

1 Predicting future athletic performance in young female
2 road cyclists based on aerobic fitness and hematological
3 variables

4 *Original Investigation*

5 Dariusz Sitkowski, Jadwiga Malczewska-Lenczowska, Ryszard
6 Zdanowicz, Michał Starczewski, Andrzej Pokrywka, Piotr
7 Źmijewski, Raphael Faiss

8

9 Dariusz Sitkowski, *Department of Physiology, Institute of Sport*
10 *– National Research Institute, Warsaw, Poland*

11 Jadwiga Malczewska-Lenczowska, *Department of Physiology*
12 *Nutrition and Dietetics, Institute of Sport – National Research*
13 *Institute, Warsaw, Poland*

14 Ryszard Zdanowicz, *Department of Physiology, Institute of*
15 *Sport – National Research Institute, Warsaw, Poland*

16 Michał Starczewski – *Department of Physiotherapy*
17 *Fundamentals, Faculty of Rehabilitation, University of Physical*
18 *Education, Warsaw, Poland*

19 Andrzej Pokrywka – *Department of Biochemistry and*
20 *Pharmacogenomics, Faculty of Pharmacy, Medical University*
21 *of Warsaw, Warsaw, Poland*

22 Piotr Źmijewski – *Department of Biomedical Sciences, Faculty*
23 *of Physical Education, University of Physical Education,*
24 *Warsaw, Poland*

25 Raphael Faiss – *Institute of Sport Sciences, University of*
26 *Lausanne, Switzerland*

27

28 Corresponding author – Raphael Faiss,
29 e-mail: raphael.faiss@unil.ch

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Purpose: This study aimed to determine whether the initial levels of aerobic fitness and hematological variables in young female road cyclists are related to their athletic performance development during their careers. Methods: Results of graded exercise tests (GXT) on a cycle ergometer and total hemoglobin mass (tHb-mass) measurements were analyzed in thirty-four female road cyclists (aged 18.6 [1.9] years). Among them, two groups were distinguished based on their competitive performance (*Union Cycliste Internationale* [UCI] world ranking) over the following eight years. Areas under the curve (AUC) in receiver operating characteristic (ROC) curves were calculated as indicators of elite performance prediction. Results: Initial GXT variables (peak power, peak oxygen uptake, and power at 4 mmol/L blood lactate) were not significantly different in elite ($n = 13$) vs. non-elite ($n = 21$) riders. In contrast, elite riders had higher tHb-mass expressed either in absolute (664 [75] vs. 596 [59] g, $P = .006$) or normalized to body mass (11.2 [0.8] vs. 10.3 [0.7] g/kg, $P = .001$) and fat-free mass (14.4 [0.9] vs. 13.1 [0.9] g/kg, $P < .001$). Also, absolute and relative erythrocyte volume were significantly higher in elite subjects (P values ranged from $< .001$ to $.006$). Of all the variables analyzed, the relative tHb-mass had the highest predictive ability to reach the elite level (AUC ranged from .82 to .85). Conclusions: Measurement of tHb-mass can be a helpful tool in talent detection to identify young female road cyclists with the potential to reach the elite level in the future.

Keywords: endurance athletes, talent identification, graded exercise test, total hemoglobin mass, intravascular volumes

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Introduction

73 Measurements of maximal and peak oxygen uptake, peak power
74 output, and power at the lactate threshold, taken during
75 incremental exercise testing in the laboratory, are considered
76 valid determinants of performance in road cycling.¹⁻³
77 Physiological assets, evaluated in the junior age category, may
78 determine a cyclists' adaptive potential and thus, their ability to
79 reach an elite level in their career development.⁴ Accordingly,
80 measures of aerobic fitness have been used for talent detection to
81 predict the professional careers of young riders, but with
82 divergent results.^{5,6}

83 It is well-documented that high-level endurance athletes
84 exhibit a higher total hemoglobin mass (tHb-mass) than untrained
85 individuals, non-endurance athletes, or when compared to less
86 successful counterparts.^{7,8} This seems reasonable since more
87 hemoglobin is directly related to improvements in oxygen
88 transport capacity in the blood.⁷ Buffering capacity in blood, and
89 nitric oxide released from red blood cells, may also facilitate
90 higher tHb-mass leading to enhanced vascular reactivity and
91 improved blood flow in working muscles.⁹ Overall, hemoglobin
92 definitely plays a crucial role in endurance exercise performance,
93 which is mainly limited by oxygen supply to working muscles in
94 trained individuals.¹⁰ Measuring tHb-mass in novice athletes was
95 therefore suggested as a valuable tool for identifying talent in
96 road cycling¹¹ while evidence to date is scarce. Nevertheless, the
97 results of a recent longitudinal study on male cross-country skiers
98 and triathletes from age 16 to 19 years suggested that tHb-mass
99 is a good predictor of national team membership later in their
100 career.¹² In contrast to hemoglobin concentration ([Hb]), tHb-
101 mass is not affected by changes in plasma volume, therefore
102 reflecting the hematological status of athletes much better than
103 [Hb]. Interestingly, during puberty, the additive effect of
104 endurance training on tHb-mass was observed only in boys, while
105 no changes were found in girls at the same time.¹³ Development
106 of tHb-mass in late adolescence in boys was reported as
107 comparably small, and not influenced by endurance training,
108 supporting the fact that tHb-mass at the age of 16 years may
109 represent an important determinant for tHb-mass as adults, and
110 possibly for attaining high-level endurance performance.¹⁴
111 Besides, only minor variations in tHb-mass (3% in average) were
112 reported in elite female cyclists during a competitive season.¹⁵
113 tHb-mass in competitive endurance athletes was also reported to
114 be stable (individual oscillation <6%) throughout the training
115 year, despite important (~25%) fluctuations in training volume.¹⁶
116 Furthermore, no increase in relative tHb-mass was observed
117 during one year of training in novice male and female cyclists

118 aged 11-15 years.¹¹ Nevertheless, tHb-mass increased by 6.0%
119 after endurance training in previously untrained individuals,¹⁰
120 and 6.5% in athletes with altitude training.⁷ Taken together, these
121 findings show that tHb-mass is relatively stable and only slightly
122 affected by age and training, in contrast to indices of exercise
123 capacity.¹⁷ Thus, we hypothesize that there is a genetic
124 predisposition of high tHb-mass in elite athletes.⁷ On the other
125 hand, some adult individuals with no history of training may have
126 a tHb-mass comparable to endurance athletes,¹⁸ confirming tHb-
127 mass depends more on the presence of genetic characteristics
128 rather than training. While genetic testing could theoretically be
129 included for talent identification purposes in road cycling,¹⁹
130 single nucleotide polymorphisms linked to tHb-mass has so far
131 only been reported in male road cyclists, but not in women.²⁰

132 Finally, recent scientific focus has been placed on
133 women's cycling with an extensive description of physical
134 requirements for elite performers. However, scarce literature has
135 described those physiological factors underlying athletic
136 performance in elite female cyclists. This study aimed to
137 determine whether the initial levels of aerobic fitness and
138 hematological variables in young female road cyclists are related
139 to athletic performance development during their sporting
140 careers. We hypothesized that the initial level of tHb-mass
141 (measured at a junior age) has some predictive value in
142 determining elite status at a senior level.

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144 Methods

145 *Subjects*

146 Thirty-seven female road cyclists (mean \pm SD: age 18.6 ± 2.1
147 years, body height 168 ± 5 cm, body mass 58.0 ± 5.5 kg, body fat
148 $21.5 \pm 3.0\%$, fat-free mass 45.4 ± 3.4 kg, and training experience
149 6.1 ± 2.6 years) participated in this study. None of them was born
150 or lived at altitudes above 700 m. While athletes were subject to
151 anti-doping tests in- or out-of-competition, none of them returned
152 any adverse analytical finding nor were sanctioned for any anti-
153 doping rule violation.

154 This study was approved by the local Committee of Ethics
155 (KEBN-22-75-DS) and performed in accordance with the
156 Declaration of Helsinki and its later amendments. All subjects,
157 and parents or legal guardians in the case of those under 18 years
158 of age, provided an informed written consent before taking part
159 in the study.

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161 *Design*

162 For this retrospective study, we used laboratory tests results
163 collected from athletes who participated in a Polish national
164 talent identification program in 2013 and 2014. Over the
165 following eight years, their performances were retrieved from the

166 *Union Cycliste Internationale* (UCI) as points scored in the world
167 ranking in the women's elite category (www.uci.org). Any athlete
168 who stopped training or competing in road cycling within three
169 years after initial testing was excluded from the analysis. Three
170 athletes stopped competing in road cycling within 3 years after
171 the initial tests and were therefore excluded from the analysis. A
172 period of eight years was considered sufficient to reach the elite
173 level in professional cycling, which was defined as reaching a
174 ranking among the top 400 in the UCI ranking. Moreover, after
175 this period, the age of the athletes who took part in our study was
176 similar to the age (27.8 ± 3.9 years) of the top 10 female road
177 cyclists in the 2014 UCI ranking.

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179 *Methodology*

180 The athletes spent the night before performing tests in a
181 dormitory located in the laboratory building. The next day they
182 underwent a sequence of tests after a standardized breakfast (in
183 the order listed below): blood sampling, anthropometric
184 measurements, medical examination, graded exercise test, and
185 measurements of total hemoglobin mass and intravascular
186 volumes.

187 *Blood sampling*

188 Between 7.30 and 8.00 a.m., and after 15-min rest in a sitting
189 position, 10 ml blood samples (Vacuette EDTA K2 tubes,
190 Greiner Bio-One, Austria) were collected by trained
191 phlebotomists from an antecubital vein to perform a complete
192 blood count (Advia 120, Bayer, Germany).

193 *Anthropometric measurements*

194 Body height, body mass (BM), and skinfold thicknesses at four
195 sites: biceps, triceps, subscapular, and supra-iliac, were measured
196 after urination. BM was measured with an accuracy of 50 g using
197 an electronic scale (Dolphin, CAS Corp., South Korea); the
198 athletes wore only underwear for this measurement. The percent
199 of body fat and fat-free mass (FFM) were calculated based on
200 skinfold thickness measurements.²¹

201 *Medical examination*

202 All athletes had current medical certificates confirming their
203 ability to practice competitive sports and reported no health
204 problems on the day of the tests and the preceding 2 weeks.
205 Nevertheless, they underwent (at least 75 minutes after
206 breakfast) a medical examination that included a medical history,
207 electrocardiography, auscultation of the heart and lungs, and
208 blood pressure measurement.

209 *Graded exercise test*

210 A graded exercise test (GXT) was performed on a personal
211 bicycle frame mounted on a cycle ergometer (Cyclus2,

212 Avantronic, Germany). The initial load was 1.5 W/kg body mass
213 and thereafter increased every 3 min by 0.65 W/kg until
214 volitional exhaustion (or cycling cadence <70 rpm). Peak power
215 (P_{peak}) was calculated as $P_{compl} + P_{incr} \times (t/180)$; where
216 P_{compl} was power at the last fully completed step, P_{incr} was
217 power increase, and t was a time in seconds completed at the
218 final step.¹⁰ During the GXT, oxygen uptake ($\dot{V}O_2$) was recorded
219 breath-by-breath at 15-s sampling intervals (MetaMax 3B
220 analyzer; Cortex, Germany). The average value of the two
221 highest consecutive 15-s $\dot{V}O_2$ values was taken as $\dot{V}O_{2peak}$.
222 Within the last 15 s of every load increment, capillary blood (20
223 μ l) was collected from fingertips for assessing lactate
224 concentration ($[La]$) using a dedicated analyzer (Super GL2, Dr.
225 Müller, Germany). Power corresponding to a $[La]$ of 4 mmol/l
226 (PAT4) was determined.

227 *Total hemoglobin mass and intravascular volumes*

228 At least two hours after the exercise test and after the athletes
229 were instructed in detail on how to perform the required
230 rebreathing procedure, total hemoglobin mass (tHb-mass) and
231 intravascular volumes (erythrocyte volume [EV], plasma volume
232 [PV], and blood volume [BV]) were determined using an
233 optimized carbon monoxide rebreathing method well-described
234 elsewhere.²² The administered gas mixture consisted of 99.97%
235 chemically pure CO (0.8 ml/kg BM) and 99.5% O₂ (3 l bag). A
236 CO sensor (Pac 7000, Dräger, Germany) was used to check
237 potential leaks in exhaled air before and after the test. Blood
238 (~105 μ l) was sampled from the hyperemized (Finalgon,
239 Boehringer Ingelheim, Germany) earlobe in the following order:
240 just before the test (4 samples), and in the 6th (2 samples) and 8th
241 minutes (3 samples) from the beginning of inhalation of the gas
242 mixture (lasting 2 min). The samples served to determine the
243 percentage value of carboxyhemoglobin (HbCO%) using a CO
244 oximeter (ABL 80 Flex, Radiometer, Denmark). A dedicated
245 software (Blood Volume Measurements: SpiCO; Blood tec,
246 Bayreuth, Germany) allowed to calculate tHb-mass and
247 intravascular volumes. All measurements were performed by the
248 same experienced laboratory technicians. A typical error of 2.5%
249 for tHb-mass was determined for the measurement during this
250 study based on duplicate measurements in 11 athletes
251 participating in this study.

252 *Statistical Analysis*

253 Descriptive data are presented as arithmetic means \pm SD. The
254 normality of the data distribution and the homogeneity of
255 variance were examined by the Shapiro-Wilk and Levene's tests,
256 respectively. Differences between mean values in the groups
257 were tested using the Student's t-test or Mann-Whitney U test,
258 where appropriate. Receiver operating characteristic (ROC)
259 curve analysis with the area under ROC curve (AUC)

260 determination (DeLong's method) was used to evaluate the
261 predictive value of GXT and hematological variables. The
262 following ranges of AUC were used to describe the goodness of
263 discrimination: = .5 – no discrimination, $> .5 < .7$ – poor
264 discrimination, $\geq .7 < .8$ – acceptable discrimination, $\geq .8 < .9$
265 excellent discrimination, $\geq .9$ outstanding discrimination.²³
266 The Youden index was adopted to set the cut-off points.
267 Additionally, Pearson's correlation coefficients were used to
268 determine associations between selected variables. PQStat
269 statistical software ver. 1.8.4 (PQStat Software Company,
270 Poznan, Poland) and JASP ver. 0.17.1 (University of Amsterdam,
271 Netherlands) were employed. In all analyses, the level of
272 significance was set at $P < .05$.

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Results

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Over the course of the eight-years of systematic training following the initial tests, 13 women out of the 34 riders (38%) reached the elite level (EL), ranking between 5th and 400th, whereas 21 (62%) were not ranked in the UCI classification, and were included in the non-elite group (NEL).

No significant differences were observed between the groups for anthropometric or training variables (Table 1).

Table 1 somewhere here

From the initial GXT results, no between-group difference was observed for any of the variables. The analysis of hematological variables indicated that EL had significantly higher tHb-mass and EV (both in absolute values and per kg of BM or FFM) than NEL as illustrated in Table 2. Nevertheless, none of the variables was significantly correlated with the UCI ranking in the EL group ($r < .364$, $P > .222$).

Table 2 somewhere here

Figure 1 somewhere here

All GXT variables had poor discriminatory ability (AUC ranged from .61 to .69). Among hematological variables, tHb-mass and EV normalized to kg BM and FFM had an excellent discriminatory ability with AUC ranging from .81 to .85 (Table 3).

Table 3 somewhere here

No significant difference was found between the AUC of tHb-mass/BM and tHb-mass/FFM ($P = .448$). In addition, sensitivity and specificity did not differ substantially between variables (i.e., .92 and .67, respectively).

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Figure 2 somewhere here

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Discussion

312 In the present study, we investigated whether initial levels of
313 aerobic fitness and hematological status among young female
314 road cyclists were related to athletic performance development in
315 the following 8 years. The most important finding is that selected
316 hematological variables (i.e., total hemoglobin mass [tHb-mass]
317 and erythrocyte volume [EV]) were able to discriminate between
318 those riders who later reached an elite level from those who did
319 not achieve the same developmental level.

320 tHb-mass and EV, normalized to BM and FFM,
321 demonstrated the highest predictive ability in terms of future
322 athletic development (see Table 3). Such findings confirm earlier
323 published work^{11,12,14} that tHb-mass in young endurance-trained
324 athletes, including cyclists, may be an important predictor of
325 athletic performance trajectories, and its measurement may
326 provide a valuable tool for talent identification or training
327 potential. Furthermore, EV was found to be a robust predictor of
328 attaining elite level status. However, EV being calculated from
329 tHb-mass, [Hb], and hematocrit, contains a greater measurement
330 uncertainty compared to the more accurate measurement of tHb-
331 mass.²⁴

332 The GXT variables did not differ between the EL and
333 NEL cyclists (see Table 2) and thus, were unable to discriminate
334 career development in our study cohort. Furthermore, when
335 evaluating the predictive ability of GXT variables (Table 3),
336 P_{peak} or PAT₄ were not better predictors of future athletic
337 performance compared to VO_{2peak}. The poor discriminatory
338 ability of the GXT variables may, at least in part, be due to
339 individual variation in training content between riders, despite all
340 being well-trained during the period of testing. Seasonal variation
341 in performance during incremental testing on a cycle ergometer
342 was reported in male cyclists,¹⁷ but no such variation in GXT
343 variables was observed in young girls (aged 13–16) involved in
344 mountain biking.²⁵ Nevertheless, our results confirmed the AUC
345 values for VO_{2peak}/BM (.69) reported by other authors, who
346 examined the usefulness of using aerobic fitness indices for
347 predicting the professional career of young male cyclists.⁵

348 Research on the physiological determinants underpinning
349 athletic performance in women's road cycling is scarce.
350 However, a study on female riders, who represented a wide range
351 of athletic abilities, found that relative VO_{2max} determined on a
352 cycle ergometer may account for as much as 82% of performance
353 in multi-day road racing³. Conversely, VO_{2peak} emerged as a
354 poor predictor of a successful professional career in a more
355 homogenous group of young male cyclists⁵. Although a high

356 VO₂max would seem a prerequisite for competing at a high level
357 in road cycling, actual endurance performance can vary among
358 individuals with similar levels of VO₂max.²⁶ Another study on
359 trained female cyclists reported that power at blood lactate
360 transition thresholds and peak aerobic power during incremental
361 exercise testing are better predictors of endurance performance
362 than VO₂peak². In addition, a study on 18-year-old male road
363 cyclists⁶ showed that power at the final minute of a ramp cycle
364 ergometer test, but not VO₂max, was able to distinguish between
365 cyclists who later became World Tour riders and those who did
366 not.

367 Taken together with the current results, we suggest that
368 tHb-mass offers an important indicator of long-term aerobic
369 potential among young female cyclists. Changes in tHb-mass
370 during childhood and adolescence, as reported in the literature,
371 show that relative tHb-mass increases around (and after) puberty
372 in males, while tHb-mass is largely unchanged in females, even
373 those undergoing regular endurance training.^{13,14,27} This sex
374 difference in tHb-mass trajectories with pubertal onset could be
375 explained by a marked increase in testosterone levels in boys,
376 being a strong stimulator of erythropoiesis, besides the
377 concomitant positive changes in fat free mass among boys.²⁸
378 Some authors, however, failed to find a direct effect of
379 testosterone on erythropoiesis during adolescence.²⁷

380 Although the studied cohort was homogeneous in terms
381 of body composition, the predictive ability of the tHb-mass/FFM
382 (AUC = .85) appears to be slightly greater than that observed for
383 the tHb-mass/BM (AUC = .82) (Table 3). Still, the ROC curve
384 comparison showed that this difference is negligible (Fig. 2). In
385 addition, the determination of FFM may be associated with some
386 measurement error, hence the tHb-mass/FFM does not appear to
387 be a more useful indicator than tHb-mass/BM in female road
388 cyclists, who themselves are generally characterized by having a
389 low degree of fat mass.

390 The current findings suggest a higher initial tHb-mass
391 amongst those young female road cyclists who also achieved elite
392 status later in their careers. Determining an absolute threshold
393 (for tHb-mass) to become an elite women cyclist does, however,
394 remain difficult. A value of 10.3 g of tHb-mass/BM was
395 considered a target for identifying talent in female riders, based
396 on a linear regression in novice cyclists aged 11–15.¹¹ Our results
397 suggest 10.6 g/kg as a possible threshold to reach the elite level,
398 with the cut-off point obtained from the ROC curve analysis and
399 the Youden index (Table 3). Interestingly, the average tHb-
400 mass/BM in the EL group was 11.2 ± 0.8 g/kg (Table 2), which
401 is consistent with other data on highly-trained female cyclists
402 (11.1 ± 0.6 g/kg),²⁹ but lower than world-class female cyclists
403 from the Australian National Team (12.3 ± 0.9 g/kg).¹⁵ Overall,

404 competitive performance in elite female cyclists does appear to
405 be related to their relative tHb-mass.⁸

406 According to a published classification framework, road
407 cyclists ranked in the top 200 in the UCI ranking could be
408 classified as world-class.³⁰ Our EL group consisted of riders
409 ranked from 5th to 400th. We therefore recalculated the cut-off
410 point for tHb-mass/BM considering only those riders who were
411 ranked up to 200th (n = 7). The cut-off value remained almost
412 unchanged (10.7 g/kg), while the mean value of the tHb-
413 mass/BM was still the same (11.2 ± 1.0 g/kg) as those athletes
414 ranked up to 400th. Combined with the lack of correlation
415 between tHb-mass and UCI ranking position in the EL group, it
416 appears that a high tHb-mass remains a strong candidate for
417 reaching the elite level in women's road cycling, while other
418 performance determinants need to be tested to explain better
419 rankings in elite riders (i.e., those with a high tHb-mass).

420 The study undertaken does have inherent limitations. For
421 example, the criterion used to define elite status, the small
422 number of participants tested, and cross-sectional design (during
423 initial testing), may all introduce bias our prediction models. On
424 the other hand, we speculate that some physiological assets (e.g.,
425 a high tHb-mass) may have robust predictive power for future
426 performance in this sporting discipline, where aerobic capacity
427 is paramount, while also potentially influencing other factors
428 (e.g. sprint, technical and tactical abilities, aerodynamics, and
429 coping with stressful conditions) that were not considered
430 herein. The role of a rider within a team adds to these
431 complexities. Some cyclists with a favorable physiological
432 predisposition may sacrifice their own rankings in order to
433 support stronger riders within a team.

434 Practical applications

435 Aerobic capacity, which is fundamental to performance in road
436 cycling, is strongly related to hematological variables. Both are
437 genetically determined, but while the former can change
438 significantly under the influence of systematic training, the latter
439 can change only moderately, reflecting innate potential better
440 than aerobic capacity indices. Therefore, measurement of tHb-
441 mass can be a helpful tool to identify young female road cyclists
442 with the potential to reach the elite level. As the use of junior
443 sports performance for selection purposes has been strongly
444 questioned recently,³¹ this information may be of prime
445 importance in talent identification for sporting federations,
446 teams, and coaches.

447 Conclusions

448 Hematological variables (i.e., total hemoglobin mass [tHb-mass]
449 and erythrocyte volume) measured at junior age allowed to
450 distinguish female road cyclists who reached the elite level in the

451 following 8 years from those who did not achieve the same
452 performance level. However, initial fitness and performance in a
453 graded exercise test were poor predictors of reaching future elite
454 performance level. Overall, the relative tHb-mass at a young age
455 represented the most robust predictor of future elite performance
456 in women's road cycling.
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