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Digit Ratio (2D:4D) and Lactate Response During a Football-Specific Intermittent Field Fitness Test in Women

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Abstract

Background: Lactate, an important respiratory substrate, is generated during exercise. Digit ratio (2D:4D) is strongly associated with lactate in men over a range of exercise intensities, but in women the association has been reported as weaker and restricted to high running speeds. Here we report a replication of this finding in women employing the 30–15 Intermittent Fitness Test (30–15 IFT).

Method: The participants were amateur female football players. Digit lengths, height, body composition and VO_{2max} were measured. The 30–15 IFT involves 30-second shuttle runs between lines positioned 40 metres apart. Audio signals determine the pace, starting at 10 km/h and increasing by 0.5 km/h to a maximum of 18 km/h.

Results: Mean lactate levels (mmol/L) rose with running speed, ranging from 2.13 (±0.78) to 13.63 (±2.83). At running speeds 12km/h to 16 km/h (*n* = 28 and 26 respectively) there were positive correlations between lactate and mass and BMI and negative associations with VO_{2max}. At 18 km/h (*n* = 16) right 2D:4D was positively related and height negatively related to lactate. The former remained significant after the effect of height was removed.

Conclusion: Our findings from the 30–15 IFT in the field are similar to those reported from a laboratory treadmill test. The digit ratio, especially right 2D:4D, is positively associated with lactate levels during running in women, independent of height. In contrast previous findings in men the association applies to high running speeds only.

Keywords: Digit ratio, Lactate, Female athletes, Intermittent Fitness Test, VO_{2max}

Introduction

Lactate was once viewed as a waste product of metabolism generated in anaerobic conditions associated with vigorous exercise. This is the 'O_2 debt' model [1] which is now considered outdated, as muscles produce the largest amounts of lactate in the body [2]. Lactate is now acknowledged as a fulcrum of metabolism [3], it is created and metabolised in aerobic conditions and is a link between glycolytic and oxidative metabolism. The lactate dehydrogenase enzyme converts lactate into pyruvate [4]. Pyruvate can then be shuttled to the mitochondria of a cell, or it can also be converted into glucose and thus is a substrate for gluconeogenesis [3]. Lactate is important in respiration in muscles, the heart and in the central nervous system. In addition to its role in respiration it is now acknowledged that lactate is an important signaling molecule or exerkine. In this regard, it promotes health through its links to vascular, cardiac and pulmonary function [3, 5, 6]. Therefore, the production of lactate during vigorous exercise may be regarded as a protective response to stress.

Interestingly, the associations between lactate and vascular, cardiac, and pulmonary health closely resemble those reported for digit ratio (2D:4D, the ratio of the 2nd to 4th digit lengths).

The 2D:4D is widely regarded as a negative correlate of testosterone and a positive correlate of oestrogen exposure during the 1st trimester of development [7-9]. Several studies have reported links between 2D:4D and both exercise and heart disease. Regarding the former, in men and women high performance in endurance sports, such as distance running, is related to low 2D:4D (high prenatal testosterone and low prenatal oestrogen [10, 11]. Regarding the latter, high 2D:4D (low testosterone and high oestrogen prenatally) is associated with heart disease in men but the evidence in women is comparatively scarce, fragmented, and far less conclusive. This highlights a critical knowledge gap, since female athletes remain underrepresented in 2D:4D research despite the increasing interest in sex-specific determinants of health and performance. Thus, high 2D:4D is correlated with early myocardial infarction in men [12-15] and comparisons of patients and age-matched controls have reported higher 2D:4D in a number of studies [4, 16]. Such similarities may arise if 2D:4D is a strong correlate of the production of lactate [17].

The relationship between 2D:4D and lactate production during an incremental cardiopulmonary exercise test has been reported for both female [18] and male footballers [17]. For the former, the mean of blood lactate levels (mmol/l) increased with increasing running speed: from 1.511 (0.515) at 6 km/h to 7.927 (1.918) at 14 km/h. A similar increase in blood lactate was reported for the latter: 1.533 (.406) at 8 km/h to 10.467 (2.786) at 16 km/h. Our focus here is the relationship between digit ratio (right 2D:4D; left 2D:4D and right-left 2D:4D [Dr-l]) and blood lactate levels with an emphasis on the associations at the highest concentrations of lactate (i.e. at the fastest speeds). Regarding the male data, 2D:4D vs lactate correlations (r) were positive and varied from large for right 2D:4D (r = .616 to .780), medium for left 2D:4D (r = .416 to .567) and small for Dr-l (r = .217 to .381). The female data yielded a similar pattern with the strongest relationships present for right 2D:4D. However, effect sizes were smaller than those reported for men: right 2D:4D (r = .232 to .633), medium for left 2D:4D (r = .10 to .471) and small for Dr-l (r = .034 to .324). A further male/female difference concerns the relationships between anthropometric variables and lactate. In men, there were no significant correlations between lactate and height, body weight, body mass index (BMI) and % body fat. For women, height and digit length were negatively related to lactate production. Nevertheless, for both men and women at the highest running speed multiple regression analysis showed right 2D:4D was positively related to lactate independent of body size.

The observed positive link between right 2D:4D and lactate production in men is likely robust, given the large effect sizes and a sample of 72 participants [17]. The findings for women for right 2D:4D and body size are less secure because of weak effect sizes and a sample size of 28 participants [17]. Therefore, our aim in this study was to see whether the findings in women would be replicated using a field-based progressive intermittent test. To facilitate comparisons, we recruited female football players. Our exercise test was the 30–15 Intermittent Fitness Test (30–15 IFT), which involves 30-second shuttle runs of increasing speed. Means for both lactate and 2D:4D are dependent on ethnicity. In comparisons between male White and African runners, the latter exhibit lower lactate accumulation in endurance tests [19-21]. Regarding 2D:4D, there are differences between White and Black groups, such that the latter have lower 2D:4D than the former [22]. Therefore, it is appropriate that these ethnic differences are controlled for when considering performance in aerobic exercise. The Manning et al. [18] report included 25 White and 3 Black participants. To facilitate comparisons, we focused solely on the White group.

Accordingly, the main objective of this study was to examine the relationship between 2D:4D and blood lactate responses during the 30–15 IFT in female football players, with particular emphasis on the associations at higher running speeds. A secondary objective was to assess whether body size variables (height, digit length, VO_2max) were related to lactate responses, and to determine whether right 2D:4D remained the strongest predictor of lactate under conditions of maximal exertion.

We predict the following: lactate levels will rise steeply with increased running speed. Digit ratio (right 2D:4D, left 2D:4D and Dr-l) will be positively correlated with blood lactate with effect sizes largest for right 2D:4D and the relationship will be significant at high running speeds only. Body size variables (e.g. height and digit length) will be negatively related to lactate as will VO_{2max}. At the highest running speeds right 2D:4D will be the strongest predictor of lactate.

Material and methods

A total of 28 female amateur football players aged between 18 and 24 years (mean ± SD: 20.54 ± 2.51 years) participated in the study. The players were recruited voluntarily from local amateur women's football teams competing in regional leagues and met the inclusion and exclusion criteria described below. Testing was conducted during the seventh month of the competitive season. All participants followed a consistent weekly training programme, which

included three 90-minute football training sessions and an official match every weekend. All participants met the following inclusion criteria: (1) a minimum of six years of football training and competition experience, ensuring adequate technical and physiological adaptation to football-specific demands; (2) regular football training and competition during the six months prior to data collection, guaranteeing consistent exposure to match-related workloads and stable fitness levels; and (3) absence of injury for at least three months, to avoid confounding effects on physiological responses and performance. The exclusion criteria were as follows: (1) unfamiliarity with the tests, to ensure valid and reliable execution of the 30–15 IFT and accurate lactate measurements; and (2) training less than two days per week, to exclude participants whose fitness levels might not reflect a typical amateur football training profile. All participants were instructed to avoid strenuous physical activity for a minimum of 24 hours prior to data collection. The tests were conducted on an artificial turf, with the players wearing their usual football boots. Informed consent was obtained from all participants, and the study adhered to the ethical principles outlined in the Declaration of Helsinki. The study was approved by the Local Clinical Research Ethics Committee (XXXX).

Anthropometric and body composition analysis

Stature was recorded to the nearest 0.1 cm using a fixed wall stadiometer (SECA 214, SECA, Hamburg, Germany). Body composition was assessed using tetrapolar bioelectrical impedance analysis (body weight in kilograms and % body fat) (BC-601, Tanita, Illinois, USA). Prior to the assessment, participants followed standard preparation protocols: no food or drink (except water) 4 hours before the measurement, avoid measuring during menstruation, no strenuous exercise within the previous 24 hours, avoidance of caffeinated beverages for at least 12 hours, bladder voiding immediately before the test, and measure at a stable temperature of 20-25° [23]. BMI was calculated as body weight (in kg) divided by height in meters squared (kg/m2) [24].

Hand scans

The 2D:4D ratio was calculated following a previously validated protocol (Manning, 2002), with slight modifications. Instead of scanning the hands, a photograph-based method was used. Participants were instructed to place their right hand palm-up on a flat, white surface, with the fingers fully extended and lightly touching the surface. A photograph of the hand was then taken using an iPhone XVI mounted on a tripod at a fixed distance of 30 cm perpendicular to the hand. This setup ensured consistent angle and scale across all participants. If the image was

unclear or improperly aligned, the process was repeated. The lengths of the second (index) and fourth (ring) digits were measured twice from the photographs by the same investigator, who was blinded to any performance or physiological data. Measurements were taken from the midpoint of the basal crease to the tip of each finger, using Vernier digital callipers (Mitutoyo, D15, Japan), with an accuracy of 0.05 mm. The 2D:4D ratio was calculated as the length of the second digit divided by the length of the fourth digit.

30-15 Intermittent Fitness Test

Before completing the 30–15 Intermittent Fitness Test (30–15 IFT), all participants performed a dynamic warm-up consisting of five exercises: leg swings, walking lunges, lateral lunges, ankle bounces, and single-leg bounces. The 30–15 IFT was carried out in accordance with the original protocol described by Buchheit [25]. The test involves 30-second shuttle runs between two lines positioned 40 metres apart, followed by 15 seconds of passive recovery. The running pace was controlled by the official 30–15 Intermittent Fitness Test (30–15 IFT) mobile application, which provides audio beeps indicating when athletes must reach each line. The test began at an initial speed of 8 km/h, as defined in Buchheit's protocol, and the pace increased by 0.5 km/h at the start of each subsequent 30-second stage. This starting velocity ensured that all players began the test at the same submaximal intensity, allowing a gradual and standardised increase in speed until volitional exhaustion. The test was performed in groups of three players and verbal encouragement was provided to ensure maximum effort. The test concluded when the participant either (i) voluntarily stopped due to exhaustion or (ii) failed to reach the next 3-metre zone before the audio signal on three consecutive occasions. The test was performed on a Wednesday between 17:00 and 19:00 h under consistent environmental conditions (temperature: –23°C; humidity: –45%).

Maximal aerobic speed (MAS) and maximal oxygen uptake (VO_2max) were estimated based on the performance in the 30–15 IFT, following validated procedures [25].

The final velocity achieved during the test (VIFT, in km/h) was recorded and used as the primary performance outcome. Although VIFT reflects a combination of aerobic power, anaerobic capacity, change-of-direction ability, and intermittent recovery capacity, it is widely used as a practical proxy for MAS in team sports contexts [25].

To estimate VO_2max, VIFT was multiplied by 3.5, as proposed by Buchheit [25] providing a valid field-based prediction of maximal oxygen uptake:

VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$) = VIFT × 3.5

In addition, MAS was estimated by applying a correction factor to account for the overestimation commonly observed in intermittent testing. Following the recommendations of Buchheit and Laursen [26], MAS was calculated as:

MAS (km/h) = VIFT × 0.85

This adjustment provides a more conservative estimate of MAS that better aligns with values obtained from continuous incremental protocols.

Lactate measurements were taken periodically during the 30–15 Intermittent Fitness Test (30–15 IFT) to assess the physiological response to increasing exercise intensity. Blood sampling was conducted at the end of each 2 km/h increment, starting from the initial test velocity of 8 km/h. Consequently, lactate was collected at stages corresponding to 8, 10, 12, 14, 16, 18, and 20 km/h, although not all players completed the final stages. Capillary blood samples (0.7 μL) were obtained via finger prick from the third or fourth digit of the non-dominant hand immediately after each relevant stage. A single-use test strip was used with the Lactate Scout (Lactate Scout, SensLab GmbH, Leipzig, Germany), a validated and reliable device for field-based lactate assessment [27]. Blood lactate concentration was displayed within 13 seconds. All samples were collected during the 15-second passive recovery period immediately after each 2 km/h increment, with the participant standing.

Statistical analysis

All parameters are presented as the mean and standard deviation (SD). The normality assumption was assessed from values of skewness and kurtosis. Intra-class correlation coefficients (ICC) (absolute agreement) between the first and second 2D:4D's of the right and left digits were calculated. Pearson- product moment correlation coefficients were used to estimate associations. Correlations were calculated for a basal condition and speeds 10 to 18 km/h. Multiple regression analyses were performed for the highest speeds (16 km/h and 18 km/h). The level of significance was set at $p < .05$.

Results

Descriptive statistics

The sample consisted of 28 female White participants. Descriptive means (SD) were as follows: age 20.5 (2.5) years, height 165.5 (5.0) cm, mass 61.30 (8.23) kg, BMI 22.46 (3.08), VO_{2max} 57.96 (4.47).

Repeatability of digit ratios

The ICC's, r1, of the digit ratios were all high and significant (right 2D:4D r1 = .908, F [1,27] 20.78, p<.0001; left 2D:4D r1 = .991, F [1,27] = 218.87, p<.0001). This indicates the between-individual differences in 2D:4D were significantly higher than the measurement error. Therefore, we calculated mean digit ratios (right 2D:4D = .981[.04]; left 2D:4D = .959[.048], right-left 2D:4D (Dr-l) = .022[.055]) and used these in all subsequent analyses.

Lactate measurements

Blood lactate levels (mmol/l) were obtained from 28 participants at the basal point and at running speeds of 10 km/hr, 12 km/hr, and 14 km/hr: from 26 participants at 16 km/hr and from 16 participants at 18 km/hr.

Lactate means (SD) (mmol/l) increased with increasing speed from 2.125 (.777) at the basal point, to 4.082 (1.059) at 10 km/h, 6.96 (2.120) at 12 km/h, 8.990 (2.52) at 14 km/h,12.369 (3.699) at 16 km/h, and 13.631 (2.832) at 18 km/h. Distributions around the lactate means showed low skewness (varying from -.010 at 14 km/h to .811 at 18 km/h) and low kurtosis (varying from -.165 at 10 km/h to -.836 at 14 km/h).

Correlates of lactate levels

Product-moment correlations were calculated between lactate level at different speeds and the following 10 variables: right 2D:4D; left 2D:4D, right-left 2D:4D (Dr-l), age, height, mass, BMI, mean of right digit length, mean of left digit length, and VO2max (Table 1).

There were no significant associations between age and lactate at any of the running speeds. Most correlations were positive, but *p* values varied from .07 (12 km/h) to .99 (10 km/h).

Regarding the 2D:4D variables, the largest effect sizes were seen for right 2D:4D with positive correlations with lactate for speeds of 12km/h through to 18 km/hr. The strongest associations were present at the highest speeds (16 km/h, $n = 26$, $r = .432$, $p = .03$; 18 km/h, $n = 16$, $r =$

.668, *p* .005: Figure 1). There were weak non-significant negative and positive associations between left 2D:4D and lactate. As with right 2D:4D, Dr-l showed a pattern of positive correlations with lactate for speeds of 12km/hr through to 18 km/hr. However, none of these correlations reached significance.

*** Figure 1 here***

Regarding body size variables, height was consistently negatively associated with lactate, but correlations were significant for the highest running speed only (18 km/h, $n = 16$, $r = -516$, $p = .04$: Figure 1). In contrast mass and BMI were positively correlated with lactate for speeds 12 km/hr through to 16 km/h (mass; 14 km/h, $n = 28$, $r = .451$, $p = .02$, 16 km/h n =26, $r = .444$, $p = .02$: BMI; 12 km/h, $n = 28$. $r = .445$, $p = .02$, 14 km/h, n = 28, $r = .551$, $p = .002$, 16 km/h, $n = 26$, $r = ,563$, $p = .003$). There were no significant associations between mean digit lengths of the right or left hands and lactate levels at ay running speed (*p* values varied from .35 to .99).

Considering VO_{2max}, there were significant negative correlations with lactate at 12 km/h, $n = 28$, $r = -.387$, $p = .04$, 14 km/h, $n = 28$, $r = -.586$, $p = .001$, 16 km/h, $n = 26$, r = -.497, $p = .01$.

Focussing on associations with lactate at the highest running speeds (i.e. 16 km/h and 18 km/h), we have four significant correlations at 16 km/hr (positive for right 2D:4D; mass; BMI and negative for VO_{2max}) and two significant correlations at 18 km/hr (positive for right 2D:4D; negative for height). We performed two multiple regression analyses. Analysis number one, dependent variable lactate at 16 km/h and independent variables right 2D:4D, mass, BMI and VO_{2max}. Analysis number two, dependent variable lactate at 18 km/hr and independent variable right 2D:4D ad height. Regarding the former, there were no significant relationships between the independent variables and lactate. Of the four variables VO_{2max} (standardised coefficient -.346, p = .06) and right 2D:4D (standardised coefficient .337, $p = .08$) were the strongest correlates of lactate levels (Table 2). Regarding the latter, right 2D:4D remained positively correlated with lactate (standardised coefficient .570, $p = .049$) after the influence of height was removed. Height was no longer significantly related to lactate (Table 2).

Discussion

The objective of the present study was to investigate the associations between 2D:4D and blood lactate responses during a football-specific intermittent fitness test in female players, with particular attention to whether these relationships became evident at higher running speeds. We

have found an increase in mean blood lactate (mmol/l) with increasing running speed: at the basal point 2.125 (.777) to 8.990 (2.52) at 14 km/hr and 13.631 (2.832) at 18 km/h. The pattern of increase was comparable to that reported by Manning et al. [18] from 1.511 (0.515) at 6 km/h to 7.927(1.918) at 14 km/h). This indicates that the differences in protocol (laboratory treadmill test versus field test, i.e., testing conducted on a football pitch under sport-specific conditions rather than in a controlled laboratory setting) have not resulted in major differences in lactate production.

Regarding relationships between digit ratio variables and lactate levels, we also have a similar pattern to that reported by Manning et al [18]. That is, at the higher running speeds (16 km/h and 18 km/h) right 2D:4D, left 2D:4Dnd Dr-l were positively related to lactate. However, the correlations are significant for right 2D:4D only (16 km/h, $r = .432$, $p = .03$, $n = 26$: 18 km/h, $r = .668$, $p = .005$, $n = .005$). With respect to other variables, lactate concentrations at 16 km/h were higher in participants with greater body weight and BMI together with lower VO_2max. At 18 km/h, increased lactate was associated with shorter stature and higher right-hand 2D:4D. Of these two variables multiple regression showed high 2D:4D to be positively related to lactate independent of height.

Our replication of Manning et al. [18] supports earlier findings in men and suggests that the relationships between digit ratio, lactate and vigorous exercise could differ by sex, although direct comparisons were not possible here since only female athletes were tested. In the current study, female players showed positive correlations between right 2D:4D (but not left 2D:4D or Dr-l) and lactate, but only at high running speeds. In contrast, previous research in men has reported positive correlations between digit ratios (right, left and Dr-l) and lactate across a range of speeds, with the highest effect sizes observed for right 2D:4D [18].

Brooks et al [3] list a number of positive effects of lactate associated with exercise. They include, the control of breathing, upregulation of vascular endothelial growth factor, fuelling the heart and activation of the metaboreflex, which regulates cardiac output during exercise. A number of these effects show sex differences which might have adaptive advantages in avoiding predators or in long-distance pursuits of game. In this regard, in low 2D:4D male individuals intense physical exercise or aggressive stimuli are also associated with increases in strength and testosterone which may map on to fluxes in lactate [28-31].

Regarding the limitations of our study, our observations relate to a relatively small sample, to female football players and to one ethnic group, i.e. Whites. Further work is necessary to

consider larger samples from diverse groups and other ethnicities such as East-Asian and Black women. The cross-sectional design also restricts causal inference. As the participants were amateur female football players, the generalisability of the findings to the general population and professional or elite individuals remains limited. Finally, while the field-based nature of the 30–15 IFT increases ecological validity, it may also have introduced uncontrolled influences such as surface conditions, environmental variability, or residual fatigue within the training microcycle.

In conclusion, vigorous exercise releases exerkines which promote health, resilience and longevity. The list of such factors is very long [32]. Lactate was once regarded as a metabolic waste product but now it is viewed as a powerful exerkine with diverse effects on metabolic and physiological regulation [3]. We have confirmed that the release of lactate during exercise is not uniform among female football players. High digit ratio, particularly high right 2D:4D, is a biomarker for high rates of lactate production during vigorous exercise in women.

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Table 1. Product-moment correlations (*r*) between digit ratios (right 2D:4D; left 2D:4D and right-left 2D:4D [Dr-]), age, height, mass, BMI, mean digit length right, mean digit length left, VO2max and blood lactate concentrations(mmol/l) at the following running speeds (basal, 10 km/hr, 12 km/hr, 14 km/hr, 16 km/hr, 18 km/hr). The *p* values for the values of *r* are given in parenthesis.

Trait	*Basal*	*10 km/hr*	*12 km/hr*	*14 km/hr*	*16 km/hr*	*18 km/hr*
	n = 28	*n = 28*	*n = 28*	*n = 28*	*n = 26*	*n = 16*
R2D:4D	.031	-.012	.252	.266	**.432**	**.668**
	(.87)	(.95)	(.20)	(.17)	**(.03)**	**(.005)**
L2D:4D	-.121	-.032	-.063	-.042	.118	.257
	(.54)	(.87)	(.75)	(.83)	(.57)	(.34)
Dr-l	.134	.019	.265	.259	.258	.340
	(.50)	(.93)	(.17)	(.18)	(.20)	(.20)
Age	.089	-.001	.352	.251	.145	.079
	(.65)	(.99)	(.07)	(.20)	(.48)	(.77)
Height	-.184	-.045	-.264	-.234	-.26	**-.516**
	(.35)	(.82)	(.18)	(.23)	(.20)	**(.04)**
Mass	.002	.144	.329	**.451**	**.444**	-.184
	(.99)	(.46)	(.09)	**(.02)**	**(.02)**	(.50)
BMI	.092	.157	**.445**	**.551**	**.563**	.083
	(.64)	(.43)	**(.02)**	**(.002)**	**(.003)**	(.76)
Mean R Digits	-.152	-.108	-.184	-.077	-.026	.011
	(.44)	(.58)	(.35)	(.70)	(.90)	(.97)
Mean L Digits	-.008	.0002	-.012	-.096	-.065	.099
	(.97)	(.99)	(.95)	(.63)	(.75)	(.72)
VO2max	-.008	-.299	**-.387**	**-.586**	**-.497**	.397
	(.97)	(.12)	**(.04)**	**(.001)**	**(.01)**	(.13)

R2D:4D = right-hand second-to-fourth digit ratio; L2D:4D = left-hand second-to-fourth digit ratio; Dr-l = right–left difference in 2D:4D; BMI = body mass index; VO_2max = maximal oxygen uptake; Mean R digits = mean digit length of the right hand; Mean L digits = mean digit length of the left hand; The **bolded value** represents a statistically significant result, as indicated by a p-value below 0.05.

Table 2. Two multiple regression analyses with dependent variable blood lactate level (mmol/l).

Speed 16km/h					
Trait	Coefficient	St. Error	St. Coefficient	*t*	*p*
R2D:4D	26.96	14.834	.337	1.817	.08
Weight	.009	.183	.020	.050	.96
BMI	.371	.543	.291	.683	.50
VO2max	-.340	.173	-.346	-1.961	.06
Speed 18 km/h					
Trait	Coefficient	St. Error	St. Coefficient	*t*	*p*
R2D:4D	29.594	13.643	.570	2.169	**.049**
Height	-.089	.150	-.156	-.593	.56

R2D:4D = right-hand second-to-fourth digit ratio; BMI = body mass index; VO_2max = maximal oxygen uptake. The **bolded value** represents a statistically significant result, as indicated by a p-value below 0.05.

Figures

Figure 1. The relationship at 18 km/h in 16 female footballers between (A) right 2D:4D and blood lactate concentration (the equation for the line is y = 34.647x-20.224, r^2 = .45), and (B) height and blood lactate concentration (the equation for the line is y = -.295x+62.758, r^2 = .27).