Article

Oxygen saturation behavior by pulse oximetry in female athletes. Breaking myths.

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Abstract: This study aims to demonstrate that the continuous measurement of blood oxygen saturation, through a pulse oximeter during a maximal exercise test in female athletes, is highly correlated with the second ventilatory threshold (anaerobic or VT_2). The latter can be used as an indicator of the decrease in peripheral oxygen saturation appearing in female athletes, during physical exertion, which is highly influenced and correlated by the physical fitness of the athletes. The measurements were performed with two pulse oximeters, during a maximum effort test on a cycloergometer, on a population of 27 healthy female athletes (25 Caucasian race and 2 black race), volunteers, aged (22.96 ± 6.19) (years); height (163.81 ± 6.89) and weight (57.23 ± 6.69) (kg). From the obtained results we conclude that pulse oximetry is a simple, fairly accurate, reproducible, and non-invasive method for studying the physical condition of athletes who perform physical exertion. We have observed in all the sportswomen, a common behavior of the evolution of oxygen saturation during an incremental exercise test. A relationship was observed between maximum oxygen consumption and the appearance of ventilatory thresholds, desaturation time, and total time of the test. The linear regression model of the desaturation time concerning the time of appearance of the anaerobic threshold in female athletes is capable of predicting the appearance of the anaerobic threshold or second ventilatory threshold at 86% of the time.

Keywords: Respiratory System; Sport; Oxygen; Blood Gas Monitoring; Pulse Oximeter; Saturation; Woman; Ventilatory Threshold

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1. Introduction

Women's sports history started back in the 19th century. Throughout history, women have had to fight for their self-improvement in the sports world, and have had to break down great social barriers until they have been recognized as having the right to practice different sports and to participate in competitions, including the Olympic Games [1–4].

The factors that have limited women's access to the sport have been social, political, religious, and biological. Until World War II, women's sport was considered an instrument of feminist liberation; it was seen as a marginal activity limited to a few women of masculine character [5–8]. The restriction was based in part on the idea that vigorous physical activity could impair women's health and adversely affect their reproductive capacity. These myths still survive today in some countries [2,9].

It was not until the 1970s that women began to train and compete in activities previously reserved for men. Scientific interest also began to be shown in their physiological responses to exercise or their influence on certain specific female functions such as menstruation or pregnancy [9,10]. It has always been believed that the anatomical differences between men and women, make men more suitable for strength sports and women those sports that required greater flexibility. [1,2].

However, nowadays, and for some decades, women's sport has been on the rise, dispelling myths from other times, based more on socio-cultural attitudes than on scientific research and data. Today, women's participation has reached areas previously considered exclusive to men, such as weightlifting or marathon running (until 1984 women were allowed to participate in a marathon [1,6–8,10]. In recent years, women have been breaking sporting records and are progressing faster and gaining access to new opportunities in aspirations where physical fitness is a prerequisite.

The recent progress of wearable sensors for continuous monitoring of physiological variables parameters has given evidence that using this technology to measure and quantify human responses to exercise has worthiness in improving the understanding of the exercise effects [11–13]. In particular, heart rate and oxygen saturation determination by photoplethysmography (PPG) constitute a key factor that provides relevant information to personalize training interventions [14–18]. The PPG sensor monitors differences in the light intensity between blood and the surrounding tissue [19,20]. These differences are associated with small variations in blood perfusion of the tissue providing information on the cardiovascular system, in particular, the pulse rate, oxygen saturation, blood pressure, and blood vessel stiffness [21]. The oxygen saturation (SO_2) in tissues is determined by optically quantifying the concentration of oxyhemoglobin (HbO_2) and deoxy-hemoglobin (Hb) [22].

Alterations in lung gas exchange occur during intense physical exercise [23]. This typically manifests as a decrease in arterial partial pressure of oxygen (PO_2), known as hypoxemia, and an associated increase in the alveolar-arterial O_2 difference $(A - aDO_2)$, which, potentially, can represent a significant barrier to endurance performance [24,25].

The aim of this study is to demonstrate a high temporal correlation between changes in the slope of blood oxygen saturation (SO_2), ventilatory thresholds (aerobic and anaerobic) in female athletes of different races (black and Caucasian), obtained by ergospirometry during a treadmill maximum stress test. Correlation analyzes were carried out between the time of appearance of the significant drop in continuous oxygen saturation with the aerobic threshold, anaerobic threshold, maximum oxygen consumption and the test time.

The manuscript is organized as follows...

2. Materials and Methods

2.1. Subjects

Twenty-seven active, healthy female volunteered for participation in this study and performed 2 incremental exercise tests on a treadmill in 2 separate sessions. The anthropometric and phenotype characteristics are presented in Table 1. Before admittance to the study, all subjects were evaluated for their cardiovascular health. None reported any respiratory or cardiac disease, presenting normal spirometric values. The exercise tests were performed in the Physiology Laboratory of the Professional School of Sport Medicine of the Faculty of Medicine (University Complutense of Madrid). In conformity with the review policy statement, the experimental protocol was approved by the local Ethics committee of the Hospital Clinico San Carlos (HCSC). All subjects gave written consent to participate once the procedure and risks of the study had been explained to them.

The criteria for subject selection were as follows: women aged from 18 to 55 performing regular practice of a competitive sport in national and regional tournaments for at least 2 years, prior to the study. All subjects trained 2 to 4 times a week between 1 and 3 hours/day. The volunteers maintained this sports practice until the day before the present study was carried out.

		Dark skin	Caucasian	Total
N		2	25	27
	Age	**Size (cm)**	**Weight (kg)**	**IMC**
$\overline{X}\pm$ SD	(22,96 $\pm$ 6,19)	(163,81 $\pm$ 6,90)	(57,24 $\pm$ 6,70)	(21,31 $\pm$ 1,98)
Minimum	14	155	41,7	16,7
Maximum	39	182	75,4	25,18

Table 1: Anthropometric and phenotype characteristic of the population studied. Values are expressed as mean $\pm$ standard deviation (SD).

2.2. *Protocol and Testing procedure*

The study protocol included anamnesis with clinical and training history, physical examination (cardiovascular and pulmonary auscultation, blood pressure, weight, and height measurements). It was followed by a maximal treadmill incremental exercise test with continuous electrocardiographic (ECG) recording, ergospirometry breath-by-breath gas analyzer, and continuous pulse oximetry recording during warm-up, maximal exercise, and recovery using a commercial pulse oximeter (Pulsox-3i Minolta).

During the athlete preparation, 10 ECG electrodes were placed for the 12-lead EKG reading as Fig. 1 shows, prior preparation of the area (shaving and alcohol sterilization) to ensure correct positioning of the electrodes while wearing a tubular mesh top. Subsequently, blood pressure was taken to establish a baseline measurement, and electrocardiographic readings were taken at rest in supine and standing positions. At the beginning of the test, time and data were synchronized among ergospirometry and oximeter measurements. Parameters readings and measurements during the stress test were collected every second.

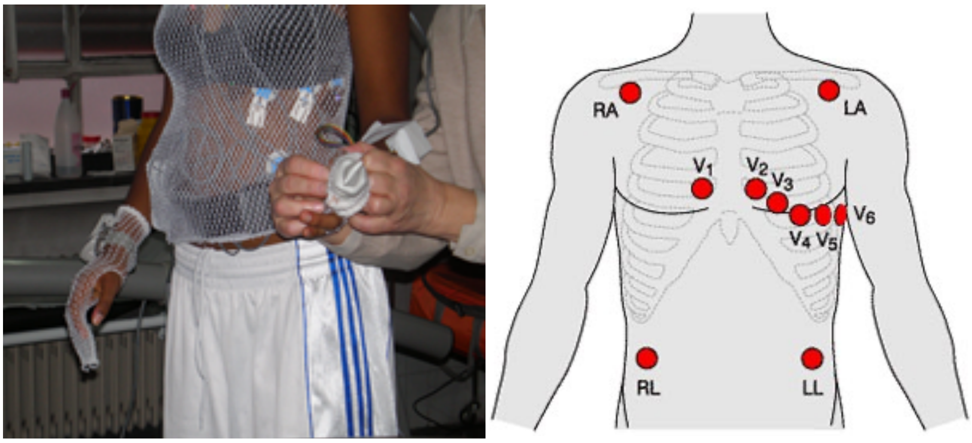

Figure 1. Placement of ECG electrodes in stress test.

Firstly, a pre-stress test forced spirometry was performed. In order to optimize the correct reading of oxygen saturation, the area where the pulse oximeter was placed (third or fourth finger of the right hand) was cleaned with hydrophilic absorbent cotton soaked in alcohol (see Fig. 2). After a minute of auto-calibration, the oximetry recording started, and ECG and oximeter heart rates were compared. A sphygmomanometer was also used to measure blood pressure during the test.

Secondly, a mask was placed over the athlete's nose and mouth to prevent air leakage and allow for proper analysis of expired gases as Fig. 3 shows. Before the stress test on a treadmill ergometer (HP Cosmos QUASAR 4.0) started, baseline data was collected while the athlete stood for one minute. In the warm-up, the athlete began to walk at 6 km/h and 1 % slope during 2 min. The athlete then started the effort phase

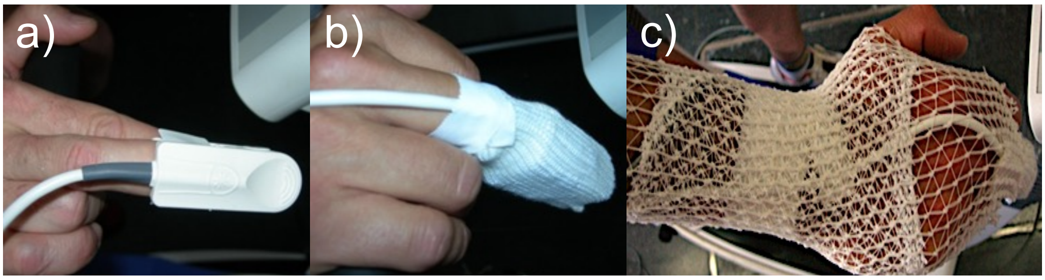

Figure 2. Positioning of the pulse oximeter sensor (a), its protection (b), and hand position (c).

running at 8 km/h and 1 % slope. When the maximum effort condition was attained, the athlete held the protective bars and jumped off the treadmill. The maximum speed achieved varied among individuals. When the speed of 14 km/h was reached, the slope was increased to 3 %. Afterward, the slope was maintained constant, while speed was increased every 2 min by 2 km/h until they were unable to continue. Active recovery was performed for 2 min at 8 km/h with a slope of 0 %. The ECG readings were taken every 10 s, averaging the last eight heartbeats. At different stages of the test, once the athlete was running at fixed speed and slope, the full-step rate (SR, in steps /min) was obtained by counting manually the number of steps in a 10 s interval, and the one-foot step was derived from that. Blood pressure was taken immediately upon test completion and at 3 and 5 minutes during the recovery period.

Every one of the athletes followed the same exercise protocol. The only difference between the tests performed by each athlete was the level of effort (stage) reached by each one, depending on their physical capacity. Upon the test's completion, the oximeter and ergospirometer were also disconnected.

Figure 3. Athlete with all sensors connected on the treadmill: oximeters attached to the hands, electrodes for electrocardiographic recording, and mouthpiece with flow analyzer

Both, personal data and obtained data from ergospirometry, oxygen saturation pulse oximetry, and lactic acid measurement devices were recorded in protocol pages and next entered into anonymized databases. Once the treadmill exercise test was performed, the ergospirometric and the commercial pulse oximeter data were compared over time by a statistical study of the variables involved.

2.3. Statistical analysis

For the statistical analysis, the quantitative variables were summarized in their mean, $\overline{X}$, and standard deviation (SD). Pearson's linear correlation coefficient and linear regression analysis were calculated to determine de relationships between the different variables. The comparison between times and independent variables of two categories was performed using Student's t-test for independent samples. The comparison of qualitative variables with more than two categories with quantitative variables was performed using one-factor analysis of variance (ANOVA). The time-independent effect of each of the evaluated parameters was studied through an analysis of covariance. Statistical significance was defined at the $p < 0.05$ level. All statistical analyses were performed using IBM SPSS Statistics software program version v.15.0 (SPSS Inc.Chicago, IL, USA).

3. Results

The results obtained from the stress test for the total population of 25 healthy Caucasian and 2 dark skin female athletes are shown in Table 2. They practiced different multi-sprint-based sports, in particular, 14 volunteers practiced 11-a-side football (aerobic-anaerobic sport), 10 long-distance athletics (aerobic sport), 1 sprint athletics (anaerobic sport), and 2 basketball (aerobic-anaerobic sport). The maximum heart rate (HR) reached, the total duration of the test (min), the time at which oxygen desaturation begins to occur (desaturation time), basal oxygen saturation values, and the total decrease in oxygen saturation observed during the test (i.e., the difference between the basal saturation and the minimum oxygen saturation values (DBMS) reached during the test) were measured following the protocol described in section 2.2 . The observed $VO_{2,max}$ ranged from 37.09 to 64.78 ml/(kg·min) (with a mean of 48.9 ml/(kg·min) and a standard deviation of 7.61 ml/(kg·min)); the maximum HR ranged between values of 168 and 205 bpm, with a mean of 189.81 bpm and a standard deviation of 8.54 bpm; the test time of between 7: 55 minutes and 14:00 minutes, with a mean of 10:45 minutes and a standard deviation of 1:27 minutes; the anaerobic threshold (AT) onset time fluctuated between 4:20 and 8:42, with a mean of 6 and a standard deviation of 0:05; the desaturation time was detected between 6:17 and 10:50, with a mean of 8:57 and a standard deviation of 1:18 (see tables 8, 9 and 10).

	$\overline{\mathbf{X}}\pm$ **SD**
HR max	189,81 ± 8,54
Test total time (min)	10,759 ±1,453
Basal VO_2 (ml/(kg·min))	4,693 ±1,527
$VO_{2,max}$ (ml/(kg·min))	48,90 ± 7,62
Desaturation time (min)	8,969 ± 1,317
Basal saturation	98,074 ±0,616
Difference basal saturation - Minimum saturation	5,815 ± 2,058

Table 2: Ergospirometry measured variables for 25 healthy caucasian and 2 dark skin female athletes obtained from the stress test.

In order to facilitate the subsequent statistical study, Tab. 3 shows the studied subjects divided according to their state of physical fitness based on their maximum oxygen consumption, as follows:

One of the objectives of this study was to assess whether variations in oxygen saturation may be related to the appearance of the aerobic threshold (AeT). For this purpose, oxygen saturation was analyzed by observing the time at which an oxygen saturation decrease occurred before the aerobic threshold, called T_1. Figure 4 shows a flowchart representing the different events of the effort test (aerobic threshold, anaerobic

Physical fitness condition	$VO_{2,max}$	Frequency	Percentage
Medium	30-40 ml/(min·kg)	4	14,8 %
Good	40-50 ml/(min·kg)	11	40,7 %
Excellent	>50 ml/(min·kg)	12	44,4 %

Table 3: Descriptive variables of the population according to physical fitness condition.

threshold), as well as the time oxygen saturation variations (desaturation prior to the aerobic threshold, desaturation time, and maximum desaturation time). T_1: time to obtain the minimum saturation before AeT. T_2: time to reach AeT from reaching the minimum oxygen saturation value. T_3: time to observe the minimum oxygen saturation after the AeT. T_4: time from obtaining the maximum oxygen saturation value observed before reaching AT.

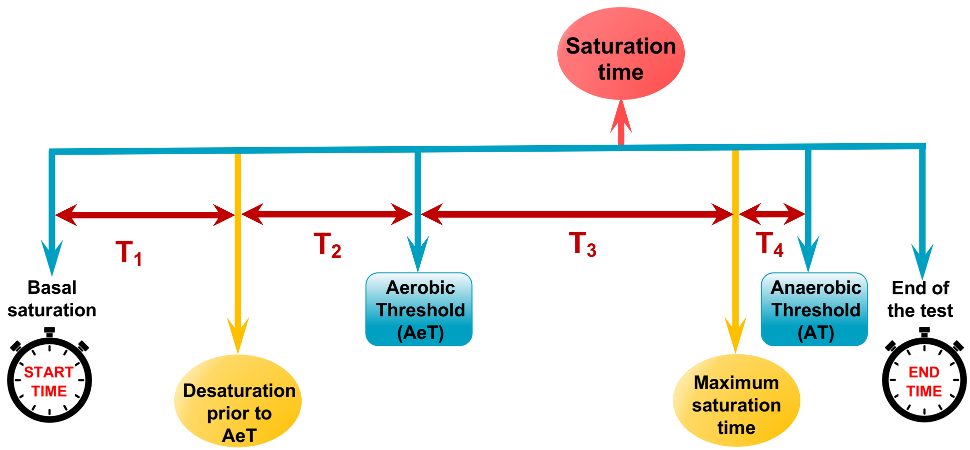

Figure 4. Flowchart representing the aerobic threshold (AeT), the anaerobic threshold (AT), and the time oxygen saturation variations (desaturation prior to the AeT, desaturation time, and maximum desaturation time). T_1: time to obtain the minimum saturation value before AeT. T_2: time to reach AeT from reaching the minimum oxygen saturation value. T_3: time to observe the minimum oxygen saturation value after the AeT. T_4: time from obtaining the maximum oxygen saturation observed before reaching AT.

Subsequently, a statistical study of the Pearson correlation coefficient between the appearance time of the desaturations and their correlations with the appearance time of both the aerobic and anaerobic threshold was carried out. For this purpose, a univariate study was made for each instant time (AeT time, AT time, desaturation time, total test time, and minimum saturation value time) and their relationship with the independent variables of the study. For the relationship between quantitative variables, Pearson's linear correlation coefficient was calculated. This coefficient has the property of being between +1 (perfect positive linear association) and -1 (perfect negative linear association). A null value does not indicate the absence of a relationship, but rather the absence of a linear association between the variables.

Table 4 shows the Pearson correlations of the appearance time of both thresholds (AeT and AT), the time of desaturation, the time of duration of the test, the total decrease in oxygen saturation in the test, the total test time, the difference between the basal saturation and the minimum oxygen saturation values (DBMS), as well as the time of the test at which the maximum level of oxygen saturation occurs versus the quantitative independent variables. The values with a significance level $p<0.05$ are marked in bold.

Table 5 shows an analysis of covariance to study the relationship of the variables with the anaerobic threshold time, aerobic threshold time, desaturation time, total test time, the difference between the basal saturation and the minimum found in the

	AeT time (min)	AT (min)	Desaturation time (min)	Total test time (min)	DBMS (min)	T_3 (min)
Age	-0,068	0,036	0,176	0,024	-0,196	0,126
Size	0,200	0,317	0,313	0,307	0,189	0,239
Weight	0,010	0,031	0,187	-0,079	0,139	0,054
IMC	-0,168	-0,24	-0,038	-0,365	-0,011	-0,138
VO_2 basal	0,096	0,025	0,050	0,132	-0,2	0,083
$VO_{2,max}$	**0,469**	**0,671**	**0,466**	**0,620**	0,366	**0,500**
HR max	-0,039	-0,190	-0,157	-0,201	-0,144	-0,058

Table 4: Pearson correlation and significance level between the variables from ergospirometry and the anthropometric variables of the study subjects. Values with a significance level p<0.05 are marked in bold.

ergospirometry, and the time at which the maximum value of oxygen saturation appears. Phenotype, physical fitness and type of sport practiced were introduced as covariables. The values with a significance level p<0.05 are marked in bold.

	AeT time (min)	AT (min)	Desaturation time (min)	Total test time (min)	DBMS (min)	T_3 (min)
Phenotype						
Caucassian	5,97 ± 0,94	9,56 ± 1,14	8,84 ± 1,28	10,65 ± 1,42	5,8 ±1,95	9,91 ± 1,62
Dark skin	7,16 ± 0,23	11,33 ± 0,47	10,58 ± 0,35	12,08 ± 1,53	6 ± 4,24	11,75 ± 1,06
Physical fitness						
Excellent	6,13 ± 0,82	**10,11 ± 1,08**	**9,08 ± 1,24**	**11,29 ± 1,22**	6,66 ± 2,18	**10,44 ± 1,59**
Good	6,34 ± 1,00	**9,84 ± 0,88**	**9,51 ± 0,94**	**10,87 ± 1,36**	5,45 ± 1,81	**10,48 ± 1,12**
Medium	5,04 ± 0,63	**8,00 ± 0,96**	**7,12 ± 0,84**	**8,83 ± 0,72**	4,25 ± 1,25	**7,66 ± 1,03**
Type of practiced sport						
Aerobic	5,80 ± 0,61	9,90 ± 1,09	8,73 ± 1,03	10,80 ± 1,18	7,2 ± 1,92	9,40 ± 0,71
Anaerobic	6,25 ± 0,84	10,00 ± 1,05	9,22 ± 1,43	11,19 ± 1,32	5,33 ± 2,06	10,72 ± 1,89
Mixed	6,07 ± 1,10	9,51 ± 1,30	8,94 ± 1,41	10,58 ± 1,61	5,56 ± 2,03	1,000 ± 1,74

Table 5: Correlations between the appearance time of the AeT, the AT, the time of desaturation, and the total duration of the test, with respect to phenotype, physical fitness condition and type of sport practiced expressed by $\overline{X}\pm$ SD. Values with a significance level p<0.05 are marked in bold.

Concerning the AT, we similarly performed the study as was done for the AeT. First, we calculated the time from the AeT to obtain the maximum value of oxygen saturation observed before the AT (T3), as well as the time from obtaining the maximum value of oxygen saturation to reach the AT (T4) as shown in Figure 4. Subsequently, the relationship of the phenotype and the physical condition with the AT appearance was studied by calculating Pearson's linear correlation coefficient and linear regression.

Table 9 shows the Pearson correlations and levels of statistical significance analyzed for the AT and the variables derived from the variations in oxygen saturation values occurring before the appearance of the AT and after the AeT. It can be observed a Pearson correlation coefficient close to 1 (0.892) statistically significant (p=0.000) when correlating the AT appearance time with the desaturation time. There also appears to be a statistically significant (p=0.048) the influence of T4 on the AT appearance of with Pearson correlation of 0.383.

In the case of T_4, the longer the T_4 is, the later the AT appears with a Pearson correlation of 0.274, without reaching values of statistical significance (p=0.167).

When comparing how both times, T_3 and T_4 could influence each other, we observed a strong negative correlation between them (-0.849), which reaches the level of statistical

		AT time (min)	T_3 (min)	T_4 (min)	Desaturation time (min)
AT time (min)	r(p)	1	-0,055	0,383*	0,892**
	p		0,785	0,048	0,000
T_3 (min)	r(p)	-0,055	1	-0,849**	-0,014
	p	0,785		0,000	0,944
T_4 (min)	r(p)	0,383*	-0,849	1	0,264
	p	0,048	0,000		0,184
Desaturation time (min)	r(p)	0,892**	-0,014	0,264	1
	p	0,000	0,944	0,184	

Table 6: Pearson correlation and significance level between AeT time and variables derived from oxygen saturation data. Data marked with * have a bilateral significance level of 0.05 while data marked with ** have a bilateral significance level of 0.01.

significance (p=0.000), i.e., as T_3 increases, T_4 decreases, as can be seen in the regression model depicted in Figure 5.

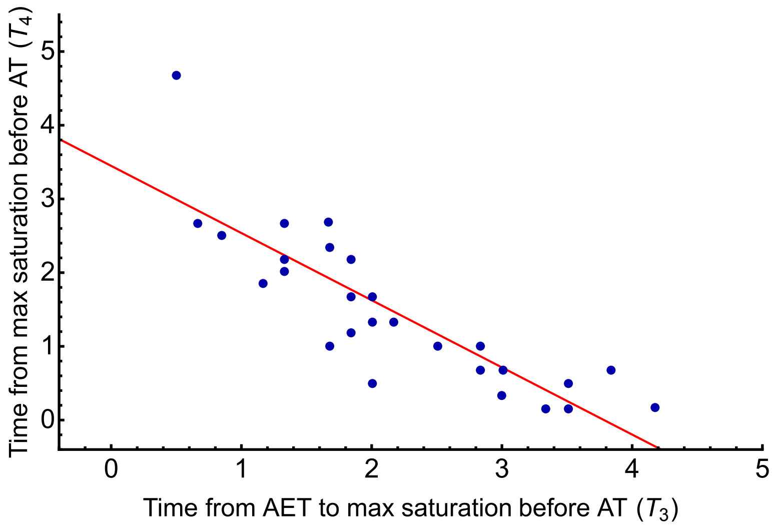

Figure 5. Regression line of the time from the AeT to obtain the maximum oxygen saturation value observed before the AT (T_3) versus the time from obtaining the maximum oxygen saturation value to reach the AT (T_4).

The linear regression model of the saturation time accurately predicts the extracted data. We found a beta coefficient of 0.812 with p=0000 that shows statistical significance. Hence, it can be prophesied that for every minute that desaturation takes to appear, the aAT takes 0.812 minutes to appear. The coefficient of determination R2 is 0.796, which shows that our line predicts 79.6% of the values, that is, it explains 79.6% of the variability of the AT, as it can be seen in Table 10. Figure 11 shows the linear regression model that relates the desaturation time versus the AT time.

4. Discussion

Studies of the evolution of (SO_2) in women during maximal exercise near sea level have shown that women have an early decrease in pulmonary gas exchange during exercise, before that of men [24]. This hypothesis is supported by the fact that the evolution of oxygen saturation during physical effort in women appears to be less than that observed in men of similar age, height, body mass and levels of effort. Wrongly considering that the lung of the adult woman has a lower vital capacity, airways of smaller diameter and a smaller diffusion surface than that of men [26–29]. Besides, maximal effort studies performed in women in both normoxic and hyperoxic environment at second ventilatory threshold (VT_2) assert that the oxygen desaturation observed in women is the main limiting factor in achieving higher $VO_{2,max}$ levels rather than the athlete's metabolic and muscular capacity [30]. Indeed, some studies indicate that women are especially vulnerable to experiencing greater exercise-induced arterial hypoxemia (EIAH) due to airflow limitation [24,31] as a consequence of relatively smaller lung size.

On the other hand, physically active women have been found to develop a decrease in oxygen saturation at markedly lower oxygen intakes compared with those observed in men [30,32]. For example, oxygen desaturation occurs only in males with $VO_{2,max} > 60$ ml/(kg·min), however, some studies have found significant desaturations in females with $VO_{2,max}$ of 40-55 ml/(kg·min) [30,32]. Although there is evidence that hypoventilation may play a role in decreased pulmonary gas exchange in women during exercise, it appears that ventilation cannot fully compensate for the increased $(A - aDO_2)$ [31]. However, no study has attempted to assess the relative contribution of the mismatch between the ventilation (V)/perfusion (P) ratio (V/Q) and the diffusion limitation to $(A - aDO_2)$ during exercise in healthy women compared with men, independent of the effects of lung size on pulmonary gas exchange [23,24,31].

The multiple inert gas elimination technique (MIGET) is a method that can be used to specify the contribution of the V/Q imbalance and O_2 diffusion limitation to pulmonary gas exchange [33]. Although there have been many studies that have used MIGET to investigate gas exchange in healthy men during exercise [34–37], only a few studies have included women [24,38,39].

The MIGET technique was used to determine whether healthy physically trained women would develop greater V/Q ratio imbalance and/or O_2 diffusion limitation during exercise compared with men [24]. The results of that study with eight women and seven men of the same age, height, and $VO_{2,max}$ during the performance of an exercise cycle under normoxic and hypoxic conditions showed that the resting lung function, as well as the arterial PO_2 desaturation, and the PCO_2 alveolo-arterial difference of O_2 $(A - aDO_2)$ were similar in both sexes. However, carbon monoxide diffusing capacity (DLCO) was lower in women ($p < 0.05$).

Other studies assert that acute ventilatory response to hypoxia (AHVR) is not related to the development of EIAH during maximal exercise in trained endurance cyclists and untrained individuals (men or woman) [39,40]. The AHVR was related to peak oxygen consumption, but not to oxygen saturation. Oxyhemoglobin saturation SO_2 values were lower in trained compared to untrained men and women $(94.4 \pm 0.8\%$ vs $94.3 \pm 0.7\%)$ $(p < 0.05)$. Trained female cyclists demonstrated EIAH to the same degree as trained male cyclists, and that some individual untrained females also exhibited EIAH. This is consistent with the possibility that healthy young women might be especially vulnerable to exercise-induced pulmonary limitation. However, it is striking that other studies attribute the presence of decreases in peripheral oxygen saturation to inconclusive problems of the respiratory system inadequacy to physical exertion, especially in women [31,41,42].

Concerning the influence on the AeT appearance, the time T2 is the one that presented statistical significance after adjusting for the 2 variables of our sample (phenotype and physical condition). Hence, we found that T2 is related to the time of the AeT appearance.

On the other hand, concerning the influence on the AT appearance, the desaturation time presented statistical significance after adjusting for the same 2 variables (phenotype and physical condition). Therefore, it was found that they are related to the time of appearance of the AT.

5. Conclusions

This section is not mandatory, but can be added to the manuscript if the discussion is unusually long or complex.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; methodology, X.X.; software, X.X.; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.", please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Funding: Please add: "This research received no external funding" or "This research was funded by NAME OF FUNDER grant number XXX." and and "The APC was funded by XXX". Check carefully that the details given are accurate and use the standard spelling of funding agency names at https://search.crossref.org/funding, any errors may affect your future funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Hospital Clinico San Carlos (HCSC).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: The authors declare that they do not have any financial interest or conflict of interest regarding the study.

Abbreviations

The following abbreviations are used in this manuscript:

VT_2	Second ventilatory threshold
PPG	Photoplethysmography
SO_2	Oxygen saturation
HbO_2	Oxyhemoglobin concentration
Hb	Deoxy-hemoglobin
PO_2	Partial pressure of oxygen
PCO_2	Partial pressure of carbon dioxide
$A - aDO_2$	Alveolar-arterial O_2 difference
$VO_{2,max}$	Maximal oxygen uptake
EIAH	Exercise-induced arterial hypoxemia
V/Q	Ventilation (V)/Perfusion (P) ratio
MIGET	Multiple inert gas elimination technique
AHVR	acute hypoxia ventilatory response

Appendix A

Appendix A.1

A statistical study of Pearson correlations was performed concerning the evolutionary and temporal parameters of oxygen saturation and the aerobic and anaerobic thresholds, obtained by ergospirometry. Table 7 shows the Pearson correlations and statistical significance levels analyzed with respect to the AeT and the variables derived from the variations in oxygen saturation values that occurred before the appearance of this threshold.

		AeT time (min)	DBMS (min)	Desaturation time (min)	T3 (min)
AeT time (min)	r(p)	1	0,044	0,375	0,274
	p		0,827	0,054	0,167
DBMS (min)	r(p)	0,044	1	0,248	-0,228
	p	0,827		0,213	0,253
Desaturation time (min)	r(p)	0,375	0,248	1	-0,789**
	p	0,054	0,213		0,000
T3 (min)	r(p)	0,274	-0,228	-0,789**	1
	p	0,167	0,253	0,000	

Table 7: Pearson correlation and significance level between AeT time and variables derived from oxygen saturation data. Data marked with ** have a bilateral significance level of 0.01.

We observed that the longer the T_1, the longer the time of appearance of the AeT (Pearson correlation of 0.375), although it does not reach values of statistical significance (p=0.054). As for T_2 the longer it lengthens, the later the AeT appears (Pearson's correlation of 0.274), without reaching values of statistical significance (p=0.167). When comparing how both times, T_1 and T_2, could influence each other, we observed a negative correlation between them (-0.789), i.e., as T_1 increases, T_2 decreases, as can be seen in Figure A1.

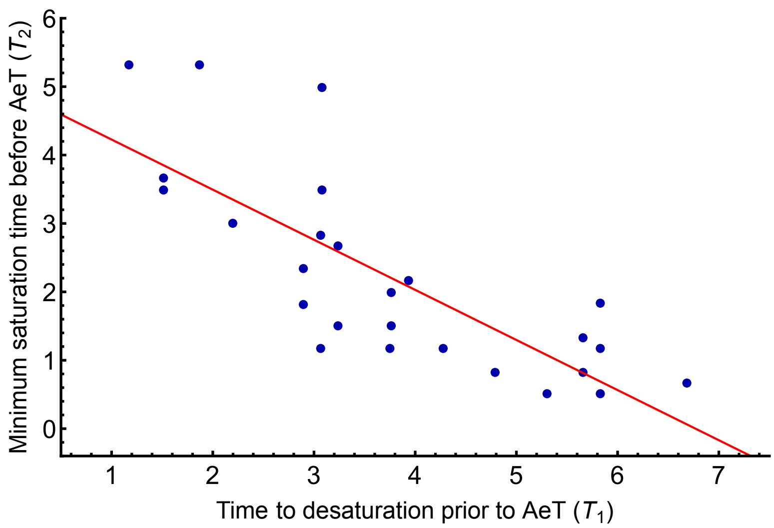

Figure A1. Regression line of the time of the test at which the lowest oxygen saturation before reaching the aerobic threshold (T_1) is observed with respect to the time elapsed from the lowest oxygen saturation before reaching the aerobic threshold to the anaerobic threshold (T_2)

A este párrafo hay que darle una vuelta, no lo entendí muy bien y no sé si la traducción está bien Since the phenotype and the maximal oxygen consumption (classified into 3 levels of physical condition) influenced the appearance of the AeT, a linear regression model was performed adjusted for the phenotype and the physical condition. When adjusting these 2 factors, the T_1 time lost statistical significance (p=0.473), with the β factor being lower than before adjusting (0.99). Therefore, if the phenotype (p=0.289) and physical condition (specifically good concerning the medium, with p=0.075; and excellent concerning the medium, with p=0.057) are taken into account, the effect of T_1 on AeT is lost, finding 31% of the variability of AeT, (R^2= 0.31) which may be mainly due to physical condition, although as can be seen in Table 8 it does not reach statistical significance.

	Unadjusted effect			
	ß coefficient	p	IC 95%	R^2
Saturation time before AeT (T1)	0,239	0,054	-0,004;0,483	0,141
	Adjusted effect			
Saturation time before AeT (T1)	0,099	0,473	-0,182; 0,380	0,310
Physical condition				
Excellent vs medium	0,951	0,075	-0,104;2,007	-
Good vs medium	1,084	0,057	-0,033;2,201	-
Phenotype				
Dark skin vs Caucassian	0,778	0,289	-0,706;2,261	-

Table 8: β and R^2 parameters and for the AeT with respect to the time of the test at which the lowest oxygen saturation is seen before the AeT is reached (T_1). Both the unadjusted and the adjusted effect for the phenotype and physical condition are detailed.

Appendix B

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled, starting with "A"—e.g., Figure A1, Figure A2, etc.

References

1. Gillet, B. *Historia del Deporte*, oikos-tau ed.; 1971; p. 126.
2. García Prieto, J.L. *Dimensión Social del Deporte.*, gráficas e ed.; 1966; p. 284.
3. S., F.N. La Educación Física en el Sistema Educativo Español: La formación del Profesorado.; 1993.
4. P., A.G. *Educación intuitiva, lecciones de cosas y excursiones escolares.*, librería d ed.; 1902; p. 317.
5. J., A. Quines han estat són les tendencies de l'EF. *Apunts Educació Física i Esports.* **1986**, *1*, 7–14.
6. Puig, N. El proceso de incorporación al deporte por parte de la mujer española (1939-1985). VV.AA. Mujer y Deporte.; Instituto de la Mujer., Ed.; , 1987; pp. 83–89.
7. Zagalaz Sánchez, M.L. *La educación física femenina en España*, uja editor ed.; 1998; p. 332.
8. Martínez-Álvarez, L. A vueltas con la historia: una mirada a la educación física escolar del sXX. *Revista de Educación* **2000**, pp. 83–112.
9. Gallo Flórez, R. Cambios fisiológicos en la mujer deportista. *Educación Física y Deporte.* **1995**, *1*, 103–9.
10. Carrero Eras, L. La actividad físico-deportiva de la mujer en España (1938-1978). PhD thesis, Universidad Politécnica de Madrid, 1995.
11. Park, Y.G.; Lee, S.; Park, J.U. Recent Progress in Wireless Sensors for Wearable Electronics. *Sensors (Basel, Switzerland)* **2019**, *19*. doi:10.3390/s19204353.
12. Cardinale, M.; Varley, M.C. Wearable Training-Monitoring Technology: Applications, Challenges, and Opportunities. *International journal of sports physiology and performance* **2017**, *12*, S255–S262. doi:10.1123/ijspp.2016-0423.
13. Seshadri, D.R.; Li, R.T.; Voos, J.E.; Rowbottom, J.R.; Alfes, C.M.; Zorman, C.A.; Drummond, C.K. Wearable sensors for monitoring the physiological and biochemical profile of the athlete. *NPJ digital medicine* **2019**, *2*, 72. doi:10.1038/s41746-019-0150-9.
14. Mucci, P.; Blondel, N.; Fabre, C.; Nourry, C.; Berthoin, S. Evidence of exercise-induced O2 arterial desaturation in non-elite sportsmen and sportswomen following high-intensity interval-training. *International journal of sports medicine* **2004**, *25*, 6–13. doi:10.1055/s-2003-45225.
15. Khan, Y.; Han, D.; Pierre, A.; Ting, J.; Wang, X.; Lochner, C.M.; Bovo, G.; Yaacobi-Gross, N.; Newsome, C.; Wilson, R.; Arias, A.C. A flexible organic reflectance oximeter array. *Proceedings of the National Academy of Sciences of the United States of America* **2018**, *115*, E11015–E11024. doi:10.1073/pnas.1813053115.
16. Pedrana, A.; Comotti, D.; Re, V.; Traversi, G. Development of a Wearable In-Ear PPG System for Continuous Monitoring. *IEEE Sensors Journal* **2020**, *20*, 14482–14490. doi:10.1109/JSEN.2020.3008479.
17. Mengelkoch, L.J.; Martin, D.; Lawler, J. A review of the principles of pulse oximetry and accuracy of pulse oximeter estimates during exercise. *Physical therapy* **1994**, *74*, 40–49. doi:10.1093/ptj/74.1.40.
18. Martin, D.; Powers, S.; Cicale, M.; Collop, N.; Huang, D.; Criswell, D. Validity of pulse oximetry during exercise in elite endurance athletes. *Journal of applied physiology (Bethesda, Md. : 1985)* **1992**, *72*, 455–458. doi:10.1152/jappl.1992.72.2.455.
19. Hertzman, A.B. THE BLOOD SUPPLY OF VARIOUS SKIN AREAS AS ESTIMATED BY THE PHOTOELECTRIC PLETHYSMOGRAPH. *American Journal of Physiology-Legacy Content* **1938**, *124*, 328–340. doi:10.1152/ajplegacy.1938.124.2.328.

20. Kamal, A.A.; Harness, J.B.; Irving, G.; Mearns, A.J. Skin photoplethysmography - a review. *Computer Methods and Programs in Biomedicine* **1989**, *28*, 257–269. doi:10.1016/0169-2607(89)90159-4.
21. Tamura, T. Current progress of photoplethysmography and SPO(2) for health monitoring. *Biomedical engineering letters* **2019**, *9*, 21–36. doi:10.1007/s13534-019-00097-w.
22. Webster, J. *Design of Pulse Oximeters*; CRC Press, 1997; p. 260. doi:https://doi.org/10.1201/9780367802592.
23. Dempsey, J.A.; Wagner, P.D. Exercise-induced arterial hypoxemia. *Journal of Applied Physiology* **1999**, *87*, 1997–2006. doi:10.1152/jappl.1999.87.6.1997.
24. Olfert, I.M.; Balouch, J.; Kleinsasser, A.; Knapp, A.; Wagner, H.; Wagner, P.D.; Hopkins, S.R. Does gender affect human pulmonary gas exchange during exercise? *The Journal of physiology* **2004**, *557*, 529–541. doi:10.1113/jphysiol.2003.056887.
25. Hopkins, S.R. Exercise induced arterial hypoxemia: the role of ventilation-perfusion inequality and pulmonary diffusion limitation. *Advances in experimental medicine and biology* **2006**, *588*, 17–30. doi:10.1007/978-0-387-34817-9_3.
26. Mead, J. Dysanapsis in normal lungs assessed by the relationship between maximal flow, static recoil, and vital capacity. *The American review of respiratory disease* **1980**, *121*, 339–342. doi:10.1164/arrd.1980.121.2.339.
27. Thurlbeck, W.M. Postnatal human lung growth. *Thorax* **1982**, *37*, 564–571. doi:10.1136/thx.37.8.564.
28. Schwartz, J.; Katz, S.A.; Fegley, R.W.; Tockman, M.S. Sex and race differences in the development of lung function. *The American review of respiratory disease* **1988**, *138*, 1415–1421. doi:10.1164/ajrccm/138.6.1415.
29. Horiuchi, M.; Kirihara, Y.; Fukuoka, Y.; Pontzer, H. Sex differences in respiratory and circulatory cost during hypoxic walking: potential impact on oxygen saturation. *Scientific reports* **2019**, *9*, 9550. doi:10.1038/s41598-019-44844-6.
30. Harms, C.A.; McClaran, S.R.; Nickele, G.A.; Pegelow, D.F.; Nelson, W.B.; Dempsey, J.A. Exercise-induced arterial hypoxaemia in healthy young women. *The Journal of physiology* **1998**, *507 (Pt 2*, 619–628. doi:10.1111/j.1469-7793.1998.619bt.x.
31. Walls, J.; Maskrey, M.; Wood-Baker, R.; Stedman, W. Exercise-induced oxyhaemoglobin desaturation, ventilatory limitation and lung diffusing capacity in women during and after exercise. *European journal of applied physiology* **2002**, *87*, 145–152. doi:10.1007/s00421-002-0600-x.
32. Harms, C.A.; McClaran, S.R.; Nickele, G.A.; Pegelow, D.F.; Nelson, W.B.; Dempsey, J.A. Effect of exercise-induced arterial O2 desaturation on VO2max in women. *Medicine and science in sports and exercise* **2000**, *32*, 1101–1108. doi:10.1097/00005768-200006000-00010.
33. Wagner, P.D. The multiple inert gas elimination technique (MIGET). *Intensive care medicine* **2008**, *34*, 994–1001. doi:10.1007/s00134-008-1108-6.
34. Rice, A.J.; Scroop, G.C.; Gore, C.J.; Thornton, A.T.; Chapman, M.A.; Greville, H.W.; Holmes, M.D.; Scicchitano, R. Exercise-induced hypoxaemia in highly trained cyclists at 40% peak oxygen uptake. *European journal of applied physiology and occupational physiology* **1999**, *79*, 353–359. doi:10.1007/s004210050520.
35. Hammond, M.D.; Hempleman, S.C. Oxygen diffusing capacity estimates derived from measured VA/Q distributions in man. *Respiration physiology* **1987**, *69*, 129–147. doi:10.1016/0034-5687(87)90022-3.
36. Elliott, A.R.; Kizhakke Puliyakote, A.S.; Tedjasaputra, V.; Pazár, B.; Wagner, H.; Sá, R.C.; Orr, J.E.; Prisk, G.K.; Wagner, P.D.; Hopkins, S.R. Ventilation-perfusion heterogeneity measured by the multiple inert gas elimination technique is minimally affected by intermittent breathing of 100% O(2). *Physiological reports* **2020**, *8*, e14488. doi:10.14814/phy2.14488.
37. Nielsen, H.B. Arterial desaturation during exercise in man: implication for O2 uptake and work capacity. *Scandinavian journal of medicine & science in sports* **2003**, *13*, 339–358. doi:10.1046/j.1600-0838.2003.00325.x.
38. Dominelli, P.B.; Foster, G.E.; Dominelli, G.S.; Henderson, W.R.; Koehle, M.S.; McKenzie, D.C.; Sheel, A.W. Exercise-induced arterial hypoxaemia and the mechanics of breathing in healthy young women. *The Journal of physiology* **2013**, *591*, 3017–3034. doi:10.1113/jphysiol.2013.252767.
39. Guenette, J.A.; Sheel, A.W. Exercise-induced arterial hypoxaemia in active young women. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* **2007**, *32*, 1263–1273. doi:10.1139/H07-122.
40. Guenette, J.A.; Diep, T.T.; Koehle, M.S.; Foster, G.E.; Richards, J.C.; Sheel, A.W. Acute hypoxic ventilatory response and exercise-induced arterial hypoxemia in men and women. *Respiratory physiology & neurobiology* **2004**, *143*, 37–48. doi:10.1016/j.resp.2004.07.004.
41. Richards, J.C.; McKenzie, D.C.; Warburton, D.E.R.; Road, J.D.; Sheel, A.W. Prevalence of exercise-induced arterial hypoxemia in healthy women. *Medicine and science in sports and exercise* **2004**, *36*, 1514–1521. doi:10.1249/01.mss.0000139898.30804.60.
42. St Croix, C.M.; Harms, C.A.; McClaran, S.R.; Nickele, G.A.; Pegelow, D.F.; Nelson, W.B.; Dempsey, J.A. Effects of prior exercise on exercise-induced arterial hypoxemia in young women. *Journal of applied physiology (Bethesda, Md. : 1985)* **1998**, *85*, 1556–1563. doi:10.1152/jappl.1998.85.4.1556.