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REVIEW

The sex difference in haemoglobin levels in adults — Mechanisms, causes, and consequences

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ABSTRACT

Men and women have different mean haemoglobin levels in health in venous blood — women have mean levels approximately 12% lower than men. A similar sex-related difference in haemoglobin levels in adult animals is found in many species of mammals, birds and reptiles, indicating that it is an important physiological phenomenon. It is probably a direct effect of sex hormones, both oestrogen and androgens, on erythropoiesis. However, since there is no difference in erythropoietin levels between the sexes, this effect most likely takes place in the kidney, rather than in the bone marrow. Oestrogens dilate and androgens constrict the renal microvasculature: dilation and vasoconstriction in vessels below 300 μm in diameter respectively increase and decrease the haematocrit in blood in arterioles, capillaries and venules, altering the oxygen delivery per unit red cell mass, and providing a mechanism for varying the red cell mass without compensatory changes in erythropoiesis.

1. Introduction

The long phylogenetic history of the sex difference in haemoglobin levels in vertebrates indicates that males and females evolved different mean venous haemoglobin levels for different purposes, or under different selection pressures. How and why these differences are maintained, and their relevance in medical practice, have not been fully defined to date, and are the subjects of this review.

Adult men and adult women have different haemoglobin levels in health [1–4]. This sex difference is independent of iron status — iron replete premenopausal women have mean haemoglobin levels approximately 12% lower than age & race matched men [1,4]. The mean circulating erythropoietin (Epo) level does not differ between men and women, and in women does not differ between pre and postmenopausal women [5,6], indicating that the sex difference is constitutive, and that women do not attempt to achieve male levels in health [5,7]. The sex difference in adult haemoglobin levels is conserved throughout *Mammalia* — a higher adult male haemoglobin level occurs in almost all mammal species studied to date, including non-menstruating and non-placental species: chimpanzees [8], rhesus macaques [9], vervet [10], cynomolgus [11] & capuchin monkeys [12], baboons [13], rodents [14], dogs [15], marsupials & monotremes [16], and in seals [17]. It also occurs in adults in many bird species [18], and in at least some reptiles at some stages of reproduction [19]. Whether the phenomenon occurs even further back in phylogenetics remains to be determined, but it is an ancient or recurring feature in evolution, which raises profound questions of what its importance may be.

A sex difference in haemoglobin levels has not been reported in juveniles in any species of mammal, bird or reptile: after the onset of adulthood male mammals and birds diverge from the juvenile state, raising their haemoglobin level by several percentage points. Females also increase their haemoglobin levels above the juvenile level, but not to the same extent as males.

2. Mechanisms producing the sex difference in venous haemoglobin levels in adult animals

In general, in healthy humans, the venous haemoglobin level correlates to a modest extent with the red cell mass, though the correlation is different for adult men and women, as discussed extensively below. The two values are determined by largely by the same factors (the tissue demand for oxygen determined at the juxtaglomerular apparatus), but are subject to some independent modulators — genetic and physiological, and are not directly interdependent. The physiological factors may be constitutive or chronic, such as puberty and menopause, acclimatisation to altitude, level of fitness or lean body mass, or acute such as posture or level of hydration. This lack of precise correlation exists both for individuals and for populations. Thus while red cell mass is on average lower per unit mass of tissue in females, the red cell mass in individuals in either sex cannot be estimated precisely from the venous haemoglobin level. Nevertheless for most clinical purposes venous

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haemoglobin levels can be used to imply red cell mass in individual patients, although the imprecision in this approach becomes apparent in the management of the apparent polycythaemia of chronic hypoxic lung or cardiac disease.

The haematocrit in healthy individuals varies predictably with the haemoglobin content of the blood it is measured in, and can be used interchangeably with haemoglobin level to compare the same value in populations of healthy individuals, and within individuals over short periods of measurement, though not reliably outside that.

The sex difference in mean venous haemoglobin levels and red cell mass is generally considered to be caused by a direct stimulatory effect of androgen in men in the bone marrow in association with erythropoietin, a stimulatory effect of androgen on erythropoietin production in the kidney, and an inhibitory effect of oestrogen on the bone marrow in women [20,21]. These effects have been demonstrated in vitro [22–24], and also work directly in vivo — androgens raise the haemoglobin level in males and females [25–29], and oestrogen lowers it [24,30]. However these direct and indirect effects of sex hormones on either marrow erythropoiesis or renal production of erythropoietin do not account for the absence of increased erythropoietic drive in females in response to their lower mean haemoglobin levels [5,7]. Testosterone in male animals may enable them to reach a desirable haemoglobin level more easily because of the synergistic effect of androgen on the bone marrow, with for example, a lower JGA mass. However this does not explain why females would not achieve the same effect in some other way, should there be an advantage in doing so. Females – adult human females at least – can raise their venous haemoglobin levels in response to additional erythropoietic stimuli. Andean women have higher haemoglobin levels at altitude than women of the same ethnicity living at lower levels, while preserving the sex difference with males at the same altitude, as do Tibetan women living at very high altitudes [31,32]. Female athletes can elevate their haemoglobin levels in response to exogenous rhuEPO in a manner similar to males [33,34]. Men, premenopausal women and postmenopausal women have similar plasma erythropoietin levels [5], indicating that women do not attempt to compensate for their lower haemoglobin levels by increasing erythropoietic drive. These observations show that the prevailing lower haemoglobin level in females cannot be ascribed to a lack of bone marrow or renal erythropoietic capability: they indicate that adult females maintain their venous haemoglobin levels at a lower level than adult males as a physiological steady state — they do not try under physiological conditions to maintain the same levels as adult males. Of course these factors may also indicate that males set their physiological haemoglobin levels higher than females, or rather that both sexes set their mean optimum level separately and to some degree independently. Anephric patients and patients with end stage renal failure do not exhibit a sex difference in baseline haemoglobin levels [35] demonstrating that the sex difference is mediated largely at the level of the kidney, rather than by direct erythropoietic action in the bone marrow. In addition, anephric patients did not have an erythropoietic response to therapeutic doses of androgens in a prospective randomised clinical trial of 103 men and 40 women, that included 15 patients who had undergone bilateral nephrectomy [36]. Perhaps it would be surprising if this were not the case, since the red cell mass itself is determined at the level of the kidney, and not at the level of the bone marrow.

It is hardly surprising that reptiles, birds and mammals have evolved different optimum levels of red cell mass and haemoglobin levels for males and females: red cells constitute a huge biological resource – one third by number of the body's cell complement, and with a relatively high turnover – that imposes enormous demands on the organism. Different drivers in males, such as competition for mates, and in females, such as parturition, could well have determined independent optimum investment in red cell mass in the sexes over the long course of vertebrate evolution. Huge phenotypic differences have existed between the sexes since the emergence of complex life forms — different venous haemoglobin levels evolved for different purposes would hardly be revolutionary. Indeed an absence of independently evolved haemoglobin levels might be more puzzling given the profound differences in the ecology and physiology of the sexes.

Erythropoietic drive in the intact adult animal is determined in very large part by the delivery of oxygen to the juxtaglomerular apparatus (JGA) of the kidney. Other mechanisms also play a part — including a baseline marrow erythroid activity that maintains a haemoglobin level around 70 g/L in anephric patients [35,37], a direct erythropoietic effect on the marrow of angiotensin II [38], and a modulation of oxygen consumption at the JGA by glomerular function [39]. Women with end stage renal disease are Epo resistant compared to age-matched males with end stage renal disease, requiring greater doses of Epo to achieve the same (sub-physiologic) haemoglobin level, consistent with the synergistic effect of androgen on erythropoietic dynamics in the bone marrow [40]. This however emphasises that females could achieve the same levels of haemoglobin by increasing Epo production — they "choose" not to, for whatever reason. Modulation of the JGA erythropoietic response to the level of red cell mass is the probable cause of the sex difference in haemoglobin levels, since as explained above, there is no compensatory response on the part of the JGA to the constitutively lower levels in healthy adult females.

The JGA could respond differently to the same total body red cell mass through several different mechanisms acting either singly or together. Red cells in adult females could deliver more oxygen per cell than in adult males. While there are subtle sex differences in red cells [41–44], a difference in oxygen dissociation has not been shown to exist, so that a difference in oxygen unloading is unlikely to account for the effect. However differences in flow dynamics in small arterioles caused by sex-related red cell differences remain possible. In addition the ability of the microvasculature to transfer oxygen from red cells to the tissues may differ between the sexes at the JGA. For example, sex differences in microvascular wall function, independent from vasodilation, that would allow females to deliver more oxygen per unit red cell mass than males to the cells of the JGA may exist, but have been not been reported to date. In addition, sex differences may exist in the molecular pathways of oxygen response in the kidney. While these effects cannot be ruled out, there are separate lines of evidence for a sex difference in blood flow in the microvasculature: a) adult females have a higher total body haematocrit than males at the same mean venous haemoglobin level; b) adult females have a higher mean microvascular haemoglobin level in the finger pulp for each value of venous haemoglobin level. A sex difference in microvascular blood flow provides a mechanism for differences in haemoglobin levels in adults, through modulation of the signal received at the JGA at a given level of red cell mass.

2.1. The Fåhraeus effect

Over eighty years ago Robin Fåhraeus observed a fall in the haematocrit of blood flowing into narrowing tubes below 300 μm in diameter [45]. The mean haematocrit in blood vessels below this size varies in proportion to the mean diameter of the vessels [46–49] (Fig. 1). In microvascular networks this effect is augmented by a separate differential segregation of red cells and plasma at vessel bifurcations. The net effect is that blood flowing through the microvasculature has a lower unit red cell content than blood in the larger veins and arteries, and that the haematocrit at a given point in a blood vessel is affected by the diameter of the vessel at that point.

A necessary consequence of the reduction in red cell content in the blood of the microvasculature is that the whole body haematocrit, determined by the simultaneous measurement of red cell mass and plasma volume, is less than the venous haematocrit. This was initially recorded by Chaplin et al. [50], who found that the mean whole body haematocrit was 10% lower than that of the venous blood, using simultaneous measurement of red cell mass by ^{51}chromium, and plasma

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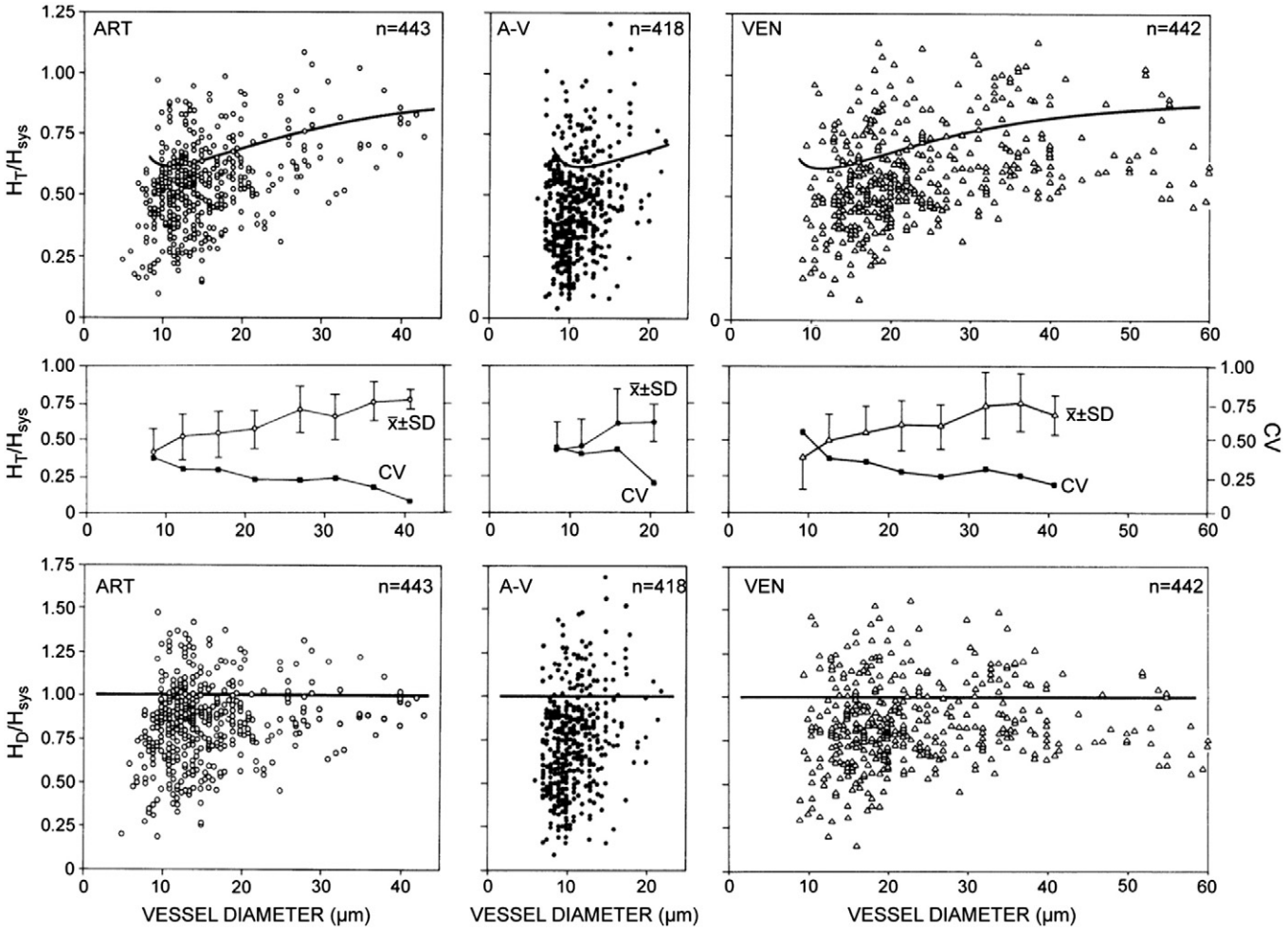

Fig. 1. Using photographic and videomicroscopy capture and analysis of blood flowing in an in vivo preparation of rat mesentery vascular networks, the authors demonstrated how the haematocrit of the blood flowing through arterioles, capillaries and venules changed in relation to the diameters of the blood vessels. The original caption reads: "Vessel segment hematocrit plotted against diameter (combined data from three networks). All hematocrits are normalized with respect to systemic hematocrit (H_{sys}). Top panels: normalised tube hematocrit (H_T/H_{sys}) for arteriolar (ART), arteriovenous (AV), and venular (VEN) segments. Solid lines represent hematocrit reduction predicted by the Fåhraeus effect as measured in vitro assuming H_D equals H_{sys}. Middle panels: upper lines give mean values and standard deviations of H_T/H_{sys}. Lower lines give corresponding coefficients of variation (CV). Bottom panel: normalized discharge hematocrit H_D/H_{sys}."
Reproduced with kind permission of the publisher from Figure 5 in. Pries AR, Ley K, Gaehtgens P. Generalization of the Fahraeus principle for microvessel networks. Am J Physiol. 1986;251: H1324–32. (Reference [46]).

volume by albumin radiolabelled with ^{131}iodine. Chaplin's initial observations on 28 subjects were repeated many times by others, and the initial ratio of 0.91 for whole body haematocrit ÷ venous haematocrit was confirmed [51], though the inter-subject variability is considerable, to the extent that the ratio has no clinical utility in calculating the red cell mass or plasma volume of individual patients. Only four subjects in Chaplin's study (3 males and one premenopausal female) were normal volunteers; the remaining 24 had various haematological abnormalities. In contrast, in a study of 22 normal individuals, 11 men and 11 premenopausal women, with mean venous haemoglobins of 140 g/L and 139 g/L respectively, and using dual isotope labelling, Karlson and Senn [52] measured the ratio at 0.91 in men and 0.974 in women, demonstrating that the red cell content of the microvasculature is higher in females than in males at the observed level of venous haemoglobin.

A similar phenomenon was reported by Tong et al. [53] using a different approach. They measured paired venous and finger pulp haemoglobin levels in 35,258 blood donors within confined haemoglobin ranges: 25,762 women with finger pulp haemoglobin levels < 125 g/L, and 10,496 men with finger pulp haemoglobin levels < 135 g/L. They reported a significant difference between the sexes in the relationship between venous and finger pulp haemoglobin levels — on average the difference between the haemoglobin levels in finger pulp and venous blood was approximately 4 g/L less in women. In addition, in women the gap between finger pulp and venous haemoglobin levels was constant until the age of 45 years, but increased sharply thereafter, whereas in men a continuous low rate of decline persisted over the entire age range [7] (Fig. 2). Cable et al. [54] reported similar sex differences in the variation between the haemoglobin content of blood from veins and finger pulp. These studies confirm that women have more red cells in their microvasculature relative to the veins (and heart and arteries) than men have. The tissues of the human finger pulp are organised as an organ of touch and heat sensitivity. The blood flow is arranged as a fan of arterioles spreading from a basal arterial arch just distal and anterior to the terminal interphalangeal joint. Blood flows distally and laterally to be collected into a venular network on the lateral side of the finger pad [55,56]. The vessels of the finger pulp are all small arterioles, capillaries and venules, below the diameter where the Fåhraeus effects operate, so that the mean red cell content of the blood will vary with the mean diameter.

The microvasculature in females contains more red cells per unit volume of blood than in males as demonstrated by the higher microvascular and whole body haematocrit and haemoglobin levels in the studies of Karlson, Tong and Cable [52–54], even though the mean red cell

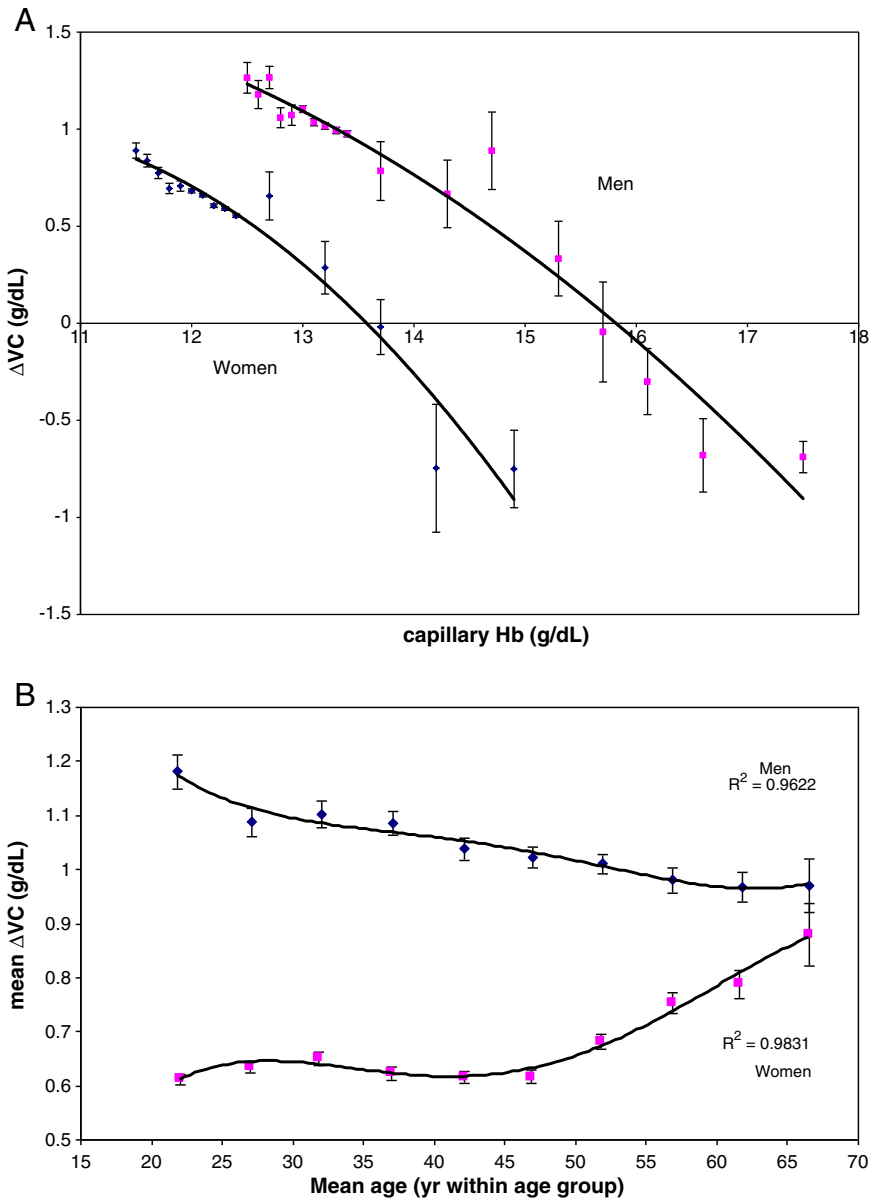

Fig. 2. Difference between venous and capillary haemoglobin levels in men and women. (A) The mean difference and standard error between venous and capillary haemoglobin levels (ΔVC) in 35,985 paired capillary and venous samples were taken from 25,557 females and 10,428 males in whom the capillary haemoglobin was >11.5 g/dL to <12.5 g/dL and >12.5 to <13.5 g/dL, respectively, and from 81 male and 74 female first time blood donors where the capillary haemoglobin levels were above the cut-off for inclusion in the original study group. There is a significant change comparing lower capillary Hb with higher venous Hb (by Kruskal–Wallis followed by Dunn post-test). The ΔVC increases in a linear manner as measured by Spearman correlation, in both females and males as the capillary haemoglobin levels in the groups decline ($r = -0.9879$, $P < 0.0001$ for the female group; $r = -0.9152$, $P = .0005$ for the male group). (B) The mean difference and standard error between venous and capillary haemoglobin levels (ΔVC) compared between age groups. The dot plot shows mean age versus mean ΔVC within each age group in men and women. Within the male cohort, there was a negative correlation; $r = -0.978$, $P = .0001$. ΔVC is lower in women and rises with increasing age. The linear correlation is much lower, and the slope is in the opposite direction; $r = 0.7818$, $P = .0102$. It is clear from the dot plot that ΔVC is stable in women until they reach their 50s, when there is a sizeable increase. By the age of 65–59 years, ΔVC standard error in women overlaps with that of men.
Reproduced with permission from Murphy WG, Tong E, Murphy C. Why do women have similar erythropoietin levels to men but lower hemoglobin levels? Blood. 2010;116:2861–2. (Reference [7]).

mass per unit body mass is lower in females. This can be ascribed to modulation of the vessel diameter — microvascular vasodilation will raise the red cell content in the vessels through the Fåhraeus effect [46–49]. The difference in the microvascular blood content between adult males and females indicates that the Fåhraeus effect is modulated differently in the sexes, in turn indicting that the mean diameter in the female microvasculature is higher than in males. Human females have higher vessel diameters than males, a direct oestrogen effect, consistent with these observations: while direct observation of relative vasodilation in vivo in females has been limited to the larger vessels, increased vascular responsiveness of microvascular beds to agonist induced-vasodilation in females [57–59] indicates increased constitutive vasodilation in the microcirculation.

Androgens are potent agents of vasoconstriction in the renal microvasculature [60–63]. This effect, which is considered to account at least in part for the sex difference in the incidence of hypertension, would be expected to reduce red cell delivery to the JGA per unit of red cell mass, and to lead to an associated relative increase in red cell mass to maintain the same renal blood flow and Epo levels. Angiotensin II blockade reduces erythropoietin levels [64], demonstrating that the fall in haemoglobin levels caused by angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers [64,65] is effected through renal

vascular pathways. Since androgens enhance angiotensin II activity in inducing hypertension [66], this is consistent with a haemoglobin-raising effect of testosterone through a direct effect on the renal microvasculature.

3. Causes: why are mean venous haemoglobin levels set at different values between males and females?

Since the red cell mass and the venous haemoglobin levels differ between the sexes, but the microcirculatory haematocrit does not, or does so to a significantly less extent, it is probable that it is the red cell mass or the venous haemoglobin level that has evolved to different levels between the sexes. Preserving the microcirculatory haematocrit at the same level by different mechanisms in the two sexes would have allowed the red cell mass, and the venous haemoglobin level, to differ between the sexes while preserving tissue oxygenation. As stated earlier, the red cell mass is an enormous resource in animals — one third of the body's cells by number in adult humans, and demands enormous effort to establish and maintain. Any reserve over and above a critical baseline level will exact a very high price in biological matériel materiel - particularly iron – to make, and in ongoing cardiorespiratory effort to keep intact and mobile in the circulation. This price must be paid for by increased fitness. However that may be done, it appears probable that the critical baseline haemoglobin level, from observations in humans, is far below the mean level observed in healthy subjects. From studies in surgical patients refusing blood transfusion, it may lie around 60 g/L [67]; from studies in chronically iron deficient children, it may even be considerably lower than that [68]. The excess red cells over the critical minimum that circulate in the large vessels (and slowly through in the spleen in some species) and that constitute half or more of the venous haemoglobin level probably provide a storage function for the reserve red cell mass for use when the need arises for increased work — fight, flight, food and reproduction. They may also act as reserves for heat exchange and iron storage. It is likely that the return in fitness from this reserve differs between the sexes, and therefore that the optimal size for maximal cost benefit also differs.

More basically perhaps, it is not clear why the prevailing haemoglobin level is set at the level it is in either sex. There is always going to be a trade-off between effort expended, and risk incurred, against benefit accrued, and it appears that this trade-off has balanced out at a lower level of haemoglobin in females. However there is nothing beyond conjecture to inform why that might be the case. Mammals have mean haemoglobin levels of 147 g/L (s.d. 20 g/L; range 101–221 g/L); birds have mean haemoglobin levels of 153 g/L (s.d. 18 g/L; range 103–193 g/L) and reptiles have mean haemoglobin levels of 85 g/L (s.d. 19 g/L; range 51–120 g/L) [18,69]. Adult mammal species without exception enucleate their mature red cells, while birds and reptiles (with the exception of some species of salamander that enucleate as much as 80% of circulating red cells [70]) do not. The microvascular dynamics and Fåhraeus effects can be expected to work differently for nucleated cells. (No one knows why red cell enucleation conveyed advantage — it does not on its own increase performance ability or thermoregulation. Perhaps it no longer conveys any persistent advantage outside the ecology in which it evolved, at the point of emergence of modern mammals.) The similarity in means and ranges of haemoglobin levels among birds and mammals suggests that this physiological equilibrium has emerged more than once by natural selection, and that it is of considerable importance and value.

There is a clear association between higher haemoglobin levels and thrombotic risk both within and above the normal range [71–75]. In addition to the epidemiological evidence, it seems very probable that increasing haemoglobin to supra-physiological levels by pharmacological means raises performance in male and female athletes, and is associated with an increased risk of thrombosis [76], at least in men. It is plausible that gains in performance above current mean physiological levels in men are associated with increased thrombosis risk, but that can hardly account for the current setting for mean levels across the animal kingdom, since if higher levels give significant advantage in the business of food and sex, selection pressure could have decreased the risk of thrombosis by some means in the timescale that it has had to do it. It may be more plausible that the competitive advantage was outstripped by the maintenance costs of a high red cell mass at the prevailing physiological level.

So if we can only guess *why* the levels are where they are in the first place, then *why* females set their levels at a value that is different than that evolved in males can also only be guessed at. Understanding *how* the effect is achieved may shed light on the reasons for the difference, and perhaps on the reasons for the base physiological level. It may also reveal useful pointers to interactions between haemoglobin levels and disease. Since the difference is driven separately by oestrogen and androgen it is reasonable to conclude that there is some advantage in the lower haemoglobin levels in females. What this may be is impossible to know — however its persistence from reptiles to birds and mammals (or its separate appearance in different lineages) implies that it has to do with fundamental properties of adult femaleness: the successful execution of the female side of reproduction. Within that scope might be included, for example, the husbanding of resources, including iron, for the prenatal or post-natal nourishing of young. Diverting some of the resource required for red cell mass to their young is a plausible reason for scaling back its demands and costs in females. During pregnancy Epo production and venous haemoglobin levels uncouple in response to very high circulating oestrogen levels suppressing the erythropoietic response to falling blood haemoglobin levels. This has the effect of diverting iron to the developing foetus even though the red cell mass increases [77]. Direct suppression by 17β-estradiol of Epo gene expression in response to hypoxia [78] may contribute to the effect of oestrogen at the JGA. This implies that there is an increased tolerance of tissue hypoxia in pregnancy that contributes to the attenuation of the increase in the red cell mass. While this is possible – it is similar to some of the adaptations of Tibetans to chronic hypoxia, though not of Andeans [31] – oestrogen-induced vasodilation in contrast results in lowering of the Epo response to a relative decline in red cell mass without a significant fall in tissue oxygen delivery. Instead the biological reserve of circulating red cells is reduced.

4. Consequences

Mechanisms that evolved to maximise survival and reproductive fitness hundreds of millions of years ago are not necessarily best fitted for longevity beyond parenting in modern humans. Hypertension and other causes of vascular degenerative diseases are major determinants of lifespan as humans age far beyond their reproductive apotheosis. Mean haemoglobin levels and red cell mass values, and their difference between the sexes, evolved at a very ancient stage in mammal phylogeny. The upper level is likely to have been determined by a trade-off between the effort, resources and risk incurred in maintaining a huge red cell mass, and the performance and reproductive advantages gained from it. Maintaining a high red cell mass beyond a necessary minimal reserve capacity in ageing humans may not serve any useful function at the individual level. Lowering the haemoglobin level and the red cell mass will reduce cardiac work. In this regard, the lowering of erythropoietic drive and haemoglobin levels caused by angiotensin II blockade [65,79–81] may be associated with the benefits of these medicines in heart failure and secondary erythrocytosis [65,82]. The haemoglobin lowering effects of enalapril and similar drugs have been ascribed to a direct suppression of erythropoiesis at the bone marrow, but the renal vasodilation that the therapy produces is a more likely cause since the anaemia is associated with a lowering of plasma Epo levels [79,82]. Whether prophylactic reduction of red cell mass in older humans will improve cardiovascular risk, and whether that would apply in both men and women remains to be seen. Women live longer, and have a lower risk of cardiovascular disease at every age; whether the

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lower cardiovascular workload associated with the lower haemoglobin level contributes to that cannot be determined or ruled out, though recent evidence that higher haemoglobin levels in young men are associated with increased long term risk of stroke [73] may be of interest. Prophylactic pharmacological phlebotomy using doses of angiotensin II blockade that induce renal vasodilation but do not reduce arterial blood pressure – essentially reproducing the premenopausal female state of affairs at the JGA – may be worth serious consideration, and may add to the therapies widely used to reduce cardiovascular risk.

The ability of transfused red blood cells to participate in and maintain the Fåhraeus effects in the microvasculature has never been deliberately assessed. It is probably essential that the cells retain their plasticity and elasticity to flow through microvascular networks in a physiological manner, so that it is unlikely that red cell stored beyond a couple of weeks after collection will be unaffected. Red cells stored for transfusion lose their plasticity as ADP and 2,3 DPG levels decline after the first week of storage [83,84]. Whether this contributes to, or causes, the putative adverse effects associated with transfusion of older blood [85,86] will be difficult to explore. However, manufacturers of red cell storage systems, and especially those attempting industrial scale, pharmaceutical grade ex vivo culture of red cells [87,88] may need to ensure that their products recapitulate the microvascular flow functions of the native system, if they are to avoid the fate of the haemoglobin solutions [89,90].

Conflict of interest

I affirm that I have no conflicts of interest in regard to the subject matter of this review.

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References

[1] Yip R, Johnson C, Dallman PR. Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. Am J Clin Nutr 1984;39:427–36.
[2] Vahlquist B. The cause of the sexual differences in erythrocyte, hemoglobin and serum iron levels in human adults. Blood 1950;5:874–5.
[3] Garn SM, Smith NJ, Clark DC. Lifelong differences in hemoglobin levels between Blacks and Whites. J Natl Med Assoc 1975;67:91–6.
[4] Ganji V, Kafai MR. Hemoglobin and hematocrit values are higher and prevalence of anemia is lower in the post-folic acid fortification period than in the pre-folic acid fortification period in US adults. Am J Clin Nutr 2009;89:363–71.
[5] Tilling L, Hunt J, Jiang B, Sanders TA, Clapp B, Chowienczyk P. Endothelial function does not relate to haemoglobin or serum erythropoietin concentrations and these do not explain the gender difference in endothelial function in healthy middle-aged men and women. Eur J Clin Invest 2013;43:225–30.
[6] Rege AB, Brookins J, Fisher JW. A radioimmunoassay for erythropoietin: serum levels in normal human subjects and patients with hemopoietic disorders. J Lab Clin Med 1982;100:829–43.
[7] Murphy WG, Tong E, Murphy C. Why do women have similar erythropoietin levels to men but lower hemoglobin levels? Blood 2010;116:2861–2.
[8] Howell S, Hoffman K, Bartel L, Schwandt M, Morris J, Fritz J. Normal hematologic and serum clinical chemistry values for captive chimpanzees (*Pan troglodytes*). Comp Med 2003;53:413–23.
[9] Buchl SJ, Howard B. Hematologic and serum biochemical and electrolyte values in clinically normal domestically bred rhesus monkeys (*Macaca mulatta*) according to age, sex, and gravidity. Lab Anim Sci 1997;47:528–33.
[10] Sato A, Fairbanks LA, Lawson T, Lawson GW. Effects of age and sex on hematologic and serum biochemical values of vervet monkeys (*Chlorocebus aethiops sabaeus*). Contemp Top Lab Anim Sci 2005;44:29–34.
[11] Xie L, Xu F, Liu S, Ji Y, Zhou Q, Wu Q, et al. Age- and sex-based hematological and biochemical parameters for *Macaca fascicularis*. PLoS One 2013;8:e64892.
[12] Wirz A, Truppa V, Riviello MC. Hematological and plasma biochemical values for captive tufted capuchin monkeys (*Cebus apella*). Am J Primatol 2008;70:463–72.
[13] Harewood WJ, Gillin A, Hennessy A, Armistead J, Horvath JS, Tiller DJ. Biochemistry and haematology values for the baboon (*Papio hamadryas*): the effects of sex, growth, development and age. J Med Primatol 1999;28:19–31.
[14] Kane JD, Steinbach TJ, Sturdivant RX, Burks RE. Sex-associated effects on hematologic and serum chemistry analytes in sand rats (*Psammomys obesus*). J Am Assoc Lab Anim Sci 2012;51:769–74.
[15] Michaelson SM, Scheer K, Gilt S. The blood of the normal beagle. J Am Vet Med Assoc 1966;148:532–4.
[16] Clark P. Haematology of Australian mammals. Collingwood: Csiro Publishing; 2004.
[17] Boily F, Beaudoin S, Measures LN. Hematology and serum chemistry of harp (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) during the breeding season, in the Gulf of St. Lawrence, Canada. J Wildl Dis 2006;42:115–32.
[18] Glomski CA, Pica A. The avian erythrocyte. Its phylogenetic odyssey. Boca Raton: CRC Press; 2011.
[19] Harris HS, Benson SR, Gilardi KV, Poppenga RH, Work TM, Dutton PH, et al. Comparative health assessment of Western Pacific leatherback turtles (*Dermochelys coriacea*) foraging off the coast of California, 2005–2007. J Wildl Dis 2011;47:321–37.
[20] Jelkmann W. Regulation of erythropoietin production. J Physiol 2011;598:1252–8.
[21] Shahani S, Braga-Basaria M, Maggio M, Basaria S. Androgens and erythropoiesis: past and present. J Endocrinol Invest 2009;32:704–16.
[22] Moriyama Y, Fisher JW. Effects of testosterone and erythropoietin on erythroid colony formation in human bone marrow cultures. Blood 1975;45:665–70.
[23] Claustres M, Sultan C. Androgen and erythropoiesis: evidence for an androgen receptor in erythroblasts from human bone marrow cultures. Horm Res 1988;29:17–22.
[24] Blobel GA, Orkin SH. Estrogen-induced apoptosis by inhibition of the erythroid transcription factor GATA-1. Mol Cell Biol 1996;16:1687–94.
[25] Nathan DG, Gardner FH. Effects of large doses of androgen on rodent erythropoiesis and body composition. Blood 1965;26:411–20.
[26] Kennedy BJ, Gilbertsen AS. Increased erythropoiesis induced by androgenic-hormone therapy. N Engl J Med 1957;256:719–26.
[27] Coviello AD, Kaplan B, Lakshman KM, Chen T, Singh AB, Bhasin S. Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. J Clin Endocrinol Metab 2008;93:914–9.
[28] Palacios A, Campfield LA, McClure RD, Steiner B, Swerdloff RS. Effect of testosterone enanthate on hematopoiesis in normal men. Fertil Steril 1983;40:100–4.
[29] Jockenhövel F, Vogel E, Reinhardt W, Reinwein D. Effects of various modes of androgen substitution therapy on erythropoiesis. Eur J Med Res 1997;2:293–8.
[30] Dukes PP, Goldwasser E. Inhibition of erythropoiesis by estrogens. Endocrinology 1961;69:21–9.
[31] Beall CM, Brittenham GM, Strohl KP, Blangero J, Williams-Blangero S, Goldstein MC, et al. Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. Am J Phys Anthropol 1998;106:385–400.
[32] Beall CM, Goldstein MC. Hemoglobin concentration of pastoral nomads permanently resident at 4,850–5,450 meters in Tibet. Am J Phys Anthropol 1987;73:433–8.
[33] Parisotto R, Wu M, Ashenden MJ, Emslie KR, Gore CJ, Howe C, et al. Detection of recombinant human erythropoietin abuse in athletes utilizing markers of altered erythropoiesis. Haematologica 2001;86:128–37.
[34] Morkeberg J, Lundby C, Nissen-Lie G, Nielsen TK, Hemmersbach P, Damsgaard R. Detection of darbepoetin alfa misuse in urine and blood: a preliminary investigation. Med Sci Sports Exerc 2007;39:1742–7.
[35] Neff MS, Goldberg J, Slifkin RF, Eiser AR, Calamia V, Kaplan M, et al. A comparison of androgens for anemia in patients on hemodialysis. N Engl J Med 1981;304:871–5.
[36] Teruel JL, Marcen R, Navarro-Antolin J, Aguilera A, Fernandez-Juarez G, Ortuño J. Androgen versus erythropoietin for the treatment of anemia in hemodialyzed patients: a prospective study. J Am Soc Nephrol 1996;7:140–4.
[37] Shaldon S, Koch KM, Oppermann F, Patyna WD, Schoeppe W. Testosterone therapy for anaemia in maintenance dialysis. Br Med J 1971;3:212–5.
[38] Mrug M, Stopka T, Julian BA, Prchal JF, Prchal JT. Angiotensin II stimulates proliferation of normal early erythroid progenitors. J Clin Invest 1997;100:2310–4.
[39] Donnelly S. Why is erythropoietin made in the kidney? The kidney functions as a critmeter. Am J Kidney Dis 2001;38:415–25.
[40] Ifudu O, Uribarri J, Rajwani I, Vlacich V, Reydel K, Delosreyes G, et al. Gender modulates responsiveness to recombinant erythropoietin. Am J Kidney Dis 2001;38:518–22.
[41] Habif S, Mutaf I, Turgan N, Onur E, Duman C, Ozmen D, et al. Age and gender dependent alterations in the activities of glutathione related enzymes in healthy subjects. Clin Biochem 2001;34:667–71.
[42] Zeltser D, Rogowski O, Berliner S, Mardi T, Justo D, Serov J, et al. Sex differences in the expression of haemorheological determinants in individuals with atherothrombotic risk factors and in apparently healthy people. Heart 2004;90:277–81.
[43] Tepper T, Sluiter WJ, Huisman RM, de Zeeuw D. Erythrocyte Na+/Li+ countertransport and Na+/K+–2Cl co-transport measurement in essential hypertension: useful diagnostic tools or failure? A meta-analysis of 17 years of literature. Clin Sci (Lond) 1998;95:649–57.
[44] Jiao Y, Okumiya T, Saibara T, Tsubosaki E, Matsumura H, Park K, et al. An enzymatic assay for erythrocyte creatine as an index of the erythrocyte life time. Clin Biochem 1998;31:59–65.
[45] Goldsmith HL, Cokelet GR, Gaehtgens P. Robin Fåhraeus: evolution of his concepts in cardiovascular physiology. Am J Physiol 1989;257(3 Pt 2):H1005–15.
[46] Pries AR, Ley K, Gaehtgens P. Generalization of the Fahraeus principle for microvessel networks. Am J Physiol 1986;251(6 Pt 2):H1324–32.
[47] Pries AR, Secomb TW, Gaehtgens P. Structure and hemodynamics of microvascular networks: heterogeneity and correlations. Am J Physiol 1995;269(5 Pt 2):H1713–22.
[48] Klitzman B, Duling BR. Microvascular hematocrit and red cell flow in resting and contracting striated muscle. Am J Physiol 1979;237:H481–90.
[49] Klitzman B, Johnson PC. Capillary network geometry and red cell distribution in hamster cremaster muscle. Am J Physiol 1982;242:H211–9.
[50] Chaplin Jr H, Mollison PL, Vetter H. The body/venous hematocrit ratio: its constancy over a wide hematocrit range. J Clin Invest 1953;32:1309–16.

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[51] Pearson TC, Guthrie DL, Simpson J, Chinn S, Barosi G, Ferrant A, et al. Interpretation of measured red cell mass and plasma volume in adults: expert panel on radionuclides of the International Council for Standardization in Haematology. Br J Haematol 1995;89:748–56.
[52] Karlson KE, Senn LY. Simultaneous determination of red cell mass and plasma volume with cr51 and i131 using a pulse height analyzer. Ann Surg 1963;158:309–18.
[53] Tong E, Murphy WG, Kinsella A, Darragh E, Woods J, Murphy C, et al. Capillary and venous haemoglobin levels in blood donors: a 42-month study of 36,258 paired samples. Vox Sang 2010;98:547–53.
[54] Cable RG, Steele WR, Melmed RS, Johnson B, Mast AE, Carey PM, et al. NHLBI Retrovirus Epidemiology Donor Study—II (REDS-II). The difference between fingerstick and venous hemoglobin and hematocrit varies by sex and iron stores. Transfusion 2012;52:1031–40.
[55] Barreiro FJJ, Valdecasas Huelin JM. Radiological studies on the vascular anatomy of the forearm and hand — recent progress. In: Tubiana R, editor. The hand, vol. 1. Philadelphia: WB Saunders; 1981. p. 310–25.
[56] Sangiorgi S, Manelli A, Congiu T, Bini A, Pilato G, Reguzzoni M, et al. Microvascularization of the human digit as studied by corrosion casting. J Anat 2004;204:123–31.
[57] Lindsey SH, Carver KA, Prossnitz ER, Chappell MC. Vasodilation in response to the GPR30 agonist G-1 is not different from estradiol in the mRen2.Lewis female rat. J Cardiovasc Pharmacol 2011;57:598–603.
[58] Ma L, Robinson CP, Thadani U, Patterson EJ. Effect of 17-beta estradiol in the rabbit: endothelium-dependent and -independent mechanisms of vascular relaxation. Cardiovasc Pharmacol 1997;30:130–5.
[59] Meyer MR, Prossnitz ER, Barton M. The G protein-coupled estrogen receptor GPER/GPR30 as a regulator of cardiovascular function. Vascul Pharmacol 2011;55:17–25.
[60] Nakagawa K, Marji JS, Schwartzman ML, Waterman MR, Capdevila JH. Androgen-mediated induction of the kidney arachidonate hydroxylases is associated with the development of hypertension. Am J Physiol Regul Integr Comp Physiol 2003;284:R1055–62.
[61] Wu CC, Schwartzman ML. The role of 20-HETE in androgen-mediated hypertension. Prostaglandins Other Lipid Mediat 2011;96:45–53.
[62] Wu CC, Cheng J, Zhang FF, Gotlinger KH, Kelkar M, Zhang Y, et al. Androgen-dependent hypertension is mediated by 20-hydroxy-5,8,11,14-eicosatetraenoic acid-induced vascular dysfunction: role of inhibitor of kappaB kinase. Hypertension 2011;57:788–94.
[63] Wang YX, Crofton JT, Bealer SL, Share L. Sexual dimorphism in regional blood flow responses to vasopressin in conscious rats. Am J Physiol 1997;273(3 Pt 2):R1126–31.
[64] Leshem-Rubinow E, Steinvil A, Zeltser D, Berliner S, Rogowski O, Raz R, et al. Association of angiotensin-converting enzyme inhibitor therapy initiation with a reduction in hemoglobin levels in patients without renal failure. Mayo Clin Proc 2012;87:1189–95.
[65] Pratt MC, Lewis-Barned NJ, Walker RJ, Bailey RR, Shand BI, Livesey J. Effect of angiotensin converting enzyme inhibitors on erythropoietin concentrations in healthy volunteers. Br J Clin Pharmacol 1992;34:363–5.
[66] Song J, Kost Jr CK, Martin DS. Androgens potentiate renal vascular responses to angiotensin II via amplification of the Rho kinase signaling pathway. Cardiovasc Res 2006;72:456–63.
[67] Spence RK, Carson JA, Poses R, McCoy S, Pello M, Alexander J, et al. Elective surgery without transfusion: influence of preoperative hemoglobin level and blood loss on mortality. Am J Surg 1990;159:320–4.
[68] Lackritz EM, Campbell CC, Ruebush II TK, Hightower AW, Wakube W, Steketee RW, et al. Effect of blood transfusion on survival among children in a Kenyan hospital. Lancet 1992;340:524–8.
[69] Hawkey CM, Bennett PM, Gascoyne SC, Hart MG, Kirkwood JK. Erythrocyte size, number and haemoglobin content in vertebrates. Br J Haematol 1991;77:392–7.
[70] Villolobos M, Leon P, Sessions SK, Kezer J. Enucleated erythrocytes in plethodontid salamanders. Herpetologica 1988;44:243–50.
[71] Anderson Jr FA, Spencer FA. Risk factors for venous thromboembolism. Circulation 2003;107(23 Suppl. 1):I9–I16.
[72] Eischer L, Tscholl V, Heinze G, Traby L, Kyrle PA, Eichinger S. Hematocrit and the risk of recurrent venous thrombosis: a prospective cohort study. PLoS One 2012;7:e38705.
[73] Toss F, Nordström A, Nordström P. Association between hematocrit in late adolescence and subsequent myocardial infarction in Swedish men. Int J Cardiol 2013;168:3588–93.
[74] Danesh J, Collins R, Peto R, Lowe GD. Haematocrit, viscosity, erythrocyte sedimentation rate: meta-analyses of prospective studies of coronary heart disease. Eur Heart J 2000;21:515–20.
[75] Pearson TC. Rheology of the absolute polycythaemias. Baillieres Clin Haematol 1987;1:637–64.
[76] Spivak JL. Erythropoietin use and abuse: when physiology and pharmacology collide. Adv Exp Med Biol 2001;502:207–24.
[77] Horiguchi H, Oguma E, Kayama F. The effects of iron deficiency on estradiol-induced suppression of erythropoietin induction in rats: implications of pregnancy-related anemia. Blood 2005;106:67–74.
[78] Mukundan H, Resta TC, Kanagy NL. 17Beta-estradiol decreases hypoxic induction of erythropoietin gene expression. Am J Physiol Regul Integr Comp Physiol 2002;283:R496–504.
[79] Cole J, Ertoy D, Lin H, Sutliff RL, Ezan E, Guyene TT, et al. Lack of angiotensin II-facilitated erythropoiesis causes anemia in angiotensin-converting enzyme-deficient mice. J Clin Invest 2000;106:1391–8.
[80] Kamper AL, Nielsen OJ. Effect of enalapril on haemoglobin and serum erythropoietin in patients with chronic nephropathy. Scand J Clin Lab Invest 1990;50:611–8.
[81] Fyhrquist F, Karppinen K, Honkanen T, Saijonmaa O, Rosenlöf K. High serum erythropoietin levels are normalized during treatment of congestive heart failure with enalapril. J Intern Med 1989;226:257–60.
[82] Gould AB, Goodman S, DeWolf R, Onesti G, Swartz C. Interrelation of the renin system and erythropoietin in rats. J Lab Clin Med 1980;96:523–34.
[83] Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion: role of red blood cell breakdown. Transfusion 2011;51:844–51.
[84] Raval JS, Waters JH, Seltsam A, Scharberg EA, Richter E, Daly AR, et al. The use of the mechanical fragility test in evaluating sublethal RBC injury during storage. Vox Sang 2010;99:325–31.
[85] Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T, et al. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008;358:1229–39.
[86] Lelubre C, Vincent JL. Relationship between red cell storage duration and outcomes in adults receiving red cell transfusions: a systematic review. Crit Care 2013;17:R66.
[87] Douay L. In vitro generation of red blood cells for transfusion: a model for regenerative medicine. Regen Med 2012;7:1–2.
[88] Mountford J, Olivier E, Turner M. Prospects for the manufacture of red cells for transfusion. Br J Haematol 2010;149:22–34.
[89] Yu B, Shahid M, Egorina EM, Sovershaev MA, Raher MJ, Lei C, et al. Endothelial dysfunction enhances vasoconstriction due to scavenging of nitric oxide by a hemoglobin-based oxygen carrier. Anesthesiology 2010;112:586–94.
[90] Natanson C, Kern SJ, Lurie P, Banks SM, Wolfe SM. Cell-free hemoglobin-based blood substitutes and risk of myocardial infarction and death: a meta-analysis. JAMA 2008;299:2304–12.

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