak cardiopulmonary exercise testing (CPET) can be applied on the Cruiser ergometer[13]. This protocol is used in lung and cardiac rehabilitation for evaluation of exercise intolerance and exercise-related symptoms which cannot be determined by means of resting pulmonary and cardiac function testing or submaximal testing[14,15]. Furthermore, on basis of such a peak exercise test the three-phase model of lactic acid accumulation potentially allows determination of a first (VT1) and second (VT2) ventilatory threshold. VT1 and VT2 are used to individually prescribe exercise training programs[16]. Training prescription based on these thresholds is expected to elicit better responses in improvement of exercise capacity after training than exercise prescription based on maximal oxygen uptake (VO_2max) or maximum heart rate (HR max)[17,18]. The VT1 and VT2 increase with age and are lower in arm exercises compared to leg exercises. Additionally they are protocol and disability specific, in which the best protocol to reach the maximal exercise capacity within 10-12 minutes has to be individually chosen[14]. It is unknown whether VT1 and VT2 can be reliably determined during peak exercise testing on the Cruiser ergometer. In addition, it is unknown whether differences exist in the level and correct determination of VT1 and VT2 between observers and if there are differences between subjects with a LLA and able bodied (AB) subjects.

The aims of the study were therefore to analyze inter-observer and intra-observer reliability of determining VT1 and VT2 in subjects with a LLA and AB subjects exercising on the Cruiser ergometer by two observers and secondly to analyze differences in VT1 and VT2 between subjects with a LLA and AB subjects.

Materials and Methods

Study design

The current study is based on re-analysis of data of two previous studies regarding standardized peak cardiopulmonary exercise tests on the Cruiser ergometer of subjects with a LLA (transtibial, knee disarticulation or transfemoral level; n=17; men14)[13] and AB subjects (n=30; men16)[10,]. Two experienced observers, a sports physician and a rehabilitation physician determined following a standardized protocol the VT1 and VT2 of both groups on two occasions at least 3 months apart.
The observers independently analyzed data were blinded for the results of each other and for their own results of the first assessment. The Medical Ethics Committee (METc) of the UMC Groningen had approved those studies (METc 2011/123 and METc 2005/237) and gave permission to re-analyze the data for this study.

Instruments and test protocol

The Cruiser ergometer[10,13] (Enraf-Nonius serial number: 3800EN014, Delft, The Netherlands) was used for the peak exercise test in both groups. The test protocol differed somewhat between the groups. Subjects with a LLA started with 3 minutes rest followed by a 3 minute warm-up at 20 W. After the warm-up the work load was increased by 10 W per minute until the point of exhaustion was reached or until the physician stopped the test. After completing the test, subjects were observed for another 3 minutes[13]. For the AB subjects the test started with 3 minutes rest, followed by a 5 minute warm-up at 50 W. After the warm-up the work load was increased by 30 W per minute for men and 20 W per minute for women until the point of exhaustion was reached or until the physician stopped the test. After the exercise test was terminated, a cooling down of 3 min was performed at 20 W[10]. Reasons to terminate testing were inability to maintain 50 rpm, pain in arms or legs or severe dyspnea. Testing was also stopped by the investigator in case of ECG abnormalities.

The feet of the user were placed against a fixed footrest on the Cruiser ergometer, which can be adjusted to the subjects' length. The subjects with a LLA performed the test without prosthesis. The residual limb rested on a support. The footrest was used to push off and move the seat backward. The handlebars are used to pull the seat forward again. In this way, arms, trunk and leg(s) overcome resistance provided by the ergometer in a cyclic multi-limb movement pattern. The ergometer was set in a constant power mode of between 35 and 60 revolutions per minute (rpm) and subjects were instructed to maintain a cadence of 50 rpm[10,13]. The accuracy of the Cruiser ergometer is within ± 10% power output (W) and ± 2 rpm for cadence. Cardiorespiratory outcomes were recorded using an Oxycon Delta (Jaeger, Bunnik, the Netherlands). Subjects wore a face mask and ventilation (VE, in l/min), oxygen uptake (VO_2, in l/min) and carbon dioxide output (VCO_2, in l/min) were measured breath by breath and plotted. Peak VO_2 and peak VCO_2 were defined as the highest average values obtained over a 30 second period. Blood pressure was measured manually at the beginning of the test, immediately after the test was completed, and after the cooling down period. Heart rate (HR in beats/min) was continuously monitored using a 12-lead electrocardiogram (ECG)[10,13].

Determination of the ventilatory thresholds

The VT1 and VT2 were determined based on ventilation plots as described by Wasserman et al.[19]. The VT1 was determined using three criteria, 1) intersection of a two line regression of the VCO_2 versus VO_2 (V-slope) graph, with a change of the slope from less than one to equal to one or greater than one, 2) first increase of VE/VO_2 versus workload (W) without a simultaneous increase in VE/VCO_2, and 3) first rise of $PETO_2$ (fraction of oxygen in the expired air), while $PETCO_2$ (fraction of CO_2 in the expired air) remains constant or is increasing. The VT2 was determined using three criteria, 1) inflection of VE versus VCO_2 (VE/ VCO_2

slope), 2) nonlinear increase of VE/VCO_2 versus W, and 3) deflection point of the end tidal $PETCO_2$[16]. The criteria are in order of preference: when the first criterion yielded adequately positioning, it was chosen; when the first criterion could not be reasonably applied, the second criterion was chosen, and so further. The observers assessed all three plots for each threshold and based their decision on the V-slope or the ventilatory equivalents; depending on which plot most clearly showed that particular VT1 or VT2. For each VT1 or VT2 determined the Oxycon Delta software calculated the VO_2 and HR. If an observer could not determine VT1 or VT2 it was recorded.

Statistical analysis

Differences between subjects with a LLA and AB subjects were analyzed by means of t-test for independent samples and Chi squared tests. Differences in determining the VT1 and VT2 between the observers for the first and second analysis was analyzed using Cochran's Q test with a post hoc pair wise with Bonferroni correction. To quantify variation in measurement results a variance component analysis (restricted maximum likelihood method) was performed for subjects with a LLA and AB subjects separately. Sources of variation included subjects (persons differ from each other) and observers (observers determine thresholds differently) and repeated analysis (results of the first vs second analysis). Negative variance components were set to 0. Based on the results of the variance components the error variance was calculated as the sum of the variances minus the variance due to subjects with a LLA. The error variance was thereafter divided by the sum of variances, resulting in a reliability coefficient. The following interpretation was used for the reliability coefficient with a minimum reliability 0.7 and clinically relevant at 0.9[20]. For AB subjects a similar procedure was followed. Limits of agreement were calculated and Bland and Altman plots were drawn for the outcomes percentage of VO_2 and HR on the VT1 and VT2. Statistical analyses were performed using SPSS (IBM SPSS Statistics 23).

Results

Baseline data of the subjects are presented in table 1. Subjects with a LLA were significantly older than the AB subjects.

Table 1. Subject characteristics

	Subjects with a LLA (n=17)	AB subjects (n=30)	Significance (p)	95 % confidence interval of the difference
Men/ women	14/3	16/14	0.06*	
Mean (SD) age in years	54.5 (18.6)	37.0 (10.0)	<0.001	(9.4; 25.7)
Mean (SD) BMI in kg/m^2	25.2 (4.0)	24.7 (2.5)	0.60	(-1.4; 2.4)
Median(IQR) time since amputation (months)	84.0 (4;144)	NA		

LLA= lower limb amputation, AB= able bodied, SD= standard deviation, BMI= Body Mass Index, NA= not applicable, IQR= interquartile range, *based on Fisher exact, all other p values based on independent sample t-test.

The number of observations in which thresholds could not be determined differed significantly between observers and tests (Cochran's Q test, p= <0.001). The number of observations in which the thresholds could not be determined was higher for VT2 than for VT1. The number of observations in which thresholds could not be determined was significantly lower for observer 1 compared to observer 2 in analysis 1 (p=0.008) and analysis 2 (p=0.001) (table 2).

Table 2. Number of observations in which observers could not determine the first ventilatory threshold (VT1) and/or the second ventilatory threshold (VT2) for all subjects (n=47)

	Observer 1		Observer 2	
	Analysis 1	Analysis 2	Analysis 1	Analysis2
VT1	2	2	3	4
VT2	6*	4#	16*	12#

*Difference between observer 1 and 2 is significant (p=0.008) # Difference between observer 1 and 2 is significant (p=0.001) Based on Cochran's Q test (P<0.001) and post hoc pair wise comparison with a Bonferroni correction for pairwise comparison.

Inter-observer and intra-observer reliability

Variation between and within observers was small (0-1.6%) compared to the total variation, for both subjects with a LLA and AB subjects (table 3). The reliability coefficients for VT1, expressed as VO_2 and HR at that threshold were respectively 0.89 and 0.75 respectively for the subjects with a LLA and 0.81 and 0.84 for the AB subjects. For VT1 expressed as VO_2 variation between observers contributed 0.0% (subjects with a LLA) and 3.9% (AB subjects) to the error variation. For the variation within observers these values were 6.6% (subjects with a LLA)

and 0.0% (AB subjects). For VT1 expressed as HR variation between observers contributed 1.2 % (subjects with a LLA) and 10.1% (AB subjects) to the error variation. For within observers variation these values were 0.0% (subjects with a LLA) and 0.0% (AB subjects) respectively. Variance components for VT2 were also calculated (table 3), but were based on a limited number of observations. Therefore these variance components estimation are difficult to interpret.
The limits of agreement for the VO_2 and HR at VT1 and VT2 for the subjects with a LLA and AB subjects are shown in table 4 and in the figures in the appendix (Fig S1-S8). There were varying differences in the limits of agreements between the subjects with a LLA and the AB subjects. In the plots there were no obvious differences between the observers (inter observer reliability) and between the analysis (intra observer reliability).
All the outcome parameters at VT1 and VT2 were significantly higher for the AB subjects than for subjects with a LLA (table 5).

Table 3. Variance component analysis (Restricted Maximum Likelihood Method) for subjects with a lower limb amputation (LLA) and able bodied subjects (AB)

Sources of variation	**LLA**	**LLA % of total var**	**LLA % of error var**	**AB**	**AB % of total var**	**AB % of error var**
VT1 VO_2 (l/min)						
Subject	114.42	88.6		192.38	81.1	
Observer	0.0*	0.0	0.0	1.77	0.7	3.9
Analysis	0.97	0.7	6.6	0.0*	0.0	0.0
Observer x Subject	5.58	4.3	37.9	0.0*	0.0	0.0
Subject x Analysis	0.0	0.0	0.0	21.08	8.9	46.9
Observer x Analysis	0.20	0.2	1.4	0.0*	0.0	0.0
Residual	7.95	6.2	54.1	22.11	9.3	49.2
Sum of var. comp.	129.12			237.34		
Reliability coeff.	0.89			0.81		
VT1 HR (beats/min)						
Subject	263.6	74.6		221.1	84.0	
Observer	1.1	0.3	1.2	4.3	1.6	10.1
Analysis	0.0*	0.0	0.0	0.0*	0.0	0.0
Observer x Subject	27.7	7.8	30.8	9.4	3.6	22.3
Subject x Analysis	6.7	1.9	7.4	7.1	2.7	16.8
Observer x Analysis	0.0*	0.0	0.0	0.0*	0.0	0.0
Residual	54.4	15.4	60.6	21.5	8.2	50.8
Sum of var. comp.	353.4			265.4		
Reliability coeff.	0.75			0.84		

Sources of variation	LLA	LLA % of total var	LLA % of error var	AB	AB % of total Var	AB % of error var
VT2 VO2 (ml/min)						
Subject	258.11	99.4		327.59	93.7	
Observer	0.0*	0.0	0.0	0.0*	0.0	0.0
Analysis	0.0*	0.0	0.0	0.0*	0.0	0.0
Observer x Subject	0.0*	0.0	0.0	4.96	1.4	22.3
Subject x Analysis	0.04	0.0	2.4	5.54	1.6	25.0
Observer x Analysis	0.0*	0.0	0.0	0.0*	0.0	0.0
Residual	1.49	0.6	97.6	11.71	3.3	52.7
Sum of var. comp.	259.64			349.80		
Reliability coeff.	0.99			0.94		
VT2 HR (beats/min)						
Subject	1028.6	99.6		181.7	82.3	
Observer	0.0*	0.0	0.0	0.0	0.0	0.0
Analysis	0.0*	0.0	0.0	1.3	0.6	3.4
Observer x Subject	0.0*	0.0	0.0	6.4	2.9	16.3
Subject x Analysis	0.0*	0.0	0.0	13.9	6.3	35.6
Observer x Analysis	0.0*	0.0	0.0	0.25	0.1	0.6
Residual	4.1	0.4	100.0	17.2	7.8	44.0
Sum of var. comp.	1032.7			220.8		
Reliability coeff.	1.00			0.82		

VT1= first ventilatory threshold, VT2= secondary ventilatory threshold, VO2= oxygen uptake, HR= heart rate, sum of var.comp= sum of variance component analysis, reliability coeff= reliability coefficient.

Table 4. Limits of agreement of the Bland and Altman plots

Limits of agreement (± 1.96 SD)	LLA subjects	AB subjects
VT1 VO_2 (l/min)	0.19	0.33
VT1 HR (beats/min)	15.08	10.04
VT2 VO_2 (l/min)	0.07	0.23
VT2 HR (beats/min)	3.36	9.19

VT1= first ventilatory threshold, VT2= second ventilatory threshold, VO_2= oxygen uptake, HR= heart rate, SD= standard deviation, LLA= lower limb amputation, AB= able bodied

Table 5. Differences of the outcome parameters between able bodied subjects and subjects with lower limb amputation

	Subjects with a LLA (n=17) Mean (SD)	AB subjects (n=30) Mean (SD)	Significance (p)	95 % confidence interval of the difference
VT1 VO_2 (l/min)	1.12 (0.35)	1.82(0.46)	<0.001	(0.45; 0.96)
VT1HR (beats/min)	117.9 (17.2)	136.9.1(15.3)	<0.001	(9.2; 28.9)
VT2 VO_2 (l/min)	1.42 (0.51)	2.33 (0.58)	<0.001	(0.56; 1.26)
VT2HR (beats/min)	130.9 (32.1)	156.2 (14.1)	0.001	(11.2; 39.3)

AB= able bodied, LLA= lower limb amputation, SD= standard deviation VT1= first ventilatory threshold, VT2= second ventilatory threshold, VO_2= oxygen uptake, HR= Heart rate

Discussion

Inter- and intra-observer reliability of determining the VT1 during CPET on the Cruiser ergometer was good for subjects with a LLA and AB subjects. For the VO_2 at VT1 the reliability coefficient for the subject with a LLA was 0.89 and for AB subjects 0.81. In contrast, the reliability coefficient for HR at VT1 was lower for the subjects with a LLA (0.75) compared to the AB subjects (0.84). The reliability coefficients of VT1 were all above the minimally required 0.7 but not above 0.9 which is a requirement for clinically application[20]. The values of VO_2 and HR at VT1 and VT2 were higher for the AB subjects than for the subjects with a LLA.

VT1 could be determined more reliably than VT2. Because VT2 could not be determined in all subjects, the number of observations involved in the variance components analysis and Bland and Altman analysis was considerably smaller (table 2) and the analysis of the inter- and intra-observer reliability of the VT2 was limited. In a similar study determining ventilatory thresholds in individuals with spinal cord injury, also about 10% of the ventilatory thresholds could not be determined, particularly for the VT2 in individuals with tetraplegia[21]. In that study intra-observer reliability for determining ventilatory thresholds was good, as in our study for the VT1. In a study determining ventilatory threshold in persons with a stroke the VO_2 at ventilatory threshold had a good inter-observer reliability with an ICC of 0.93[22]. However, in that study only VT1 was determined, on a treadmill. The VO_2 peak in that study was probably influenced by motor dysfunction after stroke resulting in low values of aerobic capacity, this effect was less pronounced for the VO_2 at ventilatory threshold[22]. In the current study the VO_2 at VT1 and VT2 was higher for the AB subjects in comparison with the subjects with a LLA. In addition to age, probably the LLA had not only an effect on VO_2peak[13], but also on ventilatory thresholds. Subjects with a LLA performed the exercise test on the Cruiser ergometer with one leg and two arms and the AB subjects with two legs and two arms. During upper body exercise ventilatory threshold can be reached at lower absolute VO_2 than during lower body exercise, whereas VT1 and VT2 occur at similar percentage of VO_2 max for both modes of exercise performed[23]. Subjects with a LLA use one leg and they had to exercise relatively more with their upper body. This difference in upper body exercise may explain lower values of VO_2 at VT1 and VT2 for subjects with a LLA compared to AB subjects.

A study limitation is the age difference between subjects with a LLA and AB subjects. As mentioned above, it was expected that the VO_2 at VT1 and VT2 was higher for the AB subjects than for subjects with a LLA because of the difference in performing the exercise test with one or two legs. However, the difference in age between the subjects with a LLA and AB subjects can also be an influencing factor on the difference in VO_2. Furthermore, the sample size of persons with a LLA was small and especially for the determining of inter- and intra-observer reliability of the VT2 the calculations were limited due to missing data (not being able to determine VT2).

Another limitation of this study was that data of performing CPET on the Cruiser ergometer twice in the same circumstances were not analyzed, so test-retest reliability of determining the VT1 and VT2 was not investigated. In addition, the outcome measure power output or work rate expressed in Watt was not used as an outcome measure because the software of the Oxycon Delta was not linked to the Cruiser ergometer and, consequently, the power output was not automatically described on VT1 and VT2. This shortcoming is a limitation, since power output can be important as an outcome measure in exercise intensity prescription, especially if the heart rate is influenced by medication[19]. Furthermore, a limitation was the difference in test protocol between the subjects with a LLA and the AB subjects. The warm-up period for subjects with a LLA was 3 minutes on 20 Watt and for AB subjects 5 minutes at 50 Watt. All the subjects with a LLA had the same test protocol, and for AB subjects there was a difference in test protocol for men and women. For some of the subjects with a LLA VT1 was determined during or soon after the warming up period of 3

minutes on 20 Watt.
For exercise prescription it is mostly recommended to use the VT1 and VT2[16]. Based on the results of this study for the Cruiser ergometer the use of only the VT1 is recommended, but further research to the clinically application is needed because the reliability coefficients were not above 0.9. To use the VT2, more research is needed to establish the reliability of determining VT2 in a larger sample of subjects with a LLA performing a CPET on the cruiser ergometer. Based on a CPET at the start of the rehabilitation after a LLA an individualized exercise intensity program can be composed to improve physical fitness, as an important cornerstone of recovery. In the present study, it was not investigated whether such an exercise intensity prescription based on VT1 or VT2 is more favorable to exercise prescription based on other variables as rate of perceived exertion, percentage of heart rate reserve or percentage of peak power output. This difference should be investigated in future research. Also training regimens based on VT1 and VT2, resulting from CPET on a Cruiser ergometer should be clinically evaluated on individual level.

Conclusion

The intra- and inter-observer reliability of determining VT1 during CPET on the Cruiser ergometer in this study is good for subjects with a LLA and AB subjects. In the current study populations determination of VT2 was less reliable. VT1 determined after an exercise test on the Cruiser ergometer can be helpful in prescribing the exercise intensity for a training program after a lower limb amputation.

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Appendix

Table S1. Results of the two analyses of the 2 observers of the first (VT1) and second (VT2) ventilatory threshold

	1st analysis				2nd analysis			
	1st observer		2nd observer		1st observer		2nd observer	
	LLA	AB	LLA	AB	LLA	AB	LLA	AB
VT1 VO_2	1.19	1.85	1.12	1.76	1.10	1.86	1.10	1.80
Mean (SD)	(0.36)	(0.51)	(0.38)	(0.45)	(0.33)	(0.50)	(0.37)	(0.49)
N valid	16	30	17	29	17	30	17	29
VT1 HR	120.3	138.4	115.7	135.0	117.8	138.1	117.2	135.7
Mean (SD)	(20.4)	(14.4)	(19.8)	(17.9)	(17.8)	(16.2)	(17.3)	(16.9)
N valid	16	29	17	27	17	28	17	26
VT2 VO_2	1.43	2.31	1.46	2.31	1.42	2.33	1.44	2.38
Mean (SD)	(0.51)	(0.54)	(0.56)	(0.62)	(0.51)	(0.60)	(0.59)	(0.67)
N valid	16	25	12	19	16	28	12	24
VT2 HR	130.8	157.2	137.5	157.7	131.0	154.2	132.8	155.6
Mean (SD)	(33.0)	(16.1)	(26.0)	(14.0)	(31.3)	(15.3)	(35.9)	(14.5)
N valid	16	25	12	19	16	27	12	23

VT1= first ventilatory threshold, VT2= second ventilatory threshold, VO_2 =oxygen uptake in l/min, HR= Heart rate in beats/min, SD= standard deviation, AB= Able bodied subjects, LLA= subjects with a lower limb amputation N valid= number of subjects who are involved in the analysis.

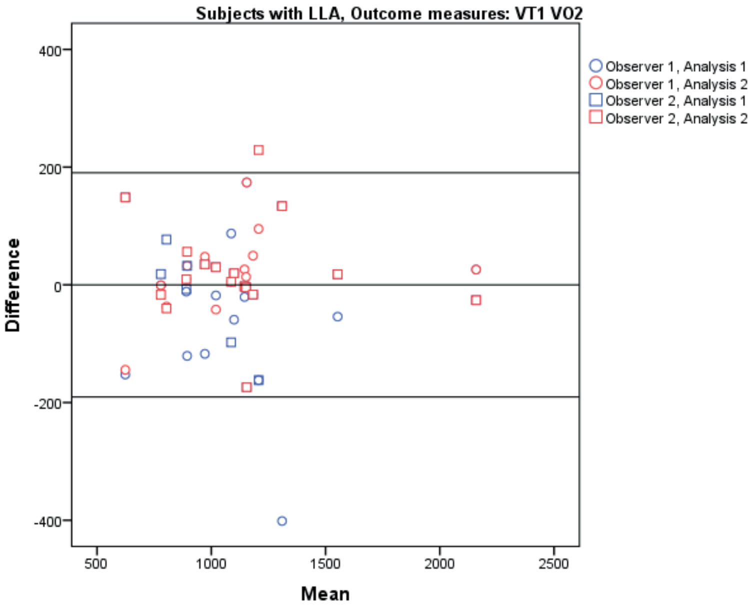

Figure S1

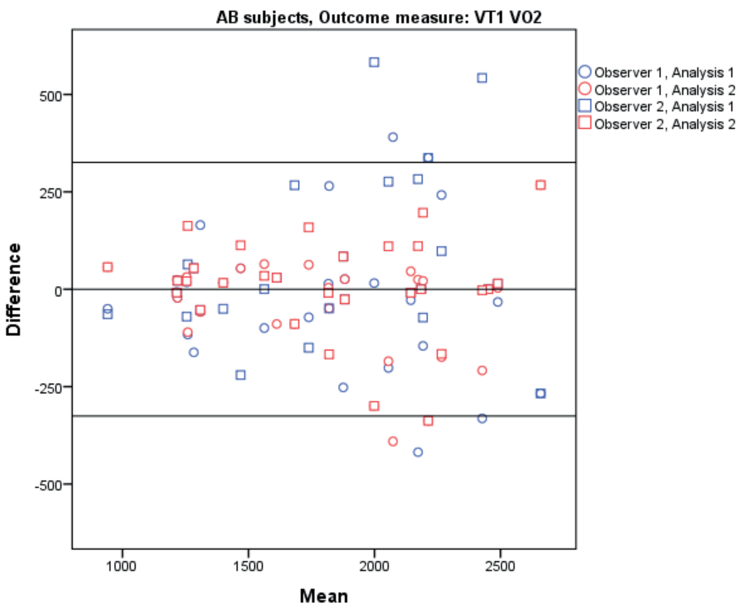

Figure S2

Figure S1 and S2: Bland and Altman plots for oxygen uptake (VO_2) at the the first ventilatory threshold (VT1) during the test on the Cruiser ergometer for subjects with a lower limb amputation (figure S1) and for the able bodied subjects (figure S2)

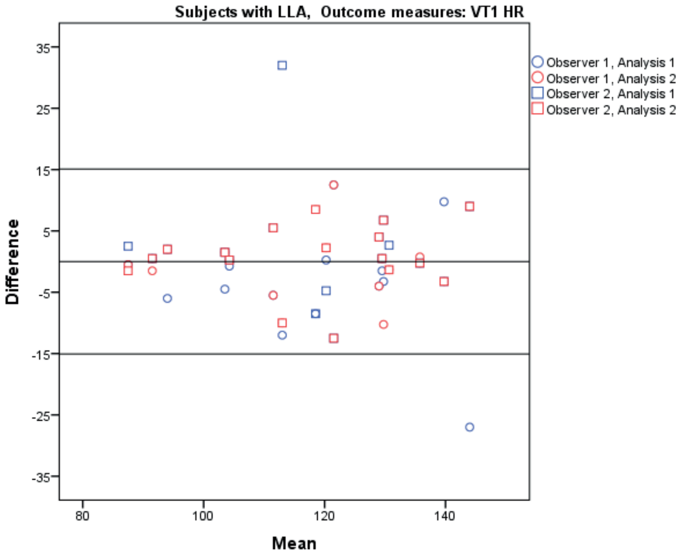

Figure S3

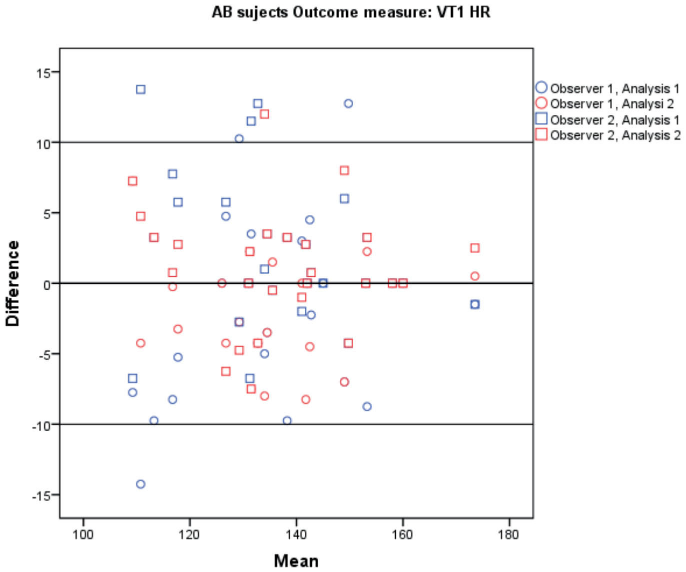

Figure S4

Figure S3 and S4: Bland and Altman plots for Heart rate(HR) at the first ventilatory threshold (VT1) during the test on the Cruiser ergometer for subjects with a lower limb amputation (figure S3) and able bodied subjects (figure S4)

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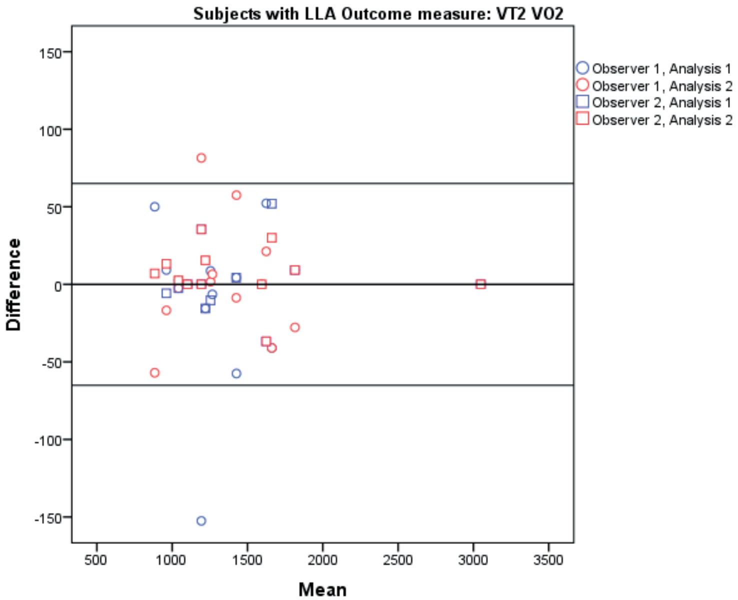

Figure S5

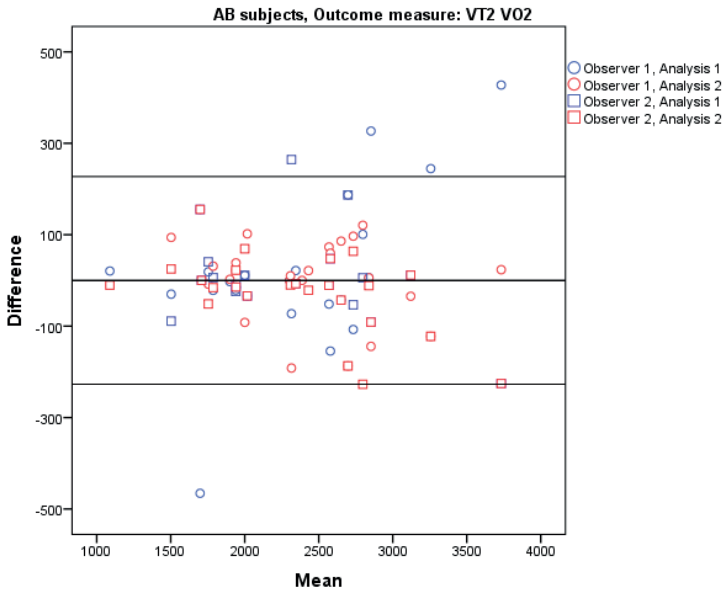

Figure S6

Figure S5 and S6: Bland and Altman plots for oxygen uptake (VO_2) at the second ventilatory threshold (VT2) on the Cruiser ergometer subjects with a lower limb amputation (figure S5) and for the able bodied subjects (figure S6).

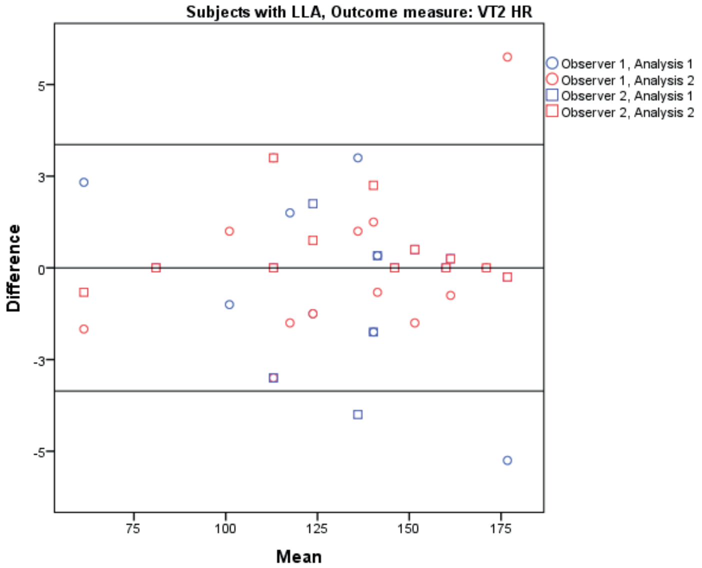

Figure S7

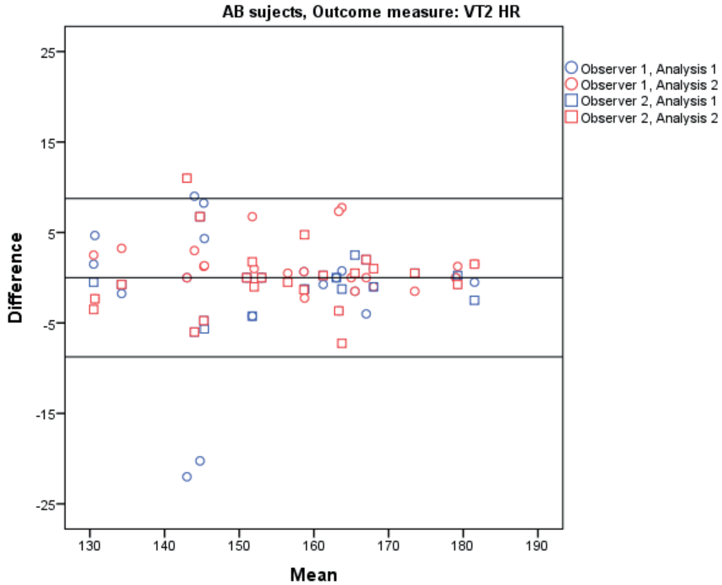

Figure S8

Figure S7 and S8: Bland and Altman plots for heart rate (HR) at the second ventilatory threshold (VT2) on the Cruiser ergometer subjects with a lower limb amputation (figure S7) and for the able bodied subjects (figure S8).