Urinary prednisolone excretion is a determinant of serum hepcidin levels in renal transplant recipients

To the Editor:
Hepcidin, which is synthesized and secreted by the liver, is considered the master regulator of iron homeostasis. Hepcidin regulates the amount of iron absorbed from the intestines and the iron release from the reticuloendothelial system by degrading ferroportin, the iron release transporter located at the duodenal enterocytes and macrophages. Circulating levels of hepcidin are known to be controlled by available iron stores, inflammation, hypoxia, insulin levels, and erythropoiesis.

Correspondence
Matheus Vescovi Gonçalves, R Dr Diogo de Faria 824, CEP 04037-002, São Paulo, SP, Brazil.
Email: Matheus.vescovi@gmail.com

REFERENCES


SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.

Received: 21 March 2017 | Accepted: 5 May 2017

DOI 10.1002/ajh.24785
Recently, hepcidin antagonists have been introduced as potential treatment to improve iron-restrictive anemia. By improving iron availability and subsequently hemoglobin levels, hepcidin antagonists might be able to improve quality of life and outcome in different patient settings. Therefore, all factors that affect serum hepcidin levels are clinically relevant specifically in populations where in the future the use of hepcidin antagonists may be considered. It has already been established that both testosterone and estrogens are associated with suppression of serum hepcidin in men. On the other hand progesterone, the anabolic steroid epitostanol, as well as the progesterone antagonist, mifepristone, are able to induce hepcidin biosynthesis in a zebrafish model.13

Synthetic glucocorticoids, like prednisone, and its active metabolite prednisolone are used in immunosuppressive regiments for renal transplant recipients (RTRs). To date, possible effects of these synthetic glucocorticoids on serum hepcidin levels in humans are unknown. Here, we report on the association of serum hepcidin with 24-h urinary glucocorticoids on serum hepcidin levels in humans are unknown.

Recently, hepcidin antagonists have been introduced as potential treatment to improve iron-restrictive anemia. By improving iron availability and subsequently hemoglobin levels, hepcidin antagonists might be able to improve quality of life and outcome in different patient settings. Therefore, all factors that affect serum hepcidin levels are clinically relevant specifically in populations where in the future the use of hepcidin antagonists may be considered. It has already been established that both testosterone and estrogens are associated with suppression of serum hepcidin in men. On the other hand progesterone, the anabolic steroid epitostanol, as well as the progesterone antagonist, mifepristone, are able to induce hepcidin biosynthesis in a zebrafish model.

Synthetic glucocorticoids, like prednisone, and its active metabolite prednisolone are often used in immunosuppressive regiments for renal transplant recipients (RTRs). To date, possible effects of these synthetic glucocorticoids on serum hepcidin levels in humans are unknown. Here, we report on the association of serum hepcidin with 24-h urinary prednisolone excretion, as a measure of 24-h prednisolone exposure.

For this study, 606 stable RTRs with a functioning graft beyond the first year after transplantation were included.4 All RTRs gave written informed consent for the study and approval by institutional review board was obtained (METc 2001/039). For the current analyses, we excluded patients with missing data on serum hepcidin (n = 45) and urinary prednisolone excretion (n = 10), resulting in 551 RTRs eligible for analyses. Serum hepcidin-25 was assessed by dual-monoclonal sandwich ELISA immunoassay. Urinary prednisolone measurements were carried out with validated high-performance liquid chromatography assay with diode-array detection after extraction with diethylether. Renal function was assessed by estimating glomerular filtration rate (eGFR) applying the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

IBM SPSS Statistics 23 was used for statistical analyses. For baseline characteristics, one-way ANOVA, Kruskal-Wallis test, and Chi square test were applied, as appropriate. Furthermore, univariate linear regression analysis was performed to assess the determinants of serum hepcidin, followed by multivariable linear regression analysis with stepwise backward procedure. P values for inclusion and exclusion were set at 0.20 and 0.10, respectively. Variables with a skewed distribution were natural log transformed for analyses. Separate linear regression analysis was performed to assess the association between serum hepcidin and hemoglobin level in RTRs after adjustment for age, sex, and eGFR.

Mean age of the 551 RTRs was 51 ± 12 years with 55% being men. Patients were included at median 6.0 (interquartile range [IQR], 2.6–11.6) years after transplantation. All RTRs used prednisolone orally ranging from 5 to 10 mg once daily. Median 24-h urinary prednisolone excretion was 758 (371–1278) pmol/24 h. The association of the individual daily prednisolone dosis with 24-h urinary prednisolone excretion was β = 0.23, P < .001. Across tertiles of 24-h urinary prednisolone excretion, RTRs within the highest tertile of prednisolone were younger, less frequently men, had lower systolic blood pressure and higher estimated glomerular filtration rate (eGFR) compared to those within the other two tertiles. Furthermore, higher hemoglobin, lower hepcidin, and lower ferritin levels were noted in RTRs in the highest tertile of prednisolone excretion compared to RTRs in the other two tertiles (Table 1).

In univariate regression analysis, serum hepcidin was found to be negatively associated with 24-h urinary prednisolone excretion (β = −0.15, P < .001). After adjustment for high-sensitivity C-reactive protein (hs-CRP), hepcidin remained associated with prednisolone (β = −0.13, P = .002). Further adjustment for eGFR did not materially alter this association (β = −0.12, P = .006). When including prednisolone in a backward multivariate model with age, sex, eGFR, hs-CRP, ferritin, hemoglobin, erythropoietin, and insulin, prednisolone remained an independent determinant of hepcidin (β = −0.10, P = .001), besides expected relationships of hepcidin with ferritin, hs-CRP, erythropoietin and insulin. In addition, serum hepcidin was found to be a determinant of hemoglobin levels (β = −0.08, P = .03) independently of age, sex, and eGFR.

In this study, we show that 24-h urinary prednisolone excretion is negatively associated with serum hepcidin in RTRs irrespective of potential confounders, including eGFR. All RTRs in our cohort used a low dose

| TABLE 1 | Baseline characteristics of 551 renal transplant recipients according to tertiles of 24-h urinary prednisolone excretion |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variables       | All patients    | Tertiles of urinary prednisolone excretion (pmol/24 h) |
|                 |                 | T1              | T2              | T3              | P value         |
| Urinary prednisolone (pmol/24 h) | 758 (371–1278) | 256 (125–371) | 755 (619–904) | 1595 (1273–2325) | <.001 |
| General characteristics |
| Age (years)    | 51 ± 12         | 53 ± 12         | 53 ± 12         | 49 ± 12         | .003 |
| Male sex      | 298 (54)        | 109 (60)        | 107 (58)        | 82 (45)         | .006 |
| eGFR (mL/min/1.73 m²) | 47 ± 16      | 41 ± 16         | 46 ± 15         | 54 ± 13         | <.001 |
| Laboratory parameters |
| Hepcidin (ng/mL) | 7.2 (3.3–13.5) | 8.6 (4.3–14.3) | 7.0 (2.8–14.3) | 5.8 (2.8–11.4) | .003 |
| Ferritin (g/L) | 13.8 ± 1.6      | 13.6 ± 1.6      | 13.8 ± 1.6      | 14.2 ± 1.4      | <.001 |
| EPO (IU/L)     | 156.0 (80.0–283.0) | 189 (102–316) | 163 (77–288) | 122 (68–233) | .001 |
| hs-CRP (mg/L)  | 17.4 (12.0–24.3) | 17.7 (11.8–25.4) | 17.9 (13.0–24.3) | 16.6 (11.4–22.9) | .28 |
| Insulin (mU/mL) | 11.1 (7.9–16.3) | 11.4 (7.8–15.7) | 11.2 (8.3–15.1) | 10.8 (7.8–15.8) | .53 |

eGFR, estimated glomerular filtration rate; EPO, erythropoietin; hs-CRP, high sensitivity C-reactive protein.
prednisolone (5–10 mg/day). Remarkably, this resulted in a broad range
of 24-h urinary prednisolone excretion and a modest association with
the daily prednisolone dose, in keeping with considerable inter-subject
pharmacokinetic variability. Twenty-4 h urinary prednisolone excretion
is considered to reflect the overall exposure to prednisolone. Previously,
it has been shown that prednisolone dose-dependently inhibits the
release of interleukin-6 (IL-6) which is known to induce hepcidin
expression. We had no data available on IL-6 levels to assess whether
effects on IL-6 is the mechanism behind the association of prednisolone
with hepcidin. The possible role of prednisolone as a direct hepcidin
antagonist and possible mechanisms linking prednisolone with hepcidin
need to be delineated in more detail in future studies.

The major strength of this report is the large cohort of RTRs with
availability of concurrent 24-h urinary prednisolone excretion and hep-
cidin data. Limitations are that it comprises a single center study, and
that we cannot exclude the possibility of residual confounding.

In conclusion, lower serum hepcidin levels are related to higher
24-h urinary prednisolone excretion in RTRs independent of clinically
relevant covariates. Our findings extend earlier data concerning effects
of other (synthetic) steroids on hepcidin regulation, and provide a
rationale to more precisely delineate direct or indirect effects of gluco-
corticoids on hepcidin regulation. From a clinical perspective, our find-
ings lend support to the possibility that prednisolone may be regarded
as a hitherto unappreciated hepcidin antagonist.

ACKNOWLEDGMENTS
This research did not receive any specific grant from funding
agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST
C.A.J.M.G. received speaking fees and research funding from Vifor
Pharma. The other authors have declared that no conflict of interest
exists.

Michele F. Eisenga1, Robin P. F. Dullaart2, Stefan P. Berger1,
Daan J. Touw3, Stephan J. L. Bakker, Carlo A. J. M. Gaillard4
1Department of Nephrology, University Medical Center Groningen,
University of Groningen, Groningen, The Netherlands
2Department of Endocrinology, University Medical Center Groningen,
University of Groningen, Groningen, The Netherlands
3Department of Clinical Pharmacy and Pharmacology, University Medical
Center Groningen, University of Groningen, Groningen, The Netherlands

Correspondence
M. F. Eisenga, Department of Internal Medicine, Division of Nephrology,
University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen,
The Netherlands.
Email: m.f.eisenga@umcg.nl

REFERENCES
din in men: a potential mechanism for testosterone-induced erythro-
component-1 regulates hepcidin biosynthesis. J Clin Invest. 2016;126:
389–401.
component of the metabolic syndrome as predictors of mortality in
and unbound prednisone and prednisolone in stable kidney transplant
dependently influences inflammation and coagulation during human

Characterization of TP53 mutations in clonal cytopenia
of undetermined significance

To the Editor:
The diagnosis of myelodysplastic syndrome (MDS) requires persist-
ent cytopenia with at least one of the following criteria: dysplasia in
at least 10% of cells in any hematopoietic lineage, increased myelo-
blasts (5–19%) in bone marrow (or 2–19% myeloblast in peripheral
blood), or MDS defining cytogenetic abnormalities. Some patients
have cytopenia and/or gene mutations, but do not meet other crite-
ria of MDS.1 These pre-MDS conditions include idiopathic cytopenia
of undetermined significance (ICUS), clonal hematopoiesis of inde-
terminate potential (CHIP) and clonal cytopenia of undetermined
significance (CCUS). The mutations frequently identified in these
pre-MDS conditions, including DNMT3A, TET2, and ASXL1, are also
the common mutations detected in MDS.2 ICUS, CHIP, and CCUS all
carry an increased risk for progression to MDS. The rate of progres-
sion to MDS varies, likely depending on the specific genes that are
mutated and their mutation burden. The role of each individual
mutation in disease progression is not well characterized.

TP53 is a tumor suppressor gene that has been studied extensively
in MDS and AML, in which the mutations are associated with a
complex karyotype and a poor prognosis. Its mutations also occur in
CHIP and CCUS.2,3 The characteristics of TP53 mutations and their
role in disease progression in these pre-MDS conditions are unknown.
In this study, we aim to characterize the clinicopathological features of
CCUS cases associated with TP53 mutations.