Eur J Appl Physiol
DOI 10.1007/s00421-009-1031-8

ORIGINAL ARTICLE

Daily regulation of serum and urinary hepcidin is not influenced by submaximal cycling exercise in humans with normal iron metabolism

Marie-Bérengère Troadec · Fabrice Lainé · Vincent Daniel · Pierre Rochcongar · Martine Ropert · Florian Cabillic · Michèle Perrin · Jeff Morcet · Olivier Loréal · Gordana Olbina · Mark Westerman · Elizabeta Nemeth · Tomas Ganz · Pierre Brissot

Accepted: 26 February 2009

Abstract Hepcidin and hemojuvelin (HJV) are two critical regulators of iron metabolism as indicated by the development of major iron overload associated to mutations in hepcidin and HJV genes. Hepcidin and HJV are highly expressed in liver and muscles, respectively. Intensive muscular exercise has been reported to modify serum iron parameters and to increase hepcidinuria. The present study aimed at evaluating the potential impact of low intensity muscle exercise on iron metabolism and on hepcidin, its key regulator. Fourteen normal volunteers underwent submaximal cycling-based exercise in a crossover design and various iron parameters, including serum and urinary hepcidin, were serially studied. The results demonstrated that submaximal ergocycle endurance exercise did not modulate hepcidin. This study also indicated that hepcidinuria did not show any daily variation whereas serum hepcidin did. The findings, by demonstrating that hepcidin concentrations are not influenced by submaximal cycling exercise, may have implications for hepcidin sampling in medical practice.

Keywords Hepcidin · Iron · Muscle exercise · Daily variations · Hemojuvelin

M.-B. Troadec · F. Lainé · M. Ropert · O. Loréal · P. Brissot
Liver Disease Unit, Inserm U-522, IFR 140,
University Hospital Pontchaillou, Rennes, France

F. Cabillic
URU Biothérapies Innovantes, Faculté de Médecine,
Université de Rennes 1 Laboratoire de cytogénétique et biologie cellulaire, University Hospital Pontchaillou, Rennes, France

F. Lainé · M. Perrin · J. Morcet
Center for Clinical Investigation, Inserm0203,
University Hospital Pontchaillou, Rennes, France

V. Daniel
Laboratory of Physiology,
University Hospital Pontchaillou, Rennes, France

V. Daniel · P. Rochcongar
Sport Medicine Unit,
University Hospital Pontchaillou, Rennes, France

G. Olbina · M. Westerman · E. Nemeth · T. Ganz
Intrinsic LifeSciences, LLC, La Jolla, CA, USA

E. Nemeth · T. Ganz
David Geffen School of Medicine,
University of California, Los Angeles, USA

Present Address:
M.-B. Troadec (✉)
Jerry Kaplan Laboratory, Department of Pathology,
University of Utah, Salt Lake City, UT 84132, USA
e-mail: marie-berengere.troadec@path.utah.edu

Introduction

The relationship between skeletal muscle and iron metabolism is well established. Skeletal muscle contains about 20% of body iron (Andrews 1999), mainly located in the hemoprotein myoglobin. Myoglobin plays a key role in coping with chronic hypoxia. Iron is also a component of muscle ferritin (Robach et al. 2007) and of the mitochondrial complex III of the respiratory electron transport chain which undergoes adaptations during chronic exercise (Hood et al. 1992; Samad et al. 2004). The recent cloning of hemojuvelin (Papanikolaou et al. 2004) suggested the potential involvement of muscle in the regulation of iron metabolism. Hemojuvelin (HJV, HFE2, or RGMc for repulsive guidance molecule c) is the third member of a gene family originally identified as playing a role in the developing and adult central nervous system (Monnier et al. 2002). HJV inactivation causes severe iron overload

Published online: 21 March 2009

both in humans (Brissot et al. 2008; Papanikolaou et al. 2004) and in mice (Huang et al. 2005) through decreasing hepatic expression of hepcidin which acts as a central iron regulatory hormone regulating iron absorption and recycling (Nicolas et al. 2001; Pigeon et al. 2001). HJV, located in the hepatocyte membrane, induces hepatic hepcidin expression through bone morphogenetic protein (BMP)-mediated signaling (Babitt et al. 2006) whereas the soluble form of HJV has the opposite effect on hepcidin expression. HJV is expressed at high levels in skeletal and heart muscle both in mice (Niederkofler et al. 2004) and humans (Papanikolaou et al. 2004), raising the possibility that the muscle tissue, through the production of soluble HJV and its effect on the BMP/SMAD (the SMAD proteins are homologs of both the drosophila protein, mothers against decapentaplegic (MAD) and the *C. elegans* protein SMA. The name is a combination of the two.) signaling pathway, acts as an upstream-regulator of hepcidin expression and consequently of systemic iron metabolism (Lin et al. 2005). So far, clinical data obtained on the impact of exercise on iron metabolism have been rather controversial (for review see Zoller and Vogel 2004). Frank iron deficiency is unusual in elite athletes although serum ferritin levels are usually low. Recently, one-third of recreational female athletes have been reported to have iron deficiency (Di Santolo et al. 2008). Mean absorption of a standard test dose of ferrous sulfate in runners also provided conflicting results (Ehn et al. 1980; Nachtigall et al. 1996). Schumacher et al. (2002) reported an increase in serum ferritin concentrations after a 45-min constant speed running test at 70% VO_2max, but attributed this variation mainly to exercise-induced changes in plasma and blood volume. Serum transferrin receptor (sTfR), known as a more reliable indicator of cellular iron content, decreased only during incremental exercise challenges to the point of exhaustion. In terms of biochemical evaluation of iron metabolism, the determination of serum and/or urinary hepcidin has recently become a key approach. Blood hepcidin concentration is known to determine the amount of plasma iron availability for the body, by controlling iron export from macrophages and enterocytes. To our knowledge, the only types of exercise which have been explored for hepcidin variations are the marathon race (more than 4 h) of female athletes and the 10-km run of male athletes (70–90% VO_2) where hepcidin concentration increased despite marked interindividual variations (Peeling et al. 2009; Roecker et al. 2005).

In order to further explore the potential interaction between muscle exercise and iron metabolism in low intense exercise, we studied the impact of submaximal cycling-based exercise on serum iron parameters, and on serum and urinary hepcidin levels in volunteers with normal iron metabolism.

Subjects and methods

Subjects selection

Fourteen healthy volunteers participated in the study. They were selected according to the following strict criteria: (i) inclusion criteria: males 18–40 years old; normal clinical examination, normal cardiac status, normal iron metabolism (including the absence of the C282Y mutation in HFE gene responsible for the most frequent genetic hemochromatosis), normal hepatic functions, and normal muscular status (from the assays of these parameters, see "Usual tests"). (ii) Non inclusion criteria: motor handicap contraindicating bicycle exercise; chronic disease; medications; alcohol consumption > 30 g alcohol per day; drug addiction; HIV, HVC or HBsAg positivity; transfusions or blood donations within the last 3 months; professional activity involving sustained muscle activity.

Protocol design

Global design

The study was a pilot, prospective, controlled, randomized, open, and cross-over study. The two cross-over sequences consisted of sequence A in which "exercise period" preceded by 14 days a "no exercise period", and sequence B in which "no exercise period" preceded by 14 days a "exercise period"). Seven subjects were included in each sequence.

Detailed procedure

Inclusion visit This visit was performed less than 1 month before the beginning of the study. It involved an ergocycle test with gradual intensity increments of 30 W and recording of maximal oxygen uptake, heart rate and extent of gas exchange by direct method (measurement of peak oxygen consumption). If eligible, the subjects were asked not to have intense physical activity within 8 days and no unusual muscle activity within 24 h preceding their admission at the Clinical Investigation Center.

Experimental periods (i) Exercise period: at day 0 (8:30 a.m. = T basal), fasting blood samples and urinary samples were collected. A 45 min ergocycle exercise was performed between 11:00 a.m. and 12:00 p.m. at 60% of the previously determined heart rate reserve (protocol close to that proposed by Schumacher et al. 2002). Blood sampling was performed immediately at the end of exercise (this time point is named T0), thereafter at 30 min, 1, 2, 4, 12 and 24 h. Urinary sampling was performed every 3 h over the 24 h period. At 8:30 a.m. (after blood sampling), 12:30 p.m. and 7:00 p.m., standardized meals were given, devoid

of iron-containing components. All samples were aliquoted and stored at −80°C after centrifugation. (ii) No exercise period: it was identical but did not comprise the ergocycle test.

Methods

Usual tests

They were performed at the Rennes University Hospital. Peripheral venous blood was obtained from all patients after an overnight fast. Fresh samples were used for the assays of: (i) iron parameters (red blood cell count and hemoglobin, serum iron using the ferene-S method (Sera-pak, Bayer, France), transferrin quantified by immunonephelemetry (BNII analyzer, Dade Behring, Paris, France), transferrin saturation calculated using the formula: % transferrin saturation = 4 × (serum iron concentration in µmol/L/ serum transferrin concentration in g/L), ferritin quantified by chemiluminescence using an ACS 180 instrument (Bayer, France), (ii) liver function tests : aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gammaglutamyl transpeptidase, and prothrombin time, (iii) inflammation tests : C-reactive protein (CRP), and (iv) muscular enzyme : creatine kinase (CK).

Specific tests

Serum IL-6 was measured on samples taken before and during exercise using commercial sandwich ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. All samples were analyzed in duplicate.

Serum and urinary hepcidin assays were performed using a validated competitive ELISA at Intrinsic LifeSciences LLC, La Jolla, California, USA. These assays detect the 25 amino-acid form of hepcidin, a biological active form of the hormone (Ganz et al. 2008). The lower limit of hepcidin detection was 5.5 ng/L. Intraassay precision yielded a coefficient of variation (CV) 5-19%. The average interassay CV precision was 12%. Normal range was 29–254 ng/mL in men. Median urinary hepcidin concentrations, normalized to urinary creatine, was 502 (71-1762) and showed good correlations with serum hepcidin concentrations (Ganz et al. 2008).

Ethical data

Every subject signed a written informed consent. The study obtained the ethical approval by CCPPRB (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Rennes), Pontchaillou Hospital, Rennes, France, on 6 July 2006 (No. 06/26-592).

Statistical analyses

Data analysis was performed using SAS V9.13 software (SAS Institute, Cary, NC, USA). The basal values of each variable were compared between groups by an ANCOVA analysis with two factors (sequence, sequence order) with the mixed procedure. Comparison of the "treatment" effects (muscular effort or not muscular effort) between groups, during each sequence, was carried out by another ANCOVA with three factors (time, treatment, sequence order) with the mixed procedure. When time × parameter of interest (such as serum iron, transferrin, transferrin saturation, serum hepcidin) interaction proved significant, treatment effect was analyzed, time by time, with Neuman–Keuls tests. When time × parameter of interest interaction was not significant, the treatment effect was globally analyzed, all times together, by ANCOVA and whenever this effect was significant, the analysis was supplemented, time by time, using Neuman–Keuls tests. For daily variations, data were grouped by 2-h period and Duncan's multiple range tests were used to make pair-wise comparisons of means. A P value lower than 0.05 was considered as statistically significant.

Results

Determination of heart rate reserve

The 14 volunteers underwent the ergocycle test with gradual increase of intensity the day of the inclusion visit. Subjects' characteristics, including age, height, weight, maximal oxygen consumption data, heart rate data and cycling performance data are presented in Table 1. Based on these records, the average programmed intensity for cycling for the day of "exercise" corresponding to 60% of the heart rate reserve was at 115 W.

Iron, liver, inflammation and muscle parameters were similar prior to "exercise" and "no exercise" periods

We first compared the values for iron metabolism, liver functions, inflammation and muscular activity prior to any "exercise" or "no exercise" periods. No statistical differences were found either the day of inclusion (data not shown) or at basal time (T basal) prior to exercise and no exercise periods for these parameters (Table 2). Two volunteers had, however, values of creatine kinase at T basal (just before the exercise or no exercise periods) above 350 IU/L in contrast to their values the day of inclusion which were

Table 1 General and aerobic fitness characteristics of the volunteers

Variables at inclusion	Mean (SD)	Median	Min–Max
Age (year)	28.1 (5.3)	29.3	19.5–34.4
Height (cm)	175.6 (7.0)	178.0	162.0–183.0
Weight (kg)	70.5 (5.5)	71.3	61.0–77.0
Body mass index	22.9 (1.4)	23.0	20.0–24.9
Room temperature at inclusion (°C)	22.0 (0.0)	22.0	22.0–22.0
Maximal power at inclusion (W)	255.7 (42.2)	260.0	170.0–320.0
Ventilation at inclusion (FR/mn)	46.1 (9.1)	43.5	30.0–71.0
Respiratory ratio at inclusion	1.2 (0.1)	1.2	1.0–1.3
Minimal heart rate at inclusion (bpm)	83.9 (14.7)	87.0	54.0–108.0
Maximal heart rate at inclusion (bpm)	190.2 (9.5)	194.0	169.0–202.0
Maximal theoric heart rate at inclusion (bpm)	186.5 (16.0)	191.0	136.0–201.0
Oxygen consumption (ml/mn)	3151.4 (528.7)	3154.5	2290.0–3993.0
Room temperature during exercise	22.8 (1.0)	23.0	21.0–24.0
Programmed power for exercise (W)	115.0 (19.9)	110.0	100.0–170.0
Minimal heart rate during exercise (bpm)	115.3 (13.7)	116.0	97.0–135.0

below 350 IU/L. These data did not impact on statistical analysis both using parametric and non parametric tests. However, given the trend toward a statistically significant difference ($P = 0.0663$) for creatine kinase at T basal for the whole group between "exercise" and "no exercise" periods, complementary analyses were adjusted to creatine kinase (CK) values.

Submaximal exercise did not modulate serum and urinary hepcidin concentrations

We then asked whether a submaximal cycling-based exercise had an effect on serum and urinary hepcidin concentrations (Fig. 1; Table 3). Statistical comparison between "exercise" and "no exercise" groups at T0 (end of the

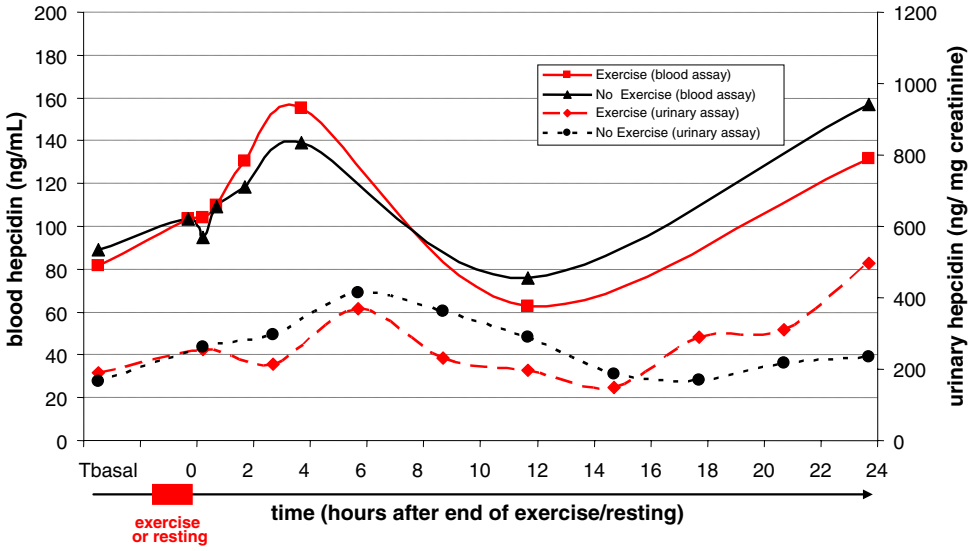

Fig. 1 No influence of muscular exercise on serum and urinary hepcidin. Serum hepcidin (*squares* and *triangles*) and urinary hepcidin (*diamonds* and *circles*) levels were measured for 24 h after a 45 min ergocycle muscular exercise (*squares* and *diamonds*) or 45 min resting period (*triangles* and *circles*). Data corresponded to mean values ($n = 14$). Muscle exercise modulated neither serum nor urinary hepcidin levels

exercise/no exercise periods) and also up to 24 h after T0 indicated that the ergocycle exercise had no significant effect on the concentrations of serum and urinary hepcidin ($P = 0.51$ and $P = 0.65$, respectively).

Submaximal exercise increased significantly but slightly serum concentrations of creatine kinase, ferritin, transferrin and iron

Statistical analyses were performed between "exercise" and "no exercise" groups from T0 up to 24 h after T0. All analyses were adjusted with sequence order, time, CK (except for analyses of CK values themselves) and interaction time-treatment. Table 3 presents mean values for T0 time point because this point appeared to show the most striking differences between "exercise" and "no exercise" groups. As expected, no effect of exercise has been shown either on red blood cells, hemoglobin, hematocrit, mean corpuscular volume, platelets and leukocytes (data not shown), or serum transaminases (AST, ALT) (Table 3). Similarly, IL6 concentrations did not vary between the end of exercise and no exercise periods (T0) (mean ± SD): 7.1 pg/mL ± 15.8 versus 3.1 pg/mL ± 3.5 ($P = 0.63$). In contrast, submaximal exercise increased significantly serum iron, serum transferrin, serum ferritin and creatine kinase (Fig. 2; Table 3). These effects were transient since essentially found at T0, i.e. at the end of the ergocyle test.

Table 3 Comparison of biochemical parameters after "exercise" and "no exercise" periods

Variables at T0	Exercise (=1)	No exercise (=2)	1 versus 2 from T0 to 24 h post-T0
	Mean (SD)	Mean (SD)	*P*
Serum hepcidin (ng/mL)	103.7 (74.1)	103.3 (103.5)	0.51
Urinary hepcidin (ng/mL)	256.8 (330.0)	261.4 (408.3)	0.65
Serum iron (µmol/L)	19.6 (8.0)	17.0 (9.6)	**0.03**
Serum transferrin (g/L)	2.69 (0.4)	2.53 (0.4)	**0.003**
Transferrin saturation (%)	29.1 (11.6)	27.6 (16.6)	0.26
Serum ferritin (µg/L)	93.9 (48.6)	84.5 (45.8)	**0.0007**
Aspartate aminotransferase (AST) IU/L	27.4 (5.6)	27.9 (4.9)	0.20
Alanine aminotransferase (ALT) IU/L	23.2 (7.9)	21.9 (12.7)	0.52
Creatine kinase (CK) IU/L	156.2 (61.7)	235.8 (216.5)	**0.0004**

This table presents mean values and standard deviation (SD) values of the 14 volunteers for T0 time point (i.e. end of exercise/no exercise period). To compare "exercise" and "no exercise" groups, however, statistical analyses were adjusted with sequence order, time, CK (except for analyses of CK values themselves) and interaction time-treatment for the whole period of time from T0 to 24 h post-T0. The *P* value indicated that result. Submaximal exercise increased significantly serum iron, serum transferrin, serum ferritin and creatine phospho kinase (*bold values*) at the end of the exercise time

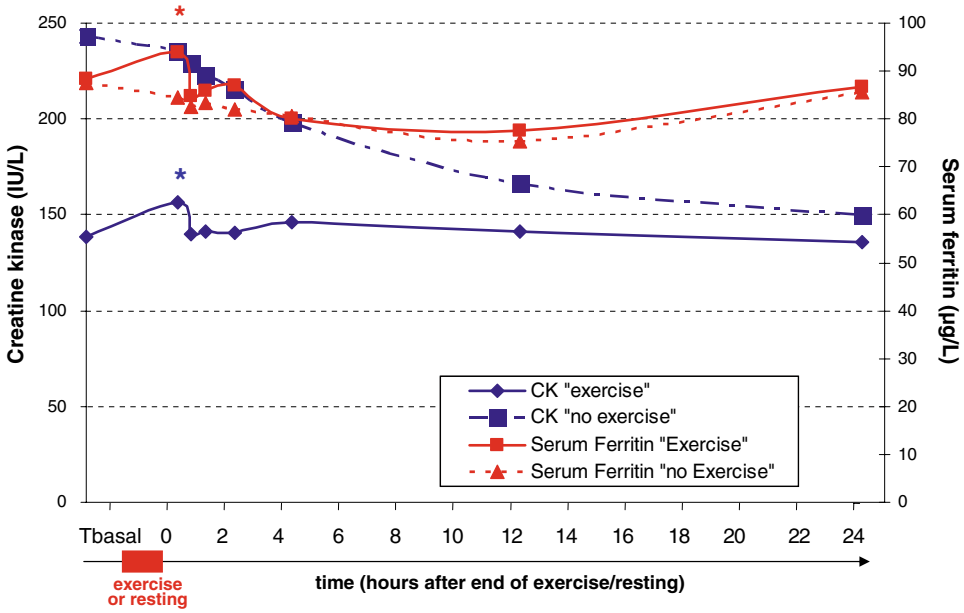

Fig. 2 Submaximal exercise increased significantly but transiently serum creatine kinase (CK) and ferritin concentrations. Serum CK and serum ferritin were measured over 24 h after a 45 min ergocycle muscular exercise. Data corresponded to mean values ($n = 14$). Muscle exercise increased CK and ferritin, but slightly and transiently (see $^*P < 0.05$)

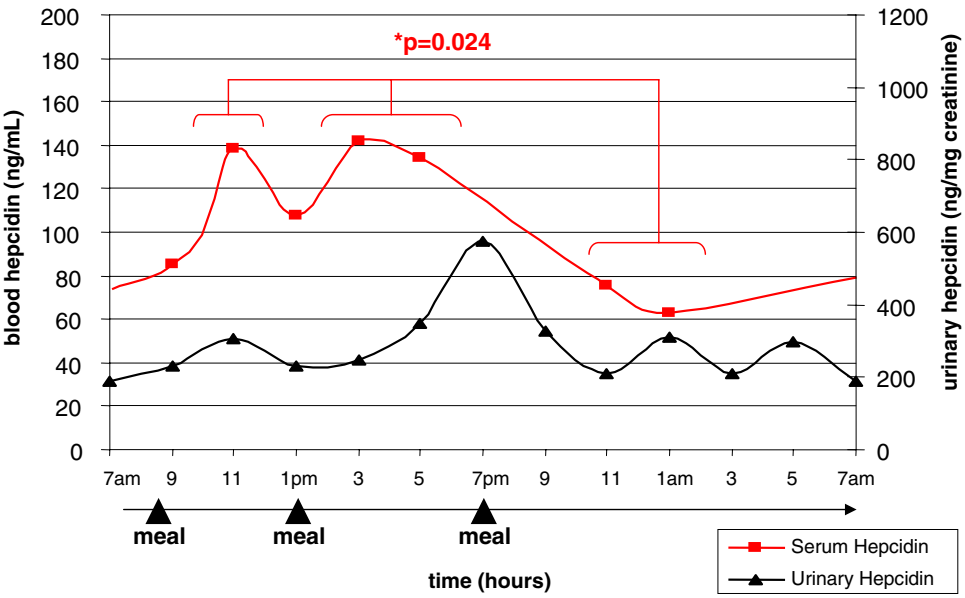

Fig. 3 Daily variations of hepcidin concentrations. Serum hepcidin (*squares*) and urinary hepcidin (*triangles*) levels were measured over 24 h on 14 healthy volunteers which received three standardized meals, devoid of iron-containing components. Data corresponded to mean values. Serum, but not urinary, hepcidin levels were significantly higher between 9:00–11:00 a.m. and 2:00–6:00 p.m. versus midnight time 10:00 p.m.–2:00 a.m. ($^*P = 0.024$, $n = 14$)

Serum hepcidin and serum iron showed daily variations but not serum ferritin or urinary hepcidin

Finally, we studied the daily variations of iron parameters from the "no exercise" groups (Figs. 3, 4). Serum hepcidin concentrations were significantly higher ($P = 0.024$) between 9:00–11:00 a.m. and 2:00–6:00 p.m. compared to midnight time 10:00 p.m.–2:00 a.m. (Fig. 3). Serum iron concentrations were significantly higher in the late morning (period 10:00 a.m.–12:00 p.m.) as compared to period between 4:00 p.m. and 2:00 a.m. ($P = 0.038$) (Fig. 4). No such variations were found for serum transferrin, serum ferritin, transferrin saturation (data not shown) or for urinary hepcidin (Fig. 3).

Discussion

Interactions between skeletal muscle system and iron metabolism have long been studied. In male and female athletes, iron depletion has been reported to develop in approximately 10 and 20–60%, respectively (Balaban et al. 1989; Gropper et al. 2006; Magnusson et al. 1984). Interpretation of the observed data was however rendered difficult by the limitations of the parameters used to evaluate iron metabolism. Serum iron, which shows marked diurnal variations, is decreased by inflammation and increased by cytolysis. Furthermore, serum ferritin becomes a reliable indicator of body iron stores only after several confounding factors causing also hyperferritinemia, such as inflammation,

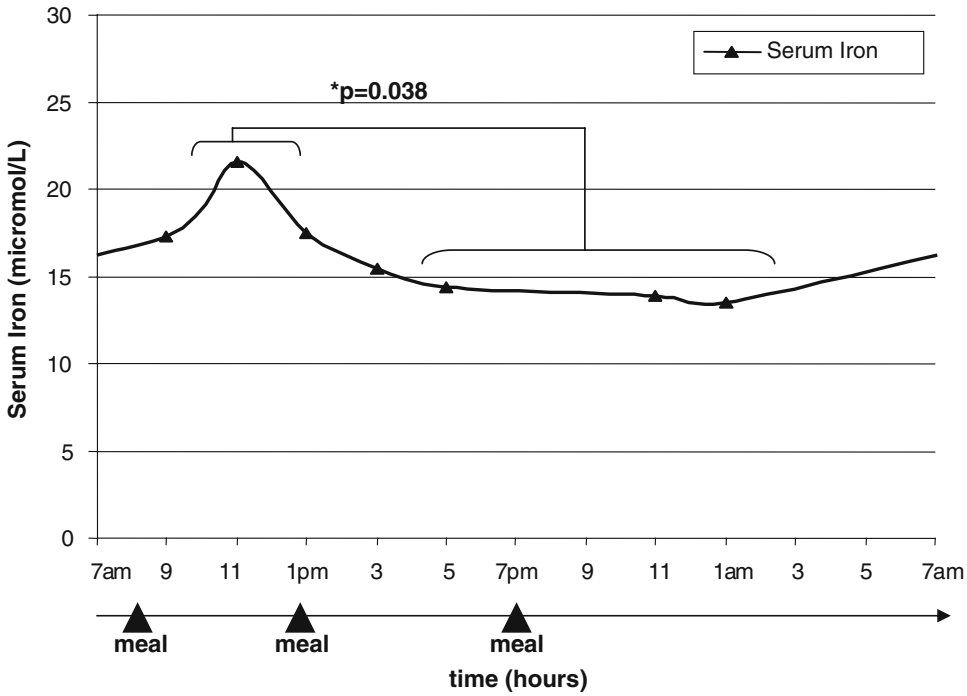

Fig. 4 Daily variations for serum iron concentrations. Serum iron was measured over 24 h on 14 healthy volunteers which received three standardized meals, devoid of iron-containing components. Data corresponded to mean values. Serum iron concentrations were significantly higher in the late morning versus the period 4:00 p.m.–2:00 a.m. (*$P = 0.038$, $n = 14$)

cytolysis, and polymetabolism have been ruled out (Aguilar Martinez et al. 2005). In the frame of exercise, variations of plasma volume (hemoconcentration immediately after short acute exercise and hemodilution after long term effort) should also be considered (Schumacher et al. 2002). In this context, serum transferrin receptor appears to remain an interesting indicator (Rocker et al. 2002; Schumacher et al. 2002). As to the mechanisms accounting for exercise-related iron deficiency, several factors have been advocated, such as inadequate iron intake, increased iron losses through sweat, urine and feces, and increased red blood cell lysis due to intravascular microtrauma involvement (Gropper et al. 2006; Peeling et al. 2008). However, among possible mechanisms responsible for iron deficiency, increasing interest focuses now on the role of exercise-related inflammation through possible increased hepcidin production. Indeed, exercise increases production of inflammatory cytokines including IL-6 (Pedersen et al. 2007; Peeling et al. 2008). IL-6 is an inflammatory signal which induces the synthesis of the iron regulatory hormone hepcidin (Nemeth et al. 2003, 2004), resulting in hypoferremia (Nemeth et al. 2004) and increased iron storage within the reticuloendothelial system (Nicolas et al. 2002). It should also be noticed that, besides increased muscular workload, psychological stress induces expression of IL-6 and therefore should be taken into account when interpreting hepcidin data (Goebel et al. 2000; Zhao et al. 2008).

However, as previously mentioned, most clinical data on the impact of exercise and iron changes have so far concerned intense muscle activity with eccentric contractions as marathon running, in which muscle damage, inflammatory response and increased iron needs for myoglobin synthesis were expected (Ahmadi et al. 2008). These data prompted us to further examine the effect of muscle exercise on iron metabolism in endurance non eccentric exercise, i.e. in a situation more relevant to usual muscular effort. Our study concerned male volunteers strictly selected in terms of iron status as explored both phenotypically (serum iron parameters in the absence of confounding factors such as inflammation and cytolysis) and genotypically (no C282Y mutation in the HFE gene). It unequivocally showed that a 45 min submaximal ergocycle exercise had no effects on serum and urinary hepcidin concentrations. This exercise, however, exerted some increasing effect on serum CK, ferritin, iron and transferrin levels. It should be pointed out that these variations, despite statistically significant, remained both moderate when considering absolute values (for instance serum ferritin rose only from 84.5 to 93.9 µg/L) and transient. As to the mechanisms accounting for these variations, some muscle damage is likely but minimal when considering that serum aspartate transaminase did not raise at all. The role of hemoconcentration is likely since the increase in ferritin and transferrin was accompanied by a significant increase of protidemia ($P = 0.045$). However, the role of inflammation can be reasonably ruled out on the fact that both CRP and IL-6 did not increase. The role of muscle hypoxia can be excluded given the low intensity of aerobic activity in our cycling exercise, especially when considering that no increase in hypoxia inducible factors and VEGF has been reported even shortly after a strenuous exercise (Kivela

et al. 2007). Notably, our study provided new insights on diurnal variations of hepcidin, and confirmed the circadian cycle for serum iron (Ganz et al. 2008; Guillygomarc'h et al. 2003; Wiltink et al. 1973) with a nadir in the evening. Two recent reports, each of them studying three healthy volunteers, described comparable diurnal variations of serum hepcidin, using a mass spectrometry approach (SELDI-TOF MS) (Kemna et al. 2007) or the same ELISA assay as in our study (Ganz et al. 2008). Our study, using a competitive ELISA to determine hepcidin concentrations in a strictly defined cohort of individuals with normal iron metabolism, further confirms blood variations of this key hormone of iron metabolism. Interestingly, in contrast to serum hepcidin, urinary hepcidin levels did not exhibit any significant diurnal changes. The reason for this discrepancy remains unclear. It is possible that the complex hepcidin handling by the kidney accounts, at least partly, for this difference: 95% of hepcidin is retained in the kidney, related either to poor glomerular filtration and/or to reabsorption and degradation in the proximal tubules like other small peptides (Ganz et al. 2008). Cycling-based exercise is usually slightly demanding in terms of eccentric contraction, therefore minimizing muscular damage as reflected by minor release of muscular enzymes (Besson et al. 1981). Consequently, these data points out that for clinical assessment, moderate non eccentric exercise preceding the serum sampling has no influence on this parameter whereas the time of sampling will be important for interpreting serum hepcidin levels.

In conclusion, a submaximal endurance ergocycle exercise, performed in volunteers with normal basal iron metabolism, did not modify serum hepcidin concentration, which maintained their diurnal variation. The data suggest that, under this setting, muscle iron needs may not be increased, therefore not eliciting significant changes in the metabolic cascade of iron regulation. These results may have useful implications for interpreting serum hepcidin values in clinical practice.

Acknowledgments This work was supported by COREC (Comité de Recherche Clinique) 2005 (University Hospital, Rennes), Association Fer et Foie, and by European Contract EuroIron1 (STREP No. 037296).

Conflict of interest statement Mark Westerman is Co-founder and President of Intrinsic Life Sciences. Tomas Ganz and Elizabeta Nemeth are Co-founders and Members of the Advisory Board for Intrinsic Life Sciences. Olbina Gordana is a Senior Scientist from Intrinsic Life Sciences.

References

Aguilar Martinez P, Schved JF, Brissot P (2005) The evaluation of hyperferritinemia: an updated strategy based on advances in detecting genetic abnormalities. Am J Gastroenterol 100:1185–1194. doi:10.1111/j.1572-0241.2005.40998.x

Ahmadi S, Sinclair PJ, Davis GM (2008) Muscle oxygenation after downhill walking-induced muscle damage. Clin Physiol Funct Imaging 28:55–63

Andrews NC (1999) Disorders of iron metabolism. N Engl J Med 341:1986–1995. doi:10.1056/NEJM199912233412607

Babitt JL, Huang FW, Wrighting DM, Xia Y, Sidis Y, Samad TA, Campagna JA, Chung RT, Schneyer AL, Woolf CJ, Andrews NC, Lin HY (2006) Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat Genet 38:531–539. doi:10.1038/ng1777

Balaban EP, Cox JV, Snell P, Vaughan RH, Frenkel EP (1989) The frequency of anemia and iron deficiency in the runner. Med Sci Sports Exerc 21:643–648. doi:10.1249/00005768-198912000-00003

Besson C, Rochcongar P, Beauverger Y, Dassonville J, Aubree M, Catheline M (1981) Study of the valuations of serum muscular enzymes and myoglobin after maximal exercise test and during the next 24 hours. Eur J Appl Physiol Occup Physiol 47:47–56. doi:10.1007/BF00422482

Brissot P, Troadec MB, Bardou-Jacquet E, Lan CL, Jouanolle AM, Deugnier Y, Loreal O (2008) Current approach to hemochromatosis. Blood Rev 22:195–210. doi:10.1016/j.blre.2008.03.001

Di Santolo M, Stel G, Banfi G, Gonano F, Cauci S (2008) Anemia and iron status in young fertile non-professional female athletes. Eur J Appl Physiol 102:703–709. doi:10.1007/s00421-007-0647-9

Ehn L, Carlmark B, Hoglund S (1980) Iron status in athletes involved in intense physical activity. Med Sci Sports Exerc 12:61–64. doi:10.1249/00005768-198021000-00012

Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M (2008) Immunoassay for human serum hepcidin. Blood 112:4292–4297. doi:10.1182/blood-2008-02-139915

Goebel MU, Mills PJ, Irwin MR, Ziegler MG (2000) Interleukin-6 and tumor necrosis factor-alpha production after acute psychological stress, exercise, and infused isoproterenol: differential effects and pathways. Psychosom Med 62:591–598

Gropper SS, Blessing D, Dunham K, Barksdale JM (2006) Iron status of female collegiate athletes involved in different sports. Biol Trace Elem Res 109:1–14. doi:10.1385/BTER:109:1:001

Guillygomarc'h A, Christian J, Romain M, Vincent Q, Veronique D, Deugnier Y (2003) Circadian variations of transferrin saturation levels in iron-overloaded patients: implications for the screening of C282Y-linked haemochromatosis. Br J Haematol 120:359–363. doi:10.1046/j.1365-2141.2003.04070.x

Hood DA, Kelton R, Nishio ML (1992) Mitochondrial adaptations to chronic muscle use: effect of iron deficiency. Comp Biochem Physiol Comp Physiol 101:597–605. doi:10.1016/0300-9629(92)90514-Q

Huang FW, Pinkus JL, Pinkus GS, Fleming MD, Andrews NC (2005) A mouse model of juvenile hemochromatosis. J Clin Invest 115:2187–2191. doi:10.1172/JCI25049

Kemna EH, Tjalsma H, Podust VN, Swinkels DW (2007) Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications. Clin Chem 53:620–628. doi:10.1373/clinchem.2006.079186

Kivela R, Kyrolainen H, Selanne H, Komi PV, Kainulainen H, Vihko V (2007) A single bout of exercise with high mechanical loading induces the expression of Cyr61/CCN1 and CTGF/CCN2 in human skeletal muscle. J Appl Physiol 103:1395–1401. doi:10.1152/japplphysiol.00531.2007

Lin L, Goldberg YP, Ganz T (2005) Competitive regulation of hepcidin mRNA by soluble and cell-associated hemojuvelin. Blood 106:2884–2889. doi:10.1182/blood-2005-05-1845

Magnusson B, Hallberg L, Rossander L, Swolin B (1984) Iron metabolism and "sports anemia". II. A hematological comparison of elite runners and control subjects. Acta Med Scand 216:157–164

Monnier PP, Sierra A, Macchi P, Deitinghoff L, Andersen JS, Mann M, Flad M, Hornberger MR, Stahl B, Bonhoeffer F, Mueller BK

(2002) RGM is a repulsive guidance molecule for retinal axons. Nature 419:392–395. doi:10.1038/nature01041

Nachtigall D, Nielsen P, Fischer R, Engelhardt R, Gabbe EE (1996) Iron deficiency in distance runners. A reinvestigation using Fe-labelling and non-invasive liver iron quantification. Int J Sports Med 17:473–479. doi:10.1055/s-2007-972881

Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T (2003) Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 101:2461–2463. doi:10.1182/blood-2002-10-3235

Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T (2004) IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 113:1271–1276

Nicolas G, Bennoun M, Devaux I, Beaumont C, Grandchamp B, Kahn A, Vaulont S (2001) Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. Proc Natl Acad Sci USA 98:8780–8785. doi:10.1073/pnas.151179498

Nicolas G, Bennoun M, Porteu A, Mativet S, Beaumont C, Grandchamp B, Sirito M, Sawadogo M, Kahn A, Vaulont S (2002) Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. Proc Natl Acad Sci USA 99:4596–4601. doi:10.1073/pnas.072632499

Niederkofler V, Salie R, Sigrist M, Arber S (2004) Repulsive guidance molecule (RGM) gene function is required for neural tube closure but not retinal topography in the mouse visual system. J Neurosci 24:808–818. doi:10.1523/JNEUROSCI.4610-03.2004

Papanikolaou G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, Dube MP, Andres L, MacFarlane J, Sakellaropoulos N, Politou M, Nemeth E, Thompson J, Risler JK, Zaborowska C, Babakaiff R, Radomski CC, Pape TD, Davidas O, Christakis J, Brissot P, Lockitch G, Ganz T, Hayden MR, Goldberg YP (2004) Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet 36:77–82. doi:10.1038/ng1274

Pedersen BK, Akerstrom TC, Nielsen AR, Fischer CP (2007) Role of myokines in exercise and metabolism. J Appl Physiol 103:1093–1098. doi:10.1152/japplphysiol.00080.2007

Peeling P, Dawson B, Goodman C, Landers G, Trinder D (2008) Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. Eur J Appl Physiol 103:381–391. doi:10.1007/s00421-008-0726-6

Peeling P, Dawson B, Goodman C, Landers G, Wiegerinck ET, Swinkels DW, Trinder D (2009) Cumulative effects of consecutive running sessions on hemolysis, inflammation and hepcidin activity. Eur J Appl Physiol 31 (Epub ahead of print)

Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, Loreal O (2001) A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem 276:7811–7819. doi:10.1074/jbc.M008923200

Robach P, Cairo G, Gelfi C, Bernuzzi F, Pilegaard H, Vigano A, Santambrogio P, Cerretelli P, Calbet JA, Moutereau S, Lundby C (2007) Strong iron demand during hypoxia-induced erythropoiesis is associated with down-regulation of iron-related proteins and myoglobin in human skeletal muscle. Blood 109:4724–4731. doi:10.1182/blood-2006-08-040006

Rocker L, Hinz K, Holland K, Gunga HC, Vogelgesang J, Kiesewetter H (2002) Influence of endurance exercise (triathlon) on circulating transferrin receptors and other indicators of iron status in female athletes. Clin Lab (Zaragoza) 48:307–312

Roecker L, Meier-Buttermilch R, Brechtel L, Nemeth E, Ganz T (2005) Iron-regulatory protein hepcidin is increased in female athletes after a marathon. Eur J Appl Physiol 95:569–571. doi:10.1007/s00421-005-0055-y

Samad TA, Srinivasan A, Karchewski LA, Jeong SJ, Campagna JA, Ji RR, Fabrizio DA, Zhang Y, Lin HY, Bell E, Woolf CJ (2004) DRAGON: a member of the repulsive guidance molecule-related family of neuronal- and muscle-expressed membrane proteins is regulated by DRG11 and has neuronal adhesive properties. J Neurosci 24:2027–2036. doi:10.1523/JNEUROSCI.4115-03.2004

Schumacher YO, Schmid A, Konig D, Berg A (2002) Effects of exercise on soluble transferrin receptor and other variables of the iron status. Br J Sports Med 36:195–199. doi:10.1136/bjsm.36.3.195

Wiltink WF, Kruithof J, Mol C, Bos MG, van Eijk HG (1973) Diurnal and nocturnal variations of the serum iron in normal subjects. Clin Chim Acta 49:99–104. doi:10.1016/0009-8981(73)90348-3

Zhao M, Chen J, Wang W, Wang L, Ma L, Shen H, Li M (2008) Psychological stress induces hypoferremia through the IL-6-hepcidin axis in rats. Biochem Biophys Res Commun 373:90–93. doi:10.1016/j.bbrc.2008.05.166

Zoller H, Vogel W (2004) Iron supplementation in athletes–first do no harm. Nutrition 20:615–619. doi:10.1016/j.nut.2004.04.006