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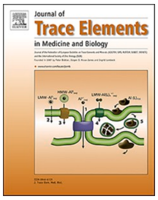

Association of iron supplementation, *HFE* and *AMPD1* polymorphisms and biochemical iron metabolism parameters in the performance of a men's World Tour cycling team: A pilot study

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ABSTRACT

Background: Nutritional strategies with iron supplementation have been shown to be effective in preventing the decline of blood biochemical parameters and sports performance. The aim of the study was to describe biochemical iron metabolism parameters in association with iron supplementation and *HFE* and *AMPD1* polymorphisms in a Union Cycliste Internationale (UCI) World Tour cycling team to evaluate performance during a whole season

Methods: Twenty-eight professional men cyclists took part in this longitudinal observational pilot study. *AMPD1* c.34 C>T (rs17602729) and *HFE* c.187 C>G (rs1799945) polymorphisms were genotyped using Single Nucleotide Primer Extension (SNPE). All the professional cyclists took oral iron supplementation throughout the season. Four complete blood analyses were carried out corresponding to UCI controls in January (1st), April (2nd), June (3rd) and October (4th). Data on participation in three-week Grand Tours, kms of competition and wins were analyzed. Results: In performance, especially in wins, there was a significant effect in *HFE* on biochemical hemoglobin ($F = 4.255$; $p = 0.021$) and biochemical hematocrit ($F = 5.335$; $p = 0.009$) and a hematocrit biochemical × genotype interaction ($F = 3.418$; $p = 0.041$), with higher values in professional cyclist with GC genotype. In *AMPD1* there were significant effects in the biochemical iron x genotype interaction in three-week Grand Tours ($F = 3.874$; $p = 0.029$) and wins ($F = 3.930$; $p = 0.028$)

Conclusions: Blood biochemical iron metabolism parameters could be related to performance in the season due to increasing hemoglobin and hematocrit concentration under iron supplementation, associated with winning in the professional cyclists with GC genotype of the *HFE* polymorphism.

1. Introduction

Professional cycling requires high levels of endurance, strength and cardiovascular fitness [1,2]. To achieve optimal performance, cyclists must pay close attention to their nutrition and training regime, and regularly monitor their blood biochemistry targets [3].

Blood biochemical markers could provide information on general health and performance, particularly in endurance sports and professional cycling [4], especially iron, serum ferritin, transferrin, hemoglobin concentration and hematocrit [5].

Iron is an essential micronutrient required for various biological processes, including erythropoiesis, oxidative metabolism, and cellular immune response [6]. Iron depletion, usually defined as a low serum ferritin level, is a common problem in athletes and is especially prevalent among those involved in endurance events [7,8]. Previous studies have reported low serum ferritin and little iron stored in the bone marrow of men distance runners [9–11]. Reduced iron stores could induce a decrease in hemoglobin concentration and iron deficiency anemia. Because hemoglobin is the main carrier of oxygen to the cells, a low hemoglobin concentration would impair aerobic metabolism [12–15]. Iron is involved in oxidative metabolism and is essential for sports performance [16]

The main mechanisms by which sport leads to iron deficiency are increased iron demand, elevated iron loss and blockage of iron

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absorption due to hepcidin bursts [17,18].

As a baseline set of blood tests, hemoglobin, hematocrit, mean cellular volume, mean cellular hemoglobin, serum ferritin and transferrin levels help monitor iron deficiency [12]. Serum ferritin is a major iron storage protein and essential to iron homeostasis. This protein is involved in a wide range of physiological and pathological processes [19]. Recently it has been shown that in healthy men athletes >15 years, serum ferritin values <35 ng/mL are equivalent to iron deficiency [20]. Even with all the published scientific evidence, the serum ferritin ranges used in research are not standardized, and due to the lack of clinical consensus, cut-off values have not been established to demonstrate the decline in performance in cohorts of elite athletes [8]. Nowadays, there is widespread belief that low serum ferritin levels, even in the context of normal hemoglobin in men (>12 g/dL), are the culprit for fatigue and poor performance in endurance athletes [21]. Iron is transported in the circulation bound to transferrin, which keeps it in an inert redox state and transports it to tissues [22]. Transferrin has multiple functions; it is the main plasma iron transport protein, sequestering iron extracellularly to deliver it to target tissues and, moreover, transferrin represents a protective mechanism against the presence of free iron in plasma, which could be extremely toxic to cells [23,24]. Each transferrin molecule has two iron binding sites and under physiological conditions transferrin saturation (TS) is only up to 30–40% [24].

To avoid the decrease in iron levels in endurance athletes, treatment of iron deficiency consists of nutritional counselling, oral iron supplementation [25] or, in specific cases, by intravenous injection [26]. Nutritional strategies with iron supplementation have been shown to be effective in preventing the decline of blood biochemical parameters such as iron, serum ferritin, hemoglobin and hematocrit in professional cyclists during the course of a three-week Grand Tour [3], showing optimal muscular recovery. However, no evidence has been presented that iron supplementation improves sports performance in endurance athletes, and there is also a lack of consensus in the research presented [8]. In this regard, prolonged daily oral or intravenous iron supplementation in the presence of normal or even elevated serum ferritin values makes no sense and may be harmful [12,27].

High percentages of hyperferritinemia has been found in professional cyclists in anti-doping tests [28]. Most elite endurance athletes take iron supplements during their active sporting life, which could aggravate hyperferritinemia. Different mutations in the human homeostatic iron regulator (*HFE*) gene has been associated with the genetic and biochemical basis of haemochromatosis *HFE* and iron overload in the general population [29], and it has been reported that the *HFE* (c.187 C>G; rs1799945 and c.845 G>A; rs1800562) polymorphisms are responsible for hemochromatosis in endurance athletes [30], defining the c.187 C>G polymorphism the status of elite endurance athlete [31,32]. Previous investigations have found that the G allele in c.187 C>G polymorphism has a higher serum ferritin levels and transferrin, associated with increased iron absorption capacity in subjects with this allelic variant [33,34], however the data are still contradictory [35,36]. The adenosine monophosphate deaminase isoform 1 (*AMPD1*) is an important regulator of energy metabolism in the muscle fiber [37–39]. Previous investigations have found that the T allele in the *AMPD1* gene (c.34 C>T; rs17602729) polymorphism might reduce the likelihood of being an elite endurance athlete [40–42] and increase the risk of muscle injuries [43].

These polymorphisms have previously been identified as markers that predispose to achieving the status of elite endurance athletes by optimizing metabolic energy and iron metabolism [44], helping to create profiles that show these markers as a genetic selection in this cohort of endurance athletes [42]. The need for knowledge of genetics and optimization of iron supplementation in the influence of sports performance of professional cyclists promotes a new area of study to personalize and individualize the performance of these cohort of athletes.

Therefore, the aim of this study was to describe blood biochemistry iron metabolism parameters in association with iron supplementation and *HFE* and *AMPD1* polymorphisms in an International Cyclist Union (UCI) World Tour professional cycling team to evaluate performance during a whole season that favors participation in three-week Grand Tour races and competition wins. This study hypothesized for the first time that genetics play a crucial role along with the blood biochemistry parameters involved in iron metabolism in the performance of professional cyclists.

2. Materials and methods

2.1. Participants

Twenty-eight men from a World Tour professional cycling team voluntarily participated in this longitudinal descriptive pilot study. All the participants belonged to a professional UCI World Tour. The study was carried out during the 2021 professional cycling season.

The baseline demographic characteristics and performance season data of the whole team are presented in Table 1.

The following inclusion criteria were established: a) professional cyclists aged >18 years; b) baseline serum ferritin values less than 35 ng/mL c) who have participated in at least 30 days of competition during the season, and d) who had performed regular exercise training during the previous 6 months. Exclusion criteria were a) professional cyclists with iron supplementation in the 3 months prior to the start of the study; b) disabling traumatic injuries for cycling training or competition in the 6 months prior to the start of the study.

Informed consent was obtained from all the participants in the investigation. The study protocol was approved by the Francisco de Vitoria University Research Ethics Committee (Institutional Review Board (IRB) UFV 32–2020)) and the confidentiality of the participants was ensured, complying with the Declaration of Helsinki 1964 (latest update 2013).

2.2. Data recording

Four complete blood analyses were carried out corresponding to UCI out-of-competition (OOC) controls in January (1st), April (2nd), June (3rd) and October (4th) during the 2021 season. Blood extraction and transportation were performed according to the UCI and World Anti-Doping Agency (WADA) guidelines (www.ama-wada.org). All samples were collected under basal conditions after a 10–12 h overnight fast. The blood samples (15 mL) were obtained at 8:00 a.m. from the antecubital vein with the subject seated in a comfortable position using ethylenediaminetetraacetic acid (EDTA) Vacutainer tubes and a tube without anticoagulant was used to quantify serum iron and transferrin.

Upon blood extraction, euhydration status was estimated through urine samples and urine specific gravity was measured with a refractometer (MASTER-S28M, Atago company, Tokyo, Japan). A urine specific gravity <1020 was a mandatory condition for validating blood biochemical iron metabolism parameters, especially hematocrit and hemoglobin.

2.3. Iron supplementation

All cyclists included received oral iron supplements on an empty stomach of Ferogradumet (Teofarma Srl, Italy); 105 mg/day (325 mg/day ferrous sulphate) in the form of fasting tablets, immediately after the results of the first analysis and were maintained throughout the season until the end of the season in October. In addition, all cyclists received Redoxon (Bayer, Germany) multivitamin pills, that included folic acid (200 μg/day), vitamin C (1000 mg/day), vitamin B12 (1000 μg/day), zinc (10 mg/day) and vitamin D (10 μg/day).

Table 1
Demographic and season performance data parameters of a professional cycling team with *HFE* and *AMPD1* polymorphisms association.

	Professional cyclists (n = 28)	*HFE* c.187 C>G; rs1799945		p value	*AMPD1* c.34 C>T; rs17602729		p value
		GC (n = 8)	CC (n = 20)		CC (n = 22)	CT (n = 6)	
Age, years	27.61 (5.42)	28.50 (7.44)	27.25 (4.57)	0.591	27.55 (4.96)	27.83 (7.44)	0.911
Height, cm	180.25 (6.71)	179.88 (8.39)	180.40 (6.16)	0.856	180.23 (6.22)	180.33 (8.98)	0.973
Weight, kg	66.82 (6.25)	68.00 (8.89)	66.35 (5.05)	0.538	67.41 (6.44)	64.67 (5.43)	0.350
BMI, kg/m^2	20.61 (3.52)	21.03 (3.73)	20.44 (3.32)	0.315	20.78 (3.66)	19.96 (3.26)	0.167
Fat mass, (%)	7.97 (0.88)	7.92 (0.64)	7.99 (0.90)	0.865	8.08 (0.95)	7.56 (0.44)	0.214
Muscle mass, kg	29.94 (2.12)	30.40 (3.08)	29.76 (1.66)	0.482	30.26 (2.19)	28.76 (1.40)	0.128
Competition days	55.75 (18.00)	57.13 (20.18)	55.20 (17.61)	0.804	56.73 (18.16)	52.17 (18.58)	0.592
Kms competition	8764.79 (1421.16)	9023.38 (1652.14)	8661.35 (1098.23)	0.776	8880.73 (1652.62)	8339.67 (1363.67)	0.699
Season Kms	34211.62 (6331.62)	34542.22 (5746.22)	34052.63 (6355.22)	0.898	34642.66 (5721.45)	34052.66 (6866.25)	0.743

BMI, body mass index; cm, centimeters; kg, kilograms; Kms, kilometers; SD, standard deviation

2.4. Deoxyribonucleic acid (DNA) extraction and genotyping

The samples were collected with SARSTED swabs by buccal smear and kept refrigerated until genotyping. Deoxyribonucleic acid (DNA) extraction from the swabs was carried out in the VIVOLabs laboratory (Madrid, Spain) by automatic extraction in QIACube equipment (QIAGEN, Venlo, Holland), yielding a DNA concentration of 25–40 ng/mL, which was kept in a solution in a volume of 100 microliters at −20ºC until genotyping.

HFE (c.187 C>G; rs1799945) and *AMPD1* (c.34 C>T; rs17602729) polymorphisms were genotyped using Single Nucleotide Primer Extension (SNPE) with the SNaPshot Multiplex Kit (Thermo Fisher Scientific, MA, USA), with analysis of the reaction result by capillary electrophoresis fragments, in an ABI3500 unit (Applied Biosystems, CA, USA) with bioinformatic analysis performed by GeneMapper 5.0 software (Applied Biosystems, CA, USA).

2.5. Data acquisition

The medical team collected biochemistry data from the professional cyclists analyzed in independent laboratories. This blood biochemistry test consisted of complete hepatic, cardiac, renal and hormonal profiles due to the requirements of UCI blood tests. These data were stored in coded form and sent for analysis anonymously. To avoid bias in the interpretation of hematocrit and hemoglobin levels, all blood biochemical tests were carried out for all cyclists one week after the last concentration at high-altitude at sea level.

The performance staff collected the data on season kilometers, days of competition, and kms in competition during the whole season through the Trainingpeaks Platform (Louisville, CO, USA).

2.6. Statistical analysis

The statistical analysis was performed using the using the Statistical Package for the Social Sciences (SPSS, IBM Company, version 25.0, IBM Corporation, Armonk, NY, USA). Data were expressed as mean and standard deviation (SD). The Hardy-Weinberg equilibrium (HWE) was tested for each polymorphism using the χ^2 test. The Shapiro-Wilk test was used to check the normality of all variables. Since all variables were normally distributed, parametric tests were applied to examine differences among conditions. A three-way Analysis of Variance (ANOVA) (biochemistry × performance × genotype) was used to compare different blood biochemistries, performance (Grand Tours participation and wins) and genotype (*HFE* and *AMPD1*). When a significant F value was obtained, an LSD post hoc analysis was performed to determine pairwise differences. Effect sizes among groups and interactions were calculated using partial eta square (η^2). The significance level was set at $p < 0.050$.

3. Results

In the *HFE* c.187 C>G polymorphism, 20 cyclists were CC genotype (71.4%) and 8 GC genotype (28.6%). In the *AMPD1* c.34 C>T polymorphism 22 cyclists were CC genotype (78.6%) and 6 CT genotype (21.4%). The polymorphisms analyzed met the HWE (all $p > 0.050$).

Fifteen cyclists (53.6%) competed throughout the season in three-week Grand Tours (Giro d'Italia, Tour de France and Vuelta a España), with four of them (26.7%) competing in two Grand Tours (Tour de France and Vuelta a España).

Only 3 riders dropped out during the Grand Tours, all of them due to crashes in the Vuelta a España, on stages 8, 18 and 20. The team competed in three-week Grand Tours an average of 26.8 days (± 9.23 days), representing 39.6% of the annual racing days of these cyclists.

None of the professional cyclists experienced side effects from iron supplementation use at the current dosage during the competitive season.

Regarding biochemical iron metabolism parameters, differences between cyclists who participated in three-week Grand Tours and those who did not, no differences were found (all $p > 0.050$), while statistical differences in hemoglobin ($p = 0.024$) were shown between cyclists who achieved season wins versus those who did not (Table 2).

No cyclists showed abnormal data in biochemical iron metabolism parameters during the season. No iron deficiency was established in the professional cyclists during the season and iron supplementation was provided ad libitum.

The cycling team undertook live high-train low (LHTL) training with

Table 2
Biochemical iron metabolism parameters at the beginning of the season among cyclists in three-week Grand Tours and wins.

	Grand Tours (n = 15), mean (SD)	**Non-Grand Tours (n = 13), mean (SD)**	**p value**
Iron, μg/dL	134.0 (22.9)	115.6 (15.1)	0.299
Serum ferritin, ng/mL	28.8 (2.5)	26.3 (2.2)	0.132
Transferrin, mg/dL	215.2 (70.3)	243.7 (34.8)	0.183
Hemoglobin, g/dL	15.1 (0.6)	14.9 (0.6)	0.892
Hematocrit, %	45.5 (2.0)	44.9 (1.9)	0.588
	Wins (n = 7), mean (SD)	**Non-wins (n = 21), mean (SD)**	**p value**
Iron, μg/dL	123.1 (19.6)	115.1 (15.2)	0.645
Serum ferritin, ng/mL	27.9 (2.3)	27.7 (2.4)	0.973
Transferrin, mg/dL	230.8 (20.0)	231.1 (62.7)	0.992
Hemoglobin, g/dL	15.5 (0.3)	14.8 (0.5)	0.024
Hematocrit, %	46.1 (1.1)	44.8 (1.9)	0.138

g/dl, gram/deciliter; mg/dl, milligram/deciliter; ng/mL, nanogram/milliliter; μg/dl, microgram/deciliter; SD, standard deviation an average of 43.2 days (± 12.66 days).

3.1. Iron

Values of serum iron during the season between Grand Tours and winning groups are shown in Fig. 1. For the *HFE* polymorphism, there was a significant effect of the biochemical iron × Grand Tour participation ($F = 3.853$; $p = 0.032$; $\mu^2 = 0.435$) interaction. The post hoc analysis revealed that, in comparison to non-Grand Tours participation, iron concentrations were higher in the first (1st) biochemistry in the GC genotype ($p = 0.013$). Regarding wins, no interactions were found in the *HFE* polymorphism (all $p > 0.050$). For the *AMPD1* polymorphism, there was a significant effect of biochemical iron × genotype ($F = 3.874$; $p = 0.029$; $\mu^2 = 0.421$) in Grand Tour participation, similar to wins, in which a biochemical iron × genotype ($F = 3.930$; $p = 0.028$; $\mu^2 = 0.424$) interaction was also shown. However, the post hoc analysis revealed that iron concentrations were similar in the GC and CC genotypes for *HFE* polymorphism and CC and CT for *AMPD1* polymorphism (all $p > 0.050$).

3.2. Serum ferritin

Serum ferritin values during the season between groups of Grand Tours and winning are presented in Fig. 2. For the *HFE* polymorphism, there were only significant effects of biochemical serum ferritin in Grand Tours participation ($F = 4.378$; $p = 0.020$; $\mu^2=0.451$) and wins ($F = 4.861$; $p = 0.014$; $\mu^2 = 0.477$) during the season. The post hoc analysis revealed that, in comparison to non-Grand Tours participation, the serum ferritin concentrations were similar in GC and CC genotypes (all $p > 0.050$). For the *AMPD1* polymorphism, no interactions were shown in Grand Tours participation, however in wins a significant effect of biochemical serum ferritin ($F = 3.551$; $p = 0.037$; $\mu^2 = 0.308$) was evident when compared to the non-winning group. The post hoc analysis revealed that the serum ferritin values were similar in the CC and CT genotypes (all $p > 0.050$).

3.3. Transferrin

Regarding transferrin, the values during the season in both groups of Grand Tours and winning are shown in Fig. 3. For the *HFE* polymorphism there was a significant effect of the biochemical transferrin × Grand Tour participation × genotype interaction ($F = 4.507$; $p = 0.019$; $\mu^2 = 0.474$) and no effects were shown in wins. The post hoc analysis revealed that ferritin values were similar in GC and CC genotypes (all $p > 0.050$). For the *AMPD1* polymorphism, no interactions were shown in Grand Tours participation and wins (all $p > 0.050$). However, in the post hoc analysis, in comparison to non-Grand Tours participation, the transferrin concentrations were higher in the 3rd and 4th biochemistries in the CT genotype (all $p < 0.050$).

3.4. Hemoglobin

The hemoglobin values between the Grand Tours and wins are presented in Fig. 4. For the *HFE* polymorphism, no interactions were shown in Grand Tours participation. However, there were significant effects of biochemical hemoglobin ($F = 8.211$; $p < 0.001$; $\mu^2 = 0.592$) and the biochemical hemoglobin × wins interaction ($F = 4.255$; $p = 0.021$; $\mu^2 = 0.429$) when compared with non-winning group. The post hoc analysis revealed that, in comparison to the non-winning group, the hemoglobin was higher in 2nd, 3rd and 4th biochemistries in the GC genotype (all $p < 0.050$). For the *AMPD1* polymorphism, no interactions were shown between Grand Tours participation and wins (all $p > 0.050$).

3.5. Hematocrit

In hematocrit values, similar results regarding hemoglobin were shown in Fig. 5. In this aspect, for the *HFE* polymorphism, no interactions were shown in Grand Tours participation. However, there were significant effects of biochemical hematocrit × genotype ($F = 3.418$; $p = 0.041$; $\mu^2 = 0.376$) and biochemical hematocrit × wins ($F = 5.335$; $p = 0.009$; $\mu^2 = 0.485$) interactions when compared to non-winning group. The post hoc analysis revealed that, in comparison to the non-winning group, the hematocrit was higher in 3rd and 4th biochemistries in the GC genotype (all $p < 0.050$). For the *AMPD1* polymorphism, no interactions were shown between Grand Tours participation and wins (all $p > 0.050$).

4. Discussion

This study describes for the first time the blood biochemistry iron metabolism parameters in association with the *HFE* and *AMPD1* polymorphisms in an UCI World Tour professional cycling team to evaluate performance during a whole season that favors participation in three-week Grand Tours races and competition wins. This was based on previous investigations that have found effects of these parameters on performance in elite endurance athletes and professional cyclists [3,28,

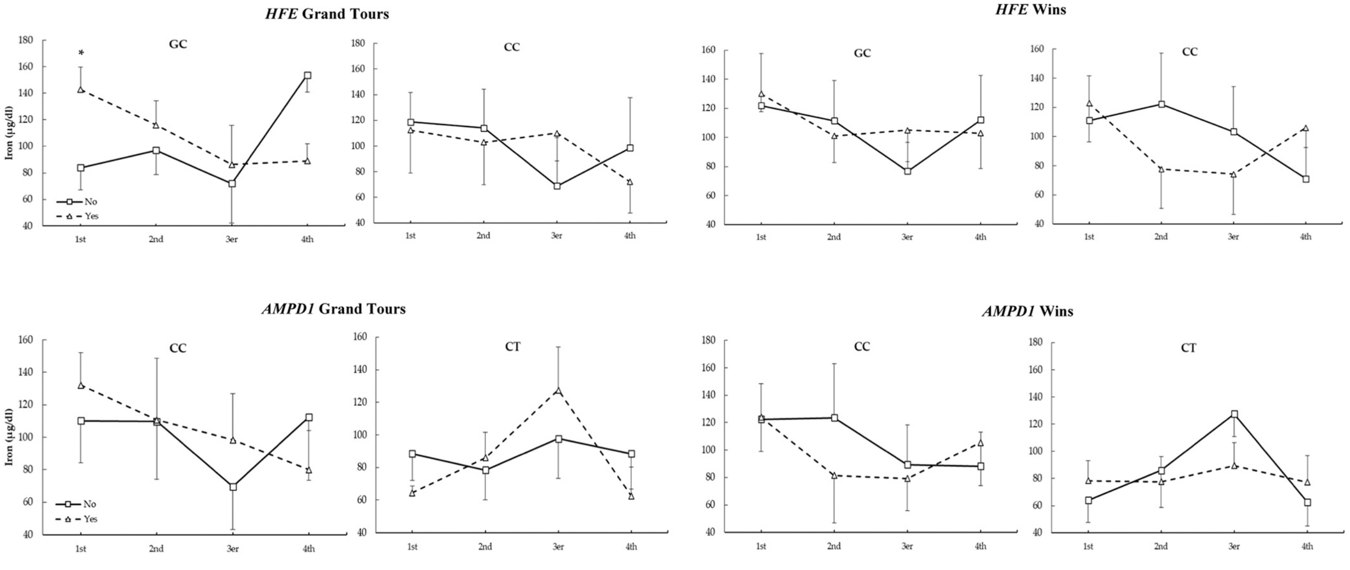

Fig. 1. Iron values in season biochemistries in professional cyclist in *HFE* and *AMPD1* in three-week Grand Tours and wins. *$p < 0.050$.

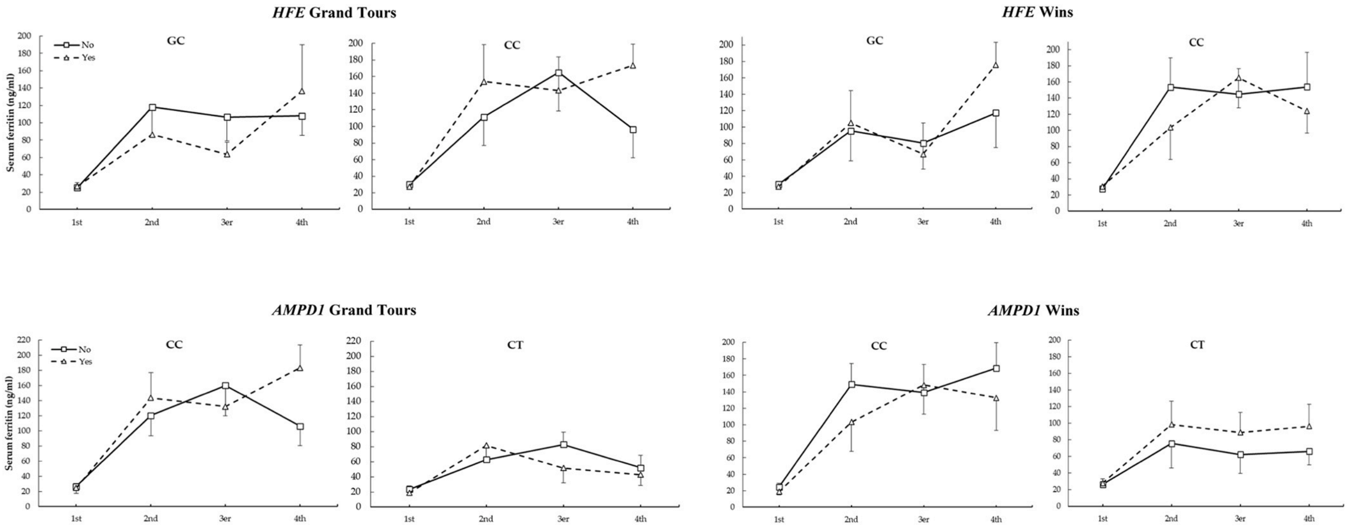

Fig. 2. Serum ferritin values in season biochemistries in professional cyclist in *HFE* and *AMPD1* in three-week Grand Tours and wins.

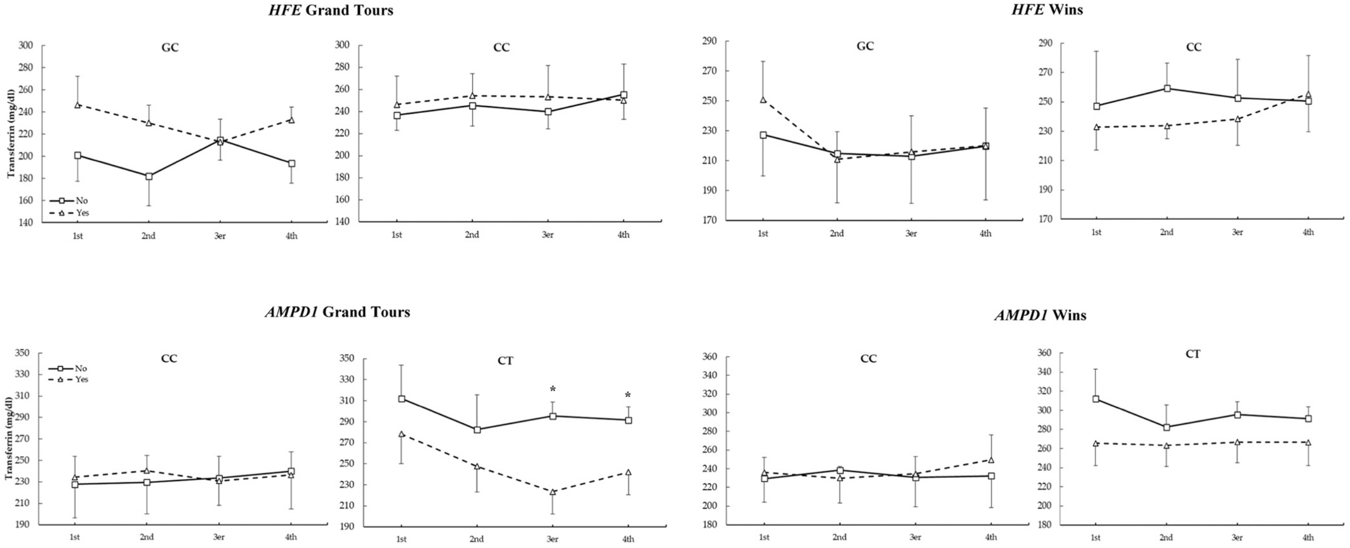

Fig. 3. Transferrin values in season biochemistries in professional cyclists in *HFE* and *AMPD1* in three-week Grand Tours and wins. *$p < 0.050$.

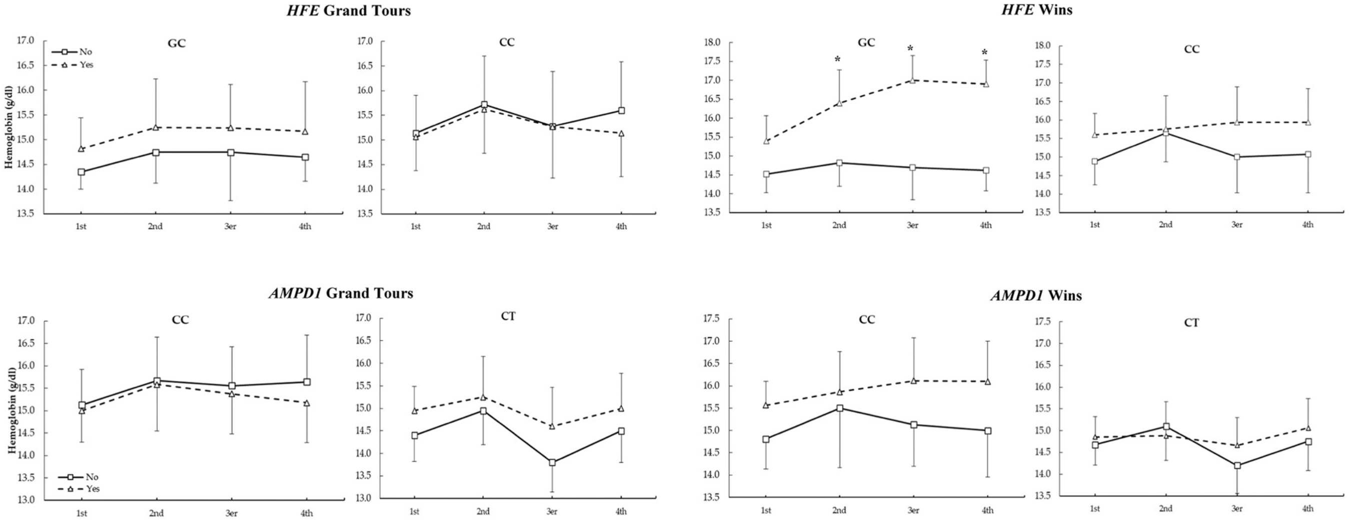

Fig. 4. Hemoglobin values in season biochemistries in professional cyclist in *HFE* and *AMPD1* in three-week Grand Tours and wins. *$p < 0.050$.

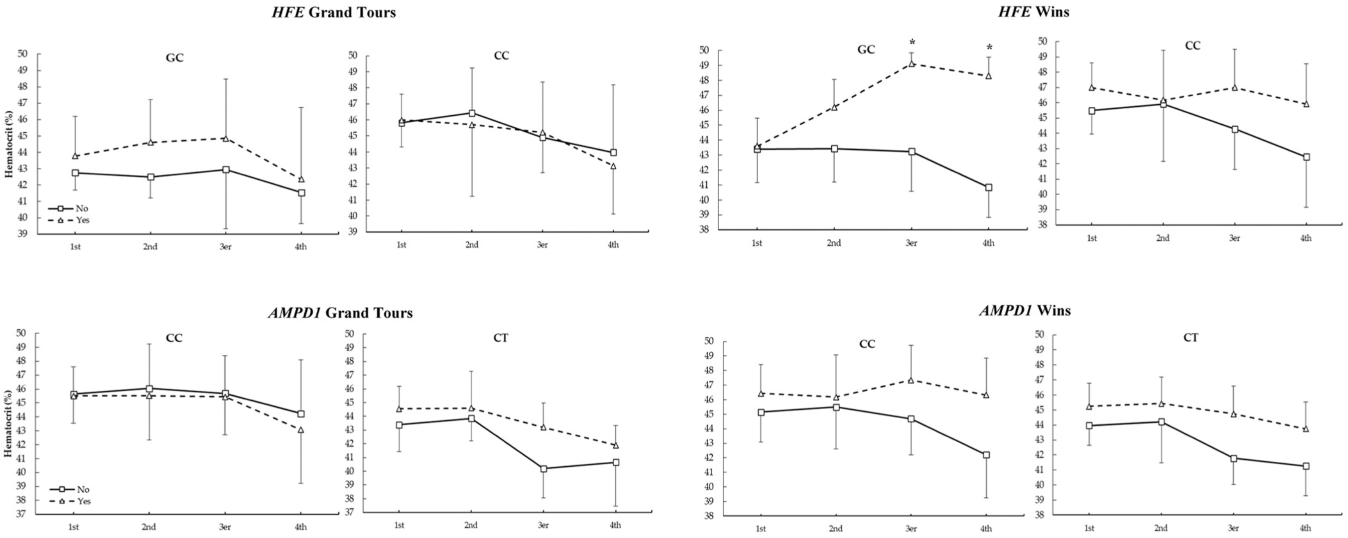

Fig. 5. Hematocrit concentration in season biochemistries in professional cyclist in *HFE* and *AMPD1* in three-week Grand Tours and wins. *p < 0.050.

45–47] and have suggested that the expectancy of having higher values may induce a performance-enhancement. The main outcomes of this investigation confirm the existence of the effect of biochemical iron metabolism parameters under iron supplementation during the season in a World Tour cycling team, especially in the increasing hemoglobin and hematocrit concentrations under iron supplementation, associated with winning in the professional cyclists with GC genotype professional cyclists in the c.187 C>G polymorphism of the *HFE*.

This research indicates for the first time that genetics could play a role in the effectiveness of achieving wins during the cycling season, especially with the *HFE* polymorphism. Specifically, *HFE*-GC genotype was associated with higher serum iron solely at the beginning of the study period in Grand-Tours participants while hemoglobin was higher in winners at the last three measurements, reaffirmed by serum ferritin during the season under iron supplementation. This research also suggests that the *HFE* and *AMPD1* polymorphisms could be used to produce greater knowledge to optimize individual nutrition in this cohort of elite athletes under iron supplementation. In turn, the study subjects took multivitamin pills that could influence iron metabolism, due to the amount of vitamin A, C, D, B12 and folic acid that increase the absorption of this mineral and its use in the organism to promote optimal recharge of iron deposits.

Iron plays a crucial role in sports performance, including cycling. Iron is an essential component of hemoglobin, a protein in red blood cells that carries oxygen to the muscles [48]. When iron levels are low, a condition known as iron deficiency or iron deficiency anemia negatively affects athletic performance. In this investigation, however, no professional cyclists presented anemia or symptoms because they intake oral iron supplementation, always monitored by the medical staff and by blood biochemical tests to avoid hyperferritinemia. The *HFE* gene encodes a protein that plays a key role in iron homeostasis, which is essential for the transport and storage of iron in the body. Mutations in the *HFE* gene can lead to disorders of iron metabolism, such as hereditary hemochromatosis, which is characterized by excessive iron absorption in the intestine and a consequent increase in iron levels in the body [49]. However, to date there is still no evidence of a direct relationship between *HFE* gene variants and athletic performance, although it is a gene that has been associated with elite endurance athlete status [42,44,50]. In this investigation, the GC genotype showed elevated values in participants in the three-week Grand Tours early in the season, which may indicate that this biochemical marker could predict optimal endurance performance, as demonstrated in previous research [51]. The *AMPD1* gene is a marker that has been studied in its association with muscle energy metabolism [44], in which the C allele is the optimal allele for correct energy metabolism, and the T allele is the cause of early fatigue, cramps or even injuries in sports practice [52–54]. For this reason, the CC genotype could be related to optimal muscle metabolism and thus act as a modulator of iron expenditure during endurance sports practice, serving as a predictor of performance. This research shows for the first time the interaction of *AMPD1* genotypes with the predisposition to participate in three-week Grand Tours, as well as in the achievement of wins. These results should be confirmed in future studies.

Serum ferritin is a useful indicator for assessing the iron status of the body. In the context of elite sport, serum ferritin levels can have an impact on the performance of endurance athletes, such as cyclists [55], although nowadays there are still discrepancies about the optimal serum ferritin levels that accompany optimal performance, especially in men athletes [8]. The results found in this investigation showed no association with serum ferritin levels throughout the season in this cohort of professional cyclists. It has been shown that excessive iron supplementation causes hyperferritinemia in elite road cyclists and persists after cessation. This excess iron can lead to long-term complications and should be eliminated, at least by the time professional cyclists retire [28, 56], and the effect of genetics should be added to monitor this iron supplementation in a more individualized way with the knowledge reported. In this professional cycling team, iron supplementation was always monitored by the medical staff and no professional cyclist presented hyperferritinemia, either during the season or during their sporting career.

Transferrin is a protein which plays an important role in the transport of iron in the blood [57]. In the context of sports performance, transferrin and iron levels in the body could have significant implications, because elevated levels of transferrin may be related to dehydration or a response to infection or physical stress [58] and decreased levels are related to iron deficiency anemia, calorie-restricted diets and several diseases [59–61]. In this investigation, an interaction was shown of ferritin with the *HFE* polymorphism and participation in Grand Tours, with the GC genotype being the one with the highest values in participation in three-week Grand Tours; an aspect that should be correlated with inflammatory markers and which, in turn, is correlated with serum iron levels throughout the season in this genotype. The CT genotype of the *AMPD1* gene also showed transferrin levels in cyclists who did not compete in three-week Grand Tours, a fact that could be related to the state of inflammation caused in the muscles due to the lack of muscular energy efficiency shown by the T allele and that would limit these subjects from participating in these competitions (Fig. 3). Due to the lack of research on the performance of this molecule and association with genetics, future studies should be performed adding biochemical inflammation data to corroborate these findings.

Total hemoglobin is a key factor in the performance of elite endurance athletes. There are numerous investigations that have shown that hemoglobin is essential for athletic performance, but this marker to date has not shown a strong association with performance [62–64]. Hemoglobin plays an important role in the transportation of oxygen for feeding the working muscles during strenuous exercise in endurance sports [64]. All professional cyclists undertook LHTL training in the season for 4–6 weeks, which was in line with the season's objectives and competitions. The *HFE* gene is not directly related to hemoglobin, but it plays a crucial role in regulating iron metabolism, which, in turn, affects iron homeostasis and therefore the production and function of hemoglobin, associated with sports performance. In this investigation, the GC genotype is shown for the first time related to sporting success due to a statistical increase in cyclists who achieved wins compared to those who did not (2nd, 3rd and 4th biochemistries) (Fig. 4). Previously, a study has shown that the G allele presented a positive correlation between iron and hemoglobin and demonstrated that changes in iron metabolism occur at an early age in *HFE* GC heterozygotes [65], with the c.187 C>G; rs1799945 polymorphism being a marker of genetic selection in sports performance as previously shown [42,44].

The increase in hematocrit is due to several metabolic pathways related to erythropoietin synthesized by continuous hypoxia conditions [66]. Increased hematocrit has drawbacks, such as blood viscosity. Hematocrit increases with acute exercise and LHTL periods, with "optimal" values being those below 50% [67,68]. Endurance sport, however, depletes the hematocrit levels, associated with a decrease in performance due to lack of oxygen transport to the muscle during high intensity efforts [69]. In this professional cycling team, no cyclist presented hematocrit levels above or below those recommended for optimal health and sports performance. The results found in the hematocrit are similar to those shown in the hemoglobin in the *HFE* gene, presenting the GC genotype with significant high values in the professional cyclists who achieved wins (3rd and 4th biochemistries) (Fig. 5). Endurance athletes often seek to increase their hemoglobin and hematocrit levels to improve their oxygen carrying capacity and therefore their endurance and performance [64]. These conditions can be achieved through external factors such as LHTL as well as internal factors that predispose to this improvement, such as genetics, previously shown as selection markers of athlete status [44].

4.1. *Practical applications*

Iron supplementation in professional cyclists could be an important strategy for improving sport performance, especially in those with iron deficiency or iron deficiency anaemia. Several practical applications of iron supplementation in professional cyclists; i) Prevention and treatment of iron deficiency anaemia: Iron deficiency anaemia is common in athletes, especially women and endurance athletes such as cyclists, due to the increased iron demands caused by intense physical activity and iron loss through sweat, urine and the gut. Iron supplementation can help prevent and treat iron deficiency anaemia, which can improve oxygen-carrying capacity and endurance [70]. ii) Improved athletic performance: Iron is essential to produce haemoglobin, which transports oxygen from the lungs to the tissues, including active muscles during cycling. By increasing iron levels, haemoglobin levels and thus oxygen-carrying capacity can be improved, which can lead to improved athletic performance, specially in endurance cycling [8] and iii) Muscle recovery: Iron is also important for muscle health and recovery after exercise. It aids in the production of myoglobin, a protein that stores and transports oxygen in muscle cells. Iron supplementation can help speed muscle recovery after intense training sessions, allowing for better adaptation to exercise and sustained athletic performance [3].

The outcomes of this investigation suggest that iron metabolism parameters could be used by the medical staff of elite cycling teams to improve performance throughout the season. To obtain this benefit, genetics should be considered as a conditioning factor to individualize the diet and optimize iron supplementation in this cohort of elite athletes.

4.2. *Strengths/limitations*

The strengths of this study are that it is the first time that genetics have been associated with biochemical iron metabolism parameters during a season in a professional World Tour cycling team. Although studies have previously presented variations in these parameters, these have only been during three-week Grand Tours; Giro d'Italia [71], Tour de France [28] and Vuelta a España [3] and without analyzing the relationship of genetics and these blood biochemical iron metabolism parameters with performance and sporting success. Because of the conflicting results of previous studies, more research on the effects of biochemical iron metabolism parameters on elite cycling performance is needed. The present study shows these biochemical iron metabolism parameters related with genetics in iron and energy metabolism in professional cyclist of a World Tour team. Also, this investigation of elite athletes provides a unique opportunity to define the upper limits of human physiology and performance and supports the need of optimizing nutritional aspects in iron metabolism according to genetics.

In spite of the numerous strengths presented, the current investigation has several limitations that should be addressed: i) in this pilot study, only one World Tour team with 28 cyclists has been included, showing a small sample to determine more powerful results, ii) only men professional cyclists have been measured, and future studies should be conducted on women professional cyclists to corroborate these results and to discover whether the menstrual cycle is related to iron loss, performance and sporting success, iii) no markers of performance have been included in this pilot study and iv) to date, few genetic factors involved in iron absorption and sports performance have been previously studied and will be the subject of future research on the associations between these markers, iron metabolism and sports performance with the inclusion of more genetic markers involved in hyperferritinemia using genetic scores.

5. Conclusion

This pilot study shows for the first time that blood biochemical iron metabolism parameters in a World Tour cycling team could be related to performance during the season under iron supplementation. The increasing hemoglobin and hematocrit concentration under iron supplementation was associated with winning in the professional cyclists with GC genotype in the c.187 C>G polymorphism of the *HFE*.

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Declaration of Competing Interest

none

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