AMR-mediated hepatic platelet clearance in vivo may represent a physiological mechanism involved in platelet homeostasis. Platelets desialylate as they circulate, thereby becoming the primary ligand for the AMR,[10] and this interaction regulates hepatocyte thrombopoietin production.[11] Desialylation also occurs when platelets are activated by several physiological stimuli, and AMR clearance may be relevant in attenuating the coagulopathy of sepsis.[12-14] Our results support the indications of international ITP guidelines,[15] which suggest that both PSSs and glycoprotein-specific antibody testing are not mandatory in ITP workup or management. However, if available on a single-center basis, these tests may help to gain insight into the prevalent mechanism underlying thrombocytopenia (increased clearance vs deficient production) in a specific patient who fails first-line therapy with glucocorticoids.

Contribution: S.C. and M.C. design the research, analyzed data, wrote the paper. M.N. performed the statistical analysis. R.C. analyzed data. L.S., S.R., M.M., and C.P. performed laboratory analysis.

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References

1. Stasi R. Immune thrombocytopenia: pathophysiologic and clinical update. *Semin Thromb Hemost.* 2012;38(5):454-462.

2. Li J, van der Wal DE, Zhu G, et al. Desialylation is a mechanism of Fc-independent platelet clearance and a therapeutic target in immune thrombocytopenia. *Nat Commun.* 2015;6:7737.

3. Ashwell G, Morell AG. The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. *Adv Enzymol Relat Areas Mol Biol.* 1974;41(0):99-128.

4. Vianelli N, Palandri F, Polverelli N, et al. Splenectomy as a curative treatment for immune thrombocytopenia: a retrospective analysis of 233 patients with a minimum follow up of 10 years. *Haematologica.* 2013;98(6):875-880.

5. Thakur ML, Walsh L, Malech HL, Gottschalk A. Indium-111-labeled human platelets: improved method, efficacy, and evaluation. *J Nucl Med.* 1981;22(4): 381-385.

6. International Committee for Standardization in Hematology. Panel on Diagnostic Applications of Radionuclides. Recommended method for indium-111 platelet survival studies. *J Nucl Med.* 1988;29(4):564-566.

7. Roca M, Muñiz-Diaz E, Mora J, et al. The scintigraphic index spleen/liver at 30 minutes predicts the success of splenectomy in persistent and chronic primary immune thrombocytopenia. *Am J Hematol.* 2011;86(11):909-913.

8. Lamy T, Moisan A, Dauriac C, Ghandour C, Morice P, Le Prise PY. Splenectomy in idiopathic thrombocytopenic purpura: its correlation with the sequestration of autologous indium-111-labeled platelets. *J Nucl Med.* 1993;34(2):182-186.

9. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood.* 2009;113(11): 2386-2393.

10. Sørensen AL, Rumjantseva V, Nayeb-Hashemi S, et al. Role of sialic acid for platelet life span: exposure of beta-galactose results in the rapid clearance of platelets from the circulation by asialoglycoprotein receptor-expressing liver macrophages and hepatocytes. *Blood.* 2009;114(8):1645-1654.

11. Grozovsky R, Begonja AJ, Liu K, et al. The Ashwell-Morell receptor regulates hepatic thrombopoietin production via JAK2-STAT3 signaling. *Nat Med.* 2015; 21(1):47-54.

12. Grewal PK, Uchiyama S, Ditto D, et al. The Ashwell receptor mitigates the lethal coagulopathy of sepsis. *Nat Med.* 2008;14(6):648-655.

13. Grewal PK, Aziz PV, Uchiyama S, et al. Inducing host protection in pneumococcal sepsis by preactivation of the Ashwell-Morell receptor. *Proc Natl Acad Sci USA.* 2013;110(50):20218-20223.

14. Ellies LG, Ditto D, Levy GG, et al. Sialyltransferase ST3Gal-IV operates as a dominant modifier of hemostasis by concealing asialoglycoprotein receptor ligands. *Proc Natl Acad Sci USA.* 2002;99(15):10042-10047.

15. Provan D, Stasi R, Newland AC, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood.* 2010;115(2):168-186.

To the editor:

The donation interval of 56 days requires extension to 180 days for whole blood donors to recover from changes in iron metabolism

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Iron deficiency (ID) is a frequently seen adverse event in whole blood donors, ultimately leading to deferral for donation because of low hemoglobin (Hb) values.[1] In accordance with guidelines of regulatory agencies and to protect whole blood donors from developing ID and anemia, many blood establishments worldwide require a minimum interval of 56 days between 2 donations.[2,3] This interval is based on studies from the 1940s and 1950s, investigating recovery of (only) Hb,[4,5] but more recently, it was reported to be too short to prevent ID.[6,7] Because Hb falls short as a marker for nonanemic ID,[8,9] and to prevent blood donors from developing ID, there is a need for additional insights into the kinetics of red cell indices and iron parameters over time after

blood donation. This may prove useful to assess the optimal donation interval and to select the parameter that is most suited to define personalized intervals.

Between March 2013 and April 2014, 24 "new" (defined as 1 or 2 donations before the start of the study) and 25 "regular" (>10 previous donations) male whole blood donors were randomly selected and included in the study (Table 1), and they were subsequently followed for 180 days after donating 500 mL blood (supplemental Figure 1, available on the *Blood* Web site). Recovery of Hb and iron parameters was tested for differences between new and regular donors in blood drawn at baseline (before donation) and at 9 time points after donation

Table 1. Baseline characteristics of new and regular male donors

Characteristics	New donors	Regular donors	P*
n	24	25	
Age, y	39.5 (7.1)	43.5 (5.8)	.034
Number of previous donations†	1 (1-2)	33 (24-52)	<.001
Days since previous donation	131 (45)	237 (138)	<.001
Blood volume (L)	5.7 (0.6)	5.7 (0.5)	.760
Blood group‡			.413
Type A⁻	2 (8.3)	0 (0)	
Type A⁺	10 (41.7)	8 (32.0)	
Type B⁺	4 (16.7)	5 (20.0)	
Type AB⁺	8 (33.3)	12 (48.0)	
Type B⁻, AB⁻, O⁻,§ or O⁺	0 (0)	0 (0)	
Smoking‡			.704
Current smoker	4 (17)	2 (8)	
Former smoker	6 (25)	6 (24)	
Never smoker	14 (58)	17 (68)	
Results of FFQ			
Total iron (mg)	31.3 (18.7)	33.5 (15.3)	.67
Heme iron (mg)	0.9 (0.5)	0.9 (0.4)	.81
Nonheme iron (mg)	30.4 (18.6)	32.6 (15.2)	.66
Calcium (mg)	1221 (876)	1402 (457)	.37

Values are given in mean (SD), unless otherwise stated.

FFQ, Food Frequency Questionnaire.

*Difference between new and regular donors by Student *t* tests, Mann-Whitney *U* test, or Fisher exact test when there were fewer than 5 observations in any cell.

†Median (p$_{25}$-p$_{75}$), in their whole donor career, but at least once in the 2 y preceding the start of the study.

‡n (%).

§Donors with blood group O⁻ were intentionally excluded because their donation frequency is higher than that of the other blood groups and therefore not representative for the (regular) donor population.

(8 hours and days 2, 4, 8, 15, 29, 57, 85, and 180). Furthermore, differences in iron absorption and erythrocyte iron incorporation between both donors groups were investigated with oral and intravenous stable iron isotopes, administered at day 8 and measured at day 29 in a subgroup of 31 donors (15 new, 16 regular), who consecutively agreed to participate. This study was approved by the Medical Ethical Committee Arnhem-Nijmegen in the Netherlands and the Ethical Advisory Council of Sanquin Blood Supply. All participants gave their written, informed consent.

In regular donors, Hb, ferritin, and hepcidin were lower, and erythropoietin (EPO) and soluble transferrin receptor (sTfR) were higher compared with new donors (Figure 1A) similar to observations in previous studies.[10-13] Iron absorption (17.0% and 21.9% of oral iron administered) and incorporation into erythrocytes (81.6% and 83.7% of intravenous iron) after donation were not statistically significantly different between new and regular donors, although the former tended to be higher in regular donors (supplemental Table 1). Interestingly, in the lower ferritin range, a trend was observed of higher iron absorption percentages for regular compared with new donors (supplemental Figure 2). Although, low numbers and high variation preclude any definitive conclusions, these data corroborate the concept of the regular donor group comprising (at least) some donors who more adequately absorb dietary iron with faster recovery from iron losses. This concept is in agreement with higher and lower prevalence of variants in *HFE* and *TMPRSS6*, respectively, in regular donors (supplemental Table 2), but needs further confirmation in larger studies.

Patterns of change in parameters over time after whole blood donation were similar for new and regular donors with an increase in EPO and decrease in Hb and hepcidin after day 2 followed by a decrease in ferritin and increase in reticulocytes, sTfR, and total iron binding capacity (TIBC) after day 4 (Figure 1A). At day 57, for only a few parameters (TIBC, Hb indices, iron, reticulocyte hemoglobin equivalent) >85% of both regular and new donors were back at predonation levels (Figure 1B; supplemental Tables 3 and 4). This percentage was particularly low for ferritin: only 25.0% of new donors and 32.0% of regular donors reached predonation levels. At day 85, >90% of donors were back at baseline levels for all parameters except for TSAT, hepcidin, sTfR-ferritin index, ferritin, and EPO, in both donor groups. At day 180, ferritin levels of all regular donors had returned to predonation levels compared with 82.6% of new donors. Importantly, for ferritin, increase in percentage of new donors that reached predonation levels was slower over time and lower at 180 days than in regular donors. This apparent faster recovery from iron losses in regular donors may be ascribed to their afore-suggested higher innate mean baseline intestinal absorption rates (for ferritin levels) in addition to previously described exponential increases in intestinal iron absorption with ferritin decreases in the low ferritin range.[14] Our observations on change of iron parameters after blood donation add detailed kinetic data to the already established theory of recovery of blood losses: (1) fast Hb decrease because of dilution to replace losses in blood volume (day 0-4), followed by (2) hypoxia-induced EPO production (days 0-4), (3) increase in EPO-induced erythropoiesis and associated increase in sTfR (days 4-29) and reticulocytes (days 2-8), (4) hepcidin decrease (day 1-8) through both signaling of increased erythropoiesis to hepatocytes[15] and decrease in body iron levels, and (5) ferritin decrease (days 1-29) through low-hepcidin induced release of stored iron into plasma. Altogether, these responses lead to increased erythropoiesis and iron availability for incorporation in newly synthesized erythrocytes, resulting in a return of Hb and iron parameters to baseline levels in time after blood donation.

In most previous studies, only recovery of Hb after blood loss was investigated and new and regular donors were not compared.[4,5] Recently, in the Recipient Epidemiology and Donor Evaluation Study–III (REDS-III) study involving fewer time points and iron parameters, investigators also observed a decrease of Hb and hepcidin and increase of reticulocyte count and EPO upon blood donation.[16] Moreover, and similar to our observations, they found that (1) absolute hepcidin levels decreased more in high ferritin donors, whereas EPO increased more in those with low ferritin, indicating that erythropoietic response to severe blood loss varies with baseline iron stores, and (2) recovery to baseline ferritin levels in high ferritin donors was less complete.

Interestingly, at baseline, 92% of new and 64% of regular donors had ferritin levels above the cutoff of 30 μg/L for ID (supplemental Figure 3). However, despite several reports on association between ferritin levels and symptoms of ID in blood donors,[17-19] it remains uncertain below which exact ferritin level complaints of ID occur. Recommended cutoffs differ between guidelines of various authorities and vary from 12 to 40 and even 100 μg/L to identify (possible) ID in the general population.[20,21] Because these values are largely based on older studies in the absence of international standards,[22,23] they are not ideally suited to define evidence-based cutoff values.

In conclusion, we provide detailed insights into changes and recovery in iron homeostasis over time until 180 days after blood donation in both regular and new whole blood donors. We conclude that for the vast majority of male donors, the donation interval of 56 days is too short to recover from donation-induced reduction in body iron stores.

To stay on the safe side, we propose, as our expert opinion, that ferritin should be kept above 30 μg/L at all times. Based on our observations, this implies a baseline ferritin at each donation of at least 50 μg/L. Furthermore, we propose ferritin as the best parameter to assess personalized donation intervals because it (1) significantly
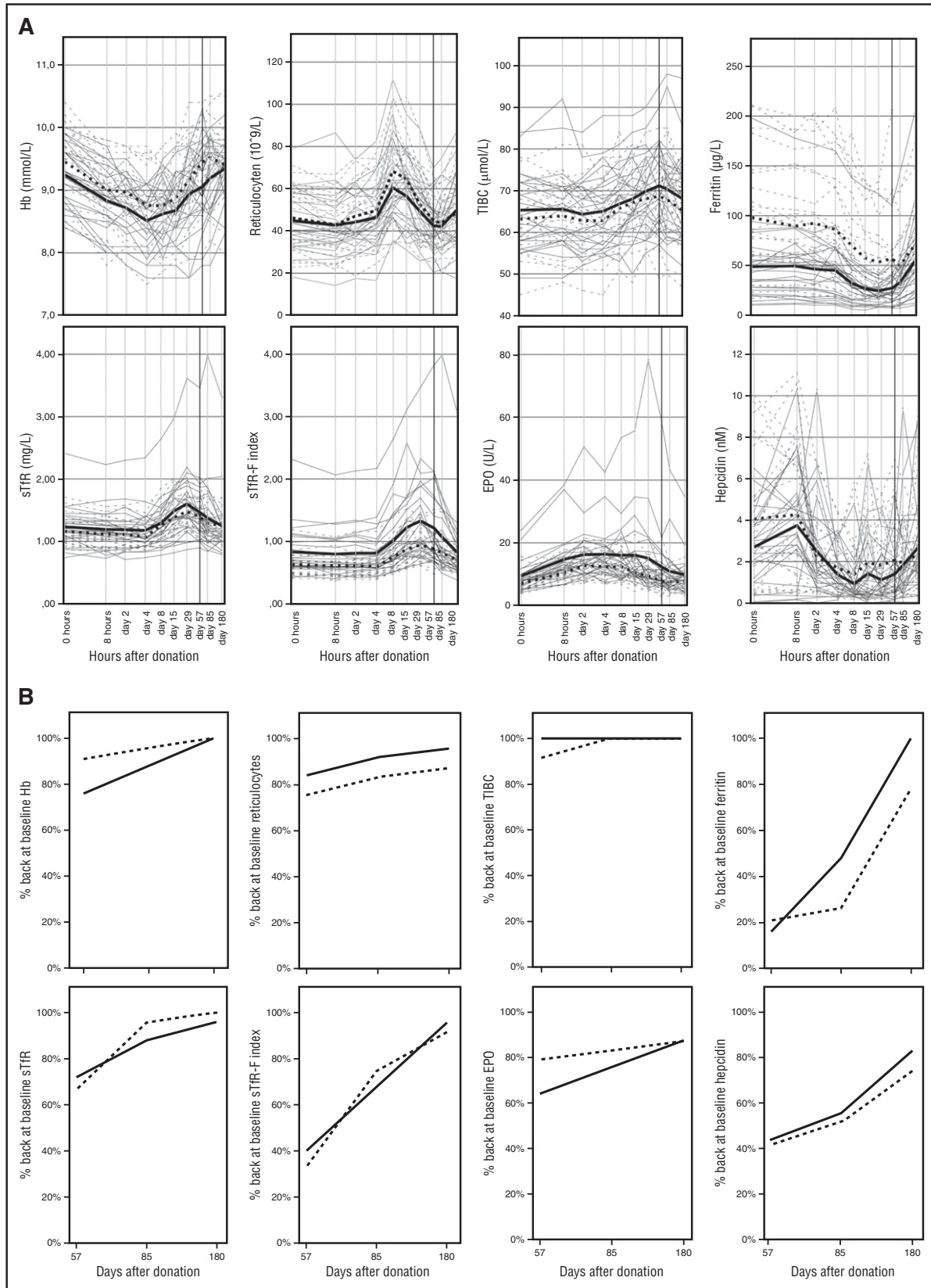
Figure 1. Recovery of Hb and iron parameters from blood donation. (A) Iron kinetics after whole blood donation for 49 whole blood donors. The vertical bold line indicates day 57, the minimum required interval between 2 donations in the Netherlands. (B) Percentage of donors back at baseline levels ($\pm 2 \times$ standard deviation [SD], in which SD = [biological + analytical coefficient of variation %] of the baseline concentration; supplemental Table 5). Solid lines indicate regular donors, and dashed lines new donors. Bold lines in panel A are the mean values for each of the groups. Mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, iron, transferrin saturation (TSAT), and zinc protoporphyrin showed no significant changes in time after blood donation and were therefore not included in this figure. Hb (mmol/L) $\times$ 1.611 = Hb (g/dL).

decreases upon blood donation in the present study and (2) has been found to be associated with symptomatic ID in blood donors.[17,18] Alternatively, and in the absence of point-of-care ferritin platforms, development of ID in donors may be prevented by (1) prolongation of donation intervals to 180 days in all donors as suggested by both the current and the REDS-III study[16] and/or (2) (low) dose iron supplementation.[24,25]

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References

1. Cable RG, Glynn SA, Kiss JE, et al; NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II). Iron deficiency in blood donors: the REDS-II Donor Iron Status Evaluation (RISE) study. *Transfusion.* 2012;52(4):702-711.

2. Canadian Blood Services. Available at: www.blood.ca. Accessed 14 June 2016.

3. European Commission. Commission directive 2004/33/EC. *Official Journal of the European Union.* 2004;L 91:25-39.

4. Fowler WM, Barer AP. Rate of hemoglobin regeneration in blood donors. *JAMA.* 1942;118(6):421-427.

5. Wadsworth GR. Recovery from acute haemorrhage in normal men and women. *J Physiol.* 1955;129(3):583-593.

6. Simon TL, Garry PJ, Hooper EM. Iron stores in blood donors. *JAMA.* 1981; 245(20):2038-2043.

7. Mast AE. Low hemoglobin deferral in blood donors. *Transfus Med Rev.* 2014; 28(1):18-22.

8. Harthoorn-Lasthuizen EJ, Lindemans J, Langenhuijsen MM. Zinc protoporphyrin as screening test in female blood donors. *Clin Chem.* 1998;44(4):800-804.

9. Hastka J, Lasserre JJ, Schwarzbeck A, Reiter A, Hehlmann R. Laboratory tests of iron status: correlation or common sense? *Clin Chem.* 1996;42(5):718-724.

10. Punnonen K, Rajamäki A. Evaluation of iron status of Finnish blood donors using serum transferrin receptor. *Transfus Med.* 1999;9(2):131-134.

11. Peffer K, Verbeek AL, Swinkels DW, Geurts-Moespot AJ, den Heijer M, Atsma F. Donation intensity and metabolic syndrome in active whole-blood donors. *Vox Sang.* 2015;109(1):25-34.

12. Salvin HE, Pasricha SR, Marks DC, Speedy J. Iron deficiency in blood donors: a national cross-sectional study. *Transfusion.* 2014;54(10): 2434-2444.

13. Milman N, Søndergaard M. Iron stores in male blood donors evaluated by serum ferritin. *Transfusion.* 1984;24(6):464-468.

14. Zimmermann MB, Troesch B, Biebinger R, Egli I, Zeder C, Hurrell RF. Plasma hepcidin is a modest predictor of dietary iron bioavailability in humans, whereas oral iron loading, measured by stable-isotope appearance curves, increases plasma hepcidin. *Am J Clin Nutr.* 2009;90(5):1280-1287.

15. Kautz L, Nemeth E. Molecular liaisons between erythropoiesis and iron metabolism. *Blood.* 2014;124(4):479-482.

16. Mast AE, Kiss JE, Cable RG, Glynn SA, Brambilla D. Effects of storage iron on erythropoietin and hepcidin responses to acute hemorrhage [abstract]. *Blood.* 2014;124(21). Abstract 1351.

17. Bryant BJ, Yau YY, Arceo SM, Hopkins JA, Leitman SF. Ascertainment of iron deficiency and depletion in blood donors through screening questions for pica and restless legs syndrome. *Transfusion.* 2013;53(8):1637-1644.

18. Spencer BR, Kleinman S, Wright DJ, et al; REDS-II RISE Analysis Group. Restless legs syndrome, pica, and iron status in blood donors. *Transfusion.* 2013;53(8):1645-1652.

19. Waldvogel S, Pedrazzini B, Vaucher P et al. Clinical evaluation of iron treatment efficiency among non-anemic but iron-deficient female blood donors: a randomized controlled trial. *BMC Med.* 2012;10:8.

20. Cook JD. Diagnosis and management of iron-deficiency anaemia. *Best Pract Res Clin Haematol.* 2005;18(2):319-332.

21. Knovich MA, Storey JA, Coffman LG, Torti SV, Torti FM. Ferritin for the clinician. *Blood Rev.* 2009;23(3):95-104.

22. Ferraro S, Mozzi R, Panteghini M. Revaluating serum ferritin as a marker of body iron stores in the traceability era. *Clin Chem Lab Med.* 2012;50(11): 1911-1916.

23. Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia: an overview. *J Gen Intern Med.* 1992;7(2): 145-153.

24. Moretti D, Goede JS, Zeder C, et al. Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. *Blood.* 2015;126(17):1981-1989.

25. Kiss JE, Brambilla D, Glynn SA, et al; National Heart, Lung, and Blood Institute (NHLBI) Recipient Epidemiology and Donor Evaluation Study–III (REDS-III). Oral iron supplementation after blood donation: a randomized clinical trial. *JAMA.* 2015;313(6):575-583.