Cardiovascular sex differences influencing microvascular exchange

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The vital role of the cardiovascular (CV) system is maintenance of body functions via the matching of exchange to tissue metabolic demand. Sex-specific differences in the regulatory mechanisms of CV function and the metabolic requirements of men and women, respectively, have been identified and appreciated. This review focuses on sex differences of parameters influencing exchange at the point of union between blood and tissue, the microvasculature. Microvascular architecture, blood pressure (hydrostatic and oncotic), and vascular permeability, therefore, are discussed in the specific context of sex in health and disorders. It is notable that when sex differences exist, they are generally subtle but significant. In the aggregate, though, they can give rise to profoundly different phenotypes. The postulated mechanisms responsible for sex differences are attributed to genomics, epigenetics, and sex hormones. Depending on specific circumstances, the effect of the combined factors can range from insignificant to lethal. Identifying and understanding key signalling mechanisms bridging genomics/sex hormones and microvascular exchange properties within the scope of this review holds significant promise for sex-specific prevention and treatment of vascular barrier dysfunction.

Keywords

Sexual dimorphism • Microvessel permeability • Hydraulic conductivity • Reproductive hormones • Volume regulatory hormones

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1. Introduction

Fluid distribution between the vascular and tissue compartments in all living beings is a controlled variable. Volume homeostasis, as proposed by Starling in 1896, is achieved when the forces governing fluid movement, set up by transmural gradients in hydrostatic (ΔP) and oncotic ($\Delta\pi$) pressures, are in balance.¹ Unstated assumptions are that the mechanisms whereby fluid homeostasis is achieved, how they vary with disease, and how they respond to treatments are the same for males and females. This brief review explores the extent to which these assumptions are true, when they appear not to be true, why we care, and what research needs to be conducted.

Features of the cardiovascular (CV) system known to differ with sex and unlikely to impact exchange directly include the smaller-sized heart and major blood vessels of women relative to men of the same race and age. In most organs, the anatomical location of the major vessels is indistinguishable among the sexes. In males and females, the same formed elements circulate in blood, the vessels are composed of the same cell types, and the union of the elements performs the same functions. Healthy males and females are in homeostasis. Consequently, the conclusion that the functions and responses of these common elements do not differ with sex is not outlandish. In

fact, notable features exist that influence basal function in men and women differently, those that induce sex-linked responses, those that are indistinguishable between the sexes, and yet others that, while appearing to differ, are without known functional consequence.

The premise of this review is that while both sexes possess the same structural elements and are in fluid balance, how those elements function to achieve homeostasis with respect to the CV system differs (from subtly to profoundly). Manifestations of these differences have real consequences relative to how and when fluid and solute exchange are disrupted, how these dysfunctions can be prevented, and the strategies for ameliorating or treating these manifestations effectively. To students of physiology, both differences and similarities are of importance as they provide fundamental insight into the mechanisms regulating the integrated functions of the intact organism.

There are a paucity of studies on humans or animals comparing fluid or solute exchange in males and females, whether animal or human. In the absence of these data, most of what is presented comes from comparisons of studies on one sex or limited studies on both sexes of a feature likely to influence exchange and then incorporated into the 'big picture'. The goal is to raise awareness, if not research interest, in missing gaps to be filled.

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Fundamentally, it is not surprising that men and women can differ. At the very least, cells from males contain a Y chromosome, express the Sry gene, and males produce testosterone (T) in the testes (Table 1). Cells from females have two X chromosomes; females produce oestrogen (oestradiol, E2) and progesterone (pregn-4-ene-3,20-dione, P4) in the ovaries. Sex-specific differences and similarities in the CV system result from more than sex hormones, *per se*. It is essential to realize that every cell of the body has 'sex' due to the presence of either XX or XY chromosomes, that sex is manifest in the womb, and that beyond behaviour, biological differences exist in the CV system before puberty.

2. Determinants of whole body fluid balance

The place to initiate investigation of where sex may influence net fluid and/or solute movement is with the governing equations.

2.1 Volume flux, J_v

Fluid movement (J_v , volume flux, mL s^{-1}) is generally described by the modern form of the Starling expression:

$$J_v = L_p S(\Delta P - \sigma \Delta \pi) = \text{CFC}(\text{NFP}) \quad (1)$$

where L_p is hydraulic conductivity ($\text{cm s}^{-1} \text{mmHg}^{-1}$, a coefficient describing barrier leakiness to water), ΔP and $\Delta \pi$ are the hydrostatic and oncotic pressure difference between the vascular and tissue spaces, respectively, and σ , a unit-less coefficient, is the osmotic reflection coefficient.

In whole organs or intact tissues, CFC (or K_f), the capillary filtration coefficient represents the L_p of N exchange microvessels of surface area S . The net filtration pressure (NFP) represents the sum of the hydrostatic and effective osmotic forces acting across the exchange vessel walls. A slight imbalance in ΔP and $\sigma \Delta \pi$ exists under basal conditions such that NFP is positive, resulting in net fluid movement (capillary filtration) into the tissue. This is offset by removal of interstitial fluid via the lymphatics. Thus, in volume balance, J_v from all perfused vessels is equal to lymph flow and interstitial fluid volume is constant.

2.2 Solute flux, J_s

Exchange involves solute and fluid movement as $\Delta \pi$ is driven by the distribution of hydrophilic molecules across the barrier. As stated by Fick's first law of diffusion, solutes move across the vascular barrier when a concentration gradient (ΔC , mmole mL^{-1}) exists:

$$J_s = P_d S(\Delta C) \quad (2)$$

Here J_s is solute flux (mmole s^{-1}) and P_d is the diffusive permeability coefficient (cm s^{-1}):

$$P_d = \frac{D_f}{\Delta x} \quad (3)$$

where D_f is the solute's free diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and Δx is barrier thickness (cm).

2.3 When fluid and solute interact, $\sigma \neq 1$

In most organs, the exchange vessels are classified as continuous and σ to proteins is near 1. The consequence is that not only can solutes

Table 1 Human reproductive sex hormone plasma levels (ranges) in males (XY) and females (XX)⁹

Hormone	Sex	Tanner stage										Adult	Phase menstrual cycle			
		1–30 day	1–7 month	1–3 years	4–9 years	10–11 years	12–13 years	I	II	III	IV	V	Follicular	Ovulatory	Luteal	Post-menopause
T (ng/mL)	M	75–400	Rises to 60–400 (2–3 month) then falls to 3–10			2–57	7–747	<20	2–149	7–762	164–854	94–783	350–1030 (20–50 years)			
	F	20–64	Fall to <10 by 7 month			2–42	6–64	<17	4–39	10–60	8–63	10–60	11–59			7–40
Sex hormone binding globulin (nmol/L)	M	13–85	70–250	50–180	28–190	13–160		26–286	22–169	13–104	11–60	11–71				
	F	14–60	60–215	60–190	33–170	11–155		30–173	16–127	12–98	14–151	23–165				
E2 (pg/mL)	M	<5–20											7.6–42.6			
	F	6–27.0											12.5–166	86–498	44–211	<5–54.7
P4 (ng/mL)	M												0.2–1.5	0.8–3.0	1.7–27	<0.2–0.8
	F			0.2–1.5	0.5–1.6			0.7–3.1	1.1–6.9	1.8–6.2	1.8–4.8	1.4–6.8				
FSH (mIU/mL)	M			1.2–5.7	0.8–3.0			0.5–5.1	2.4–8.7	3.8–8.1	1.1–9.6	2.0–7.6				
	F								0.2–2.8	1.2–3.9	0.9–4.4	1.8–5.3	3.5–12.5	4.7–21.5	1.7–7.7	25.8–134.8
LH (mIU/mL)	M	<0.5							0.1–4.1	0.2–9.2	0.7–8.6	0.5–7.3				
	F	<0.5											2.4–12.6	14–95.6	1–11.4	7.7–58.5

diffuse between the vascular and interstitial spaces, but changes in ΔP can lead to faster than expected movement when solutes with low D_f (proteins) become entrained with J_v . The importance of 'solvent drag' or convection, expressed by a hairy set of Eqs. (4–7), is that fluid movement influences macromolecule distribution and vice versa.

For completeness, J_s under these circumstances is:

$$\frac{J_s}{S\Delta C} = P_d \left(\frac{Pé}{e^{Pé} - 1} + \frac{J_v}{S(1 - \sigma)} \right) \quad (4),$$

where $Pé$ is the Péclet number:

$$Pé = \frac{[(J_v/S)(1 - \sigma)]}{P_d} \quad (5)$$

$Pé$ provides a determination of how much of J_s , at a given pressure, results from fluid movement relative to diffusive mechanisms. At $Pé < 0.2$ diffusive mechanisms predominate and Fick's first law [Eq. (2)] applies. When $Pé > 5$ (i.e. volume flux $\gg$ diffusive flux) the solute moves primarily by convection; Eq. (4) reduces to:

$$\frac{J_s}{S\Delta C} = L_p(1 - \sigma)(\Delta P - \sigma\Delta\pi) \quad (6)$$

For J_v , if microvessel pressures keep changing thus providing insufficient time for moving solute to alter the transvascular gradients the modified Starling equation [Eq. (1)] holds. Otherwise, if solute and fluid flux together the gradients will change and a steady-state condition can be achieved wherein the transvascular osmotic gradient influences steady-state filtration:

$$J_v = L_p S(\Delta P - (\sigma^2 \pi_p [e^{-Pé}](1 - \sigma e^{-Pé})^{-1})) \quad (7)$$

The steady state filtration relationship is non-linear until high pressures are reached ($Pé > 5$) and the relationship of J_v/S with P parallels Eq. (1) with a limiting slope $= L_p$. The other notable feature of this relationship is that capillaries and venules exposed to fairly constant hydrostatic pressures have from negligible to appreciable constant filtration and cannot sustain fluid re-absorption from the interstitium into the vasculature. Of note, collecting lymphatics contract spontaneously under physiological conditions and could support both transient re-absorption and filtration² by failing to achieve steady-state conditions.

The equations illustrate that multiple parameters influence exchange substantially. The extent to which they differ by sex in health and/or disease is not well defined.

2.4 The forces: hydrostatic, P , and oncotic, π , pressure

While text books refer to 'normal' arterial blood pressure (BP) as 120/80 mmHg, the BP of healthy adult human females is lower than males. Data from the second (US) National Health and Nutritional Examination Survey ($n = 22\,732$)³ show (Table 2) male/female differences in mean systolic blood pressure (SBP) for all races by 12–17 years, that are sustained until 55–64 years, and reversed in the next decade. No sex differences in diastolic blood pressure (DBP) are observed until 18–24 years (Table 2); this difference is retained until 65–74 years. Mean DBP peaks at 45–54 years in males and 55–64 in females.³ The net result is a pulse pressure (SBP–DBP), greater in males, that increases by ~ 0.4 mmHg/year and ~ 0.6 mmHg/year in males and females, respectively, between the ages of 35–59.

2.4.1 Capillary pressure, P_c

The inference from these data, given the anatomical location of exchange vessels downstream of resistance vessels, is that up to age 65, capillary pressure, P_c , is lower in women than men. Direct measurements of P_c^4 confirm lower values in pre-menopausal women than men (Table 2). The caveat is that arterial BP, SBP, DBP, or mean (MAP), *do not* relate directly with P_c .⁴ This lack of direct correlation between SBP and P_c confirms the demonstration⁵ of mechanisms protecting the exchange vasculature from changes in arterial BP.

2.4.2 Venous pressure, central venous pressure, and venous compliance

What correlates with P_c is venous pressure (VP).⁶ P_c increases to approximately match increases in VP up to 20 mmHg.⁶ A search of the current literature failed to reveal direct measurements of VP or central venous pressure (CVP) in healthy male and female animals or humans. Most human data are from surgical or medical intensive care patients and are mixed-sex populations—usually biased towards males. What has been studied, and germane to this discussion, is venous compliance. Given that the veins are capacitance vessels and distensible, changes in CVP result from changes in venous volume and/or venous smooth muscle tone. Consequently, vascular volume expansion or a reduction in venous compliance will increase VP that can elevate exchange vessel pressures. Not only does venous compliance differ between males and females but hormones and disease states can regulate venous compliance differentially by sex (Table 3). Venous capacitance is lower in age-matched females than males. After correction for fluid filtration, venous compliance at low transmural pressures is lower in women (e.g. for a given pressure, the veins of women can accommodate a greater change in volume).⁷ With respect to pressure transmission into the exchange vasculature, for a given increase in venous volume, hydrostatic pressures would be anticipated to rise more quickly in men than women.

2.4.3 Oncotic pressures and plasma protein content

The oncotic pressure (π) is a direct function of protein concentration ($\pi = \Phi nC$, where Φ is a correction coefficient, n is valence number, and C is molar concentration) in the vascular and tissue compartments, respectively. Under physiological conditions, π is presumed constant. The major determinant of π in blood is plasma protein concentration, with albumin playing a dominant role. According to the NHANES II data set,⁸ plasma protein levels averaged for all ages (6–74 years) are sex-independent (Table 2). Inspection of Figure 1A reveals a complex temporal pattern. Before age 14, serum protein levels are sex-independent. The greatest sex difference, 0.3 g/dL, occurs between 18 and 24 years; after which albumin levels in both sexes decline until they again are sex-independent for 55–74 years old. The influence of plasma protein content on π is illustrated in Figure 1B. As comprehensive a data set as NHANES does not seem to exist with measures of total serum protein. If albumin is assumed to be a constant proportion of total protein (e.g. 69% of 7 g/dL total protein plasma contains 4.8 g/dL albumin⁹), mean π is 25.7 mmHg for males and 24.9 mmHg for females for ages 6–74.

2.4.4 Net filtration pressure

The consequence of sex differences in P_c and π is that the 2.3 mmHg M/F difference in P_c observed by Shore *et al.*⁴ is offset by at least 1 mmHg M/F difference in π . Noteworthy is that when exchange P_c becomes sex-independent (>65 years), π also is sex-independent.

Table 2 Measurement of parameters influencing or indicative of vascular exchange

Feature	Male	Female	Note	Units	Species	Reference
Systolic blood pressure	114.8 ± 0.8 137.3 ± 0.9 142.3 ± 0.9	110.8 ± 0.8 137.4 ± 1 145.8 ± 1	M = F M > F M = F M < F	<11 years 12–17 years 55–64 years 65–74 years	mmHg	Human 3
Diastolic blood pressure	75.6 ± 0.4	70.2 ± 0.5	M = F M > F M = F	<17 years 18–24 years 65–74 years		
Capillary pressure	18.2 ± 2.3	15.9 ± 3.0	M > F	pre-menopause	mmHg	Human 4
Plasma protein	4.1–6.3 4.7–6.7 5.5–7.0 5.7–8.0 6.0–8.0	4.2–6.2 4.4–6.6 5.7–8.0 5.7–8.0		Birth: 31 days 1–6 months 6 months to 1 year 1–18 years 'Adult'	g/dL	Human 9
Plasma albumin	3.4–4.8 4.8 ± 0.01 Figure 1	4.7 ± 0.01	M = F M = F M > F M = F	'Adult' 6–74 years 6–14 years 18–24 years 55–74 years	g/dL g/dL	Human Human 9 8
Oncotic pressure	25.7	24.9	M > F	Calculated, 6–74 years	mmHg	Human 8
Blood volume	6.86 ± 0.53	7.84 ± 0.70	M < F	84–91 days	mL/100 g	Rat adult 32
Haematocrit	39.5 ± 3	38 ± 3	M > F		%	
Plasma volume	4.12 ± 0.32	4.86 ± 0.54	M < F		mL/100 g	
Skeletal muscle surface density	25.2 ± 2.2 15.5 ± 1.2 10.4 ± 1.7 8.5 ± 1.8	21.4 ± 2.4 17.5 ± 2.1 16.4 ± 4.2 10.9 ± 1.5	M = F M = F M < F M = F	Type I fibres <60 years Type I fibres ≥60 years Type II fibres <60 years Type II fibres ≥60 years	mm ² /mm ³	Human 76
Capillary length density	1.009 ± 104 1.240 ± 138 799 ± 75 984 ± 74	1.231 ± 189 1.237 ± 134 1.251 ± 239 1.179 ± 174	M = F M = F M < F M < F	Type I fibres <60 years Type I fibres ≥60 years Type II fibres <60 years Type II fibres ≥60 years	mm/mm ³	
[BNP]	8.0 ± 12.8	13.9 ± 18.9	M < F	M 54 ± 9; F 55 ± 9 years	pg/mL	Human 49
[ANP]	45 (1–236)	55 (11–317)	M < F	M 57.8 ± 0.2 years; F 57.7 ± 0.2 years	pg/mL	Human 77
Prorenin	221	155	M > F		mU/L	
Renin	16.1	12.3	M > F			
Aldosterone	111 (9–585)	109 (4–2954)	M = F		pg/mL	
ACE	25.4 ± 0.4	25.4 ± 0.4	M = F		U/L	
[BNP]	381.9 ± 649.6	252.0 ± 266.8	M = F	21–89 years	pg/mL	CHF human 51
[ANP]	141.2 ± 140.7	114.9 ± 101.3	M = F			

Table 3 Exchange parameters depend on microvessel environment

Parameter	Stimulus/model	Environment/phenotype	Note	Species/model	Reference
Pressure		Hypertension	M	Rat	12
			F		
	Low dose ANGII		M	Rat brain	46,47
	Low dose Aldo		M		
	Maternal protein restriction	Hypertension	M $\neq$ F	Rat	36
			M $\neq$ F		37
Venous capacitance	Lower limb	Normal	M = F	Human	7
			M > F		
Venous compliance			M > F		
Exchange surface area	Hypertension	Rarefaction	M	Rat	12
			F		
	Exercise training	Performance	M	Human	14
			F		
	Congestive heart failure	VO ₂ max, capillary density		Human <i>vastus lateralis</i>	15
	Ageing	Interstitial capillary length density (LV cap it, mus)	M = F		
	Ageing	Length of capillaries contacting fibres	M = F		
Vascular smooth muscle tone	Control	<i>In vivo</i> and isolated		Multiple	17,20
	Control	Isolated vessels	M $\neq$ F		64
	Fat restricted diet	EC-dependent dilatation	M $\neq$ F	Brachial artery juvenile human	35
	Maternal protein restricted diet		M $\neq$ F	Rat	37
Vascular Smooth Muscle growth	Control (WKY)	Culture	M > F	Rat arteriole VSM	16
	Hypertension (SHR)		M > F		
CFC	Control	<i>In vivo</i> lower Limb	M < F	Human	7
CFC	Munich Wistar/Ztm	Kidney	M > F	Rat	33
Protein excretion			M > F		
Albumin clearance	Control	<i>In vivo</i> mesentery	M = F	Rat	27
	Insulin		M $\neq$ F		
Protein clearance	Control		M = F		
	Insulin		M > F		
Peritoneal transport rate	Control	<i>In vivo</i> peritoneum	M = F	Mouse	69
Na ⁺ sieving			M > F		
AQP-1 protein			M > F		

TNF- α	Control	M = F	Adult	53
IL-6		M = F		
TNF- α	High fat meal	M < F	Excursion M < F	
IL-6		M < F		
C-reactive protein		M = F		
TNF- α	Sleep deprivation	M < F	Time of increase shorter in M	54
IL-6		M < F		
TNF- α	Coronary artery disease	M > F	Monocyte PPAR- γ levels 50-X normal	56
PPAR- γ		M > F		
Phosphodiesterases	(Culture) SKM microvessel EC	M = F		66
	PDE 2A, PDE 4D	M < F	Adult SD rat	
	PDE 3B	M < F		
	PDE1A	M > F		
SYR		M $\neq$ F	Retained by EC from M	
NC, no change.				

Because albumin is smaller than most other plasma proteins, its concentration is comparatively higher in the interstitial fluid, owing to the sieving characteristics of the microvascular barrier. Thus, extravasated albumin exerts a greater impact on π^{tissue} than other plasma proteins. Additionally, albumin functions as an antioxidant and facilitates transport of a wide variety of substances (including free fatty acids, ions, calcium, phospholipids, bilirubin, enzymes, hormones, drugs, and metabolites) owing to the presence of multiple surface-charged groups and ionic/hydrophobic binding sites.¹⁰ Thus sex differences in P_s^{albumin} (Table 4) likely influences more than π in the vascular space. It is notable that critical care physicians are discussing limiting use of plasma products from women because of adverse responses [development of acute lung injury (ALI)] in men. The pathogenesis has been related to infusion of donor (female) antibodies into the recipient (male) with subsequent development of capillary leak and ALI. Though the use of male-only plasma has reduced the incidence of antibody-mediated cases and deaths, ALI still occurs and appears to involve neutrophil-mediated endothelial cell (EC) cytotoxicity.¹¹

2.5 Vascular architecture determining exchange surface area, S, in health and disease

Surface area in addition to the ΔP and $\Delta \pi$ will limit water and solute exchange. As an architectural parameter, S is a function of microvessel number, length, and diameter. If volume homeostasis is presumed to be the same between sexes, then the parameters determining S should likewise be sex-independent.

2.5.1 Anatomical structure

This appears to be true in the absence of ‘stress’. Vascular architecture can differ significantly in response to exercise training,¹² heart failure,¹² hypertension (HTN),¹² and diabetes,¹³ to mention a few cases (Table 3). In a rat model of HTN, MAP in males rises at an earlier age and is accompanied by significant loss of skeletal muscle (SKM) microvessels (rarefaction); in females, MAP rises less and is unassociated with rarefaction.¹² In humans, exercise training improves submaximal performance in men, not women, although capillary density is increased in both sexes.¹⁴ When peak oxygen consumption and vastus lateralis capillary density were examined in chronic heart failure patients, they varied inversely for men and were unrelated for women.¹⁵ A well-delineated sexually dimorphic component influencing microvessel structure is the growth of arteriolar vascular smooth muscle (VSM) cells from normotensive and HTN rats,¹⁶ which in turn would be expected to influence vascular reactivity.

2.5.2 Vascular tone and functional structure

A more important means of varying S in the intact circulation is through mechanisms usually studied with respect to blood flow regulation and associated with arteriolar vascular tone. As outlined above, arterial pressures, *per se*, do not correlate directly with P_c . Arteriolar vasodilatation, though, will reduce pre-capillary resistance and increase vascular exchange via perfusion of additional capillaries (functional vs. anatomical change in S).

Several studies, demonstrating sex differences in vascular tone,^{17–20} provide evidence for involvement of the sex hormones E2 and T. In general, most suggest that T is a relative vasoconstrictor and E2 is a relative vasodilator via activation of endothelial nitric oxide synthase (eNOS) and cyclooxygenase. These conclusions are not absolutes as there are examples where the reproductive hormones, *per se*, do not

Figure 1 Plasma serum albumin and effective net oncotic pressure vary with sex and age. Data in (A) are mean levels of serum albumin (circles) over the age ranges (given by the horizontal lines) of men (blue) and women (red) between 3 and 74 years.⁸ (B) provides calculated plasma oncotic pressure (π , mmHg) using the data in (A) as delineated in the text.

appear to regulate tone.^{20–22} Obviously, this is an area for additional study with a need for inclusion of the roles of P4, metabolites of T, epigenetics, and *in utero* sex genome programming.

2.6 Exchange and the permeability ‘constants’

2.6.1 Assumptions in the assessment of exchange

Widely held perceptions of exchange are that (i) arterioles do not participate in exchange; (ii) capillaries, given their large number, are the primary site for volume flux; and (iii) venules, given their ‘leaky’ walls and low pressures, are the primary site for protein flux. Consequently, venule leak index assesses solute flux to tissue, measures of CFC reflect exclusively the behaviour of capillaries, and capillary L_p assesses tissue hydration.

According to Eq. (1), two other conditions that could result in net vascular to tissue space fluid movement are if CFC increases and/or σ decreases. In a study of lower limb venous compliance, CFC was determined as a sex difference could compromise the measures. Venous compliance was found to be lower and CFC higher in the females than the males.⁷ CFC can increase acutely if L_p increases or more vessels are perfused (increasing S). Likewise, compounds that elevate P_s under conditions of constant perfusion will increase exchange as will those that change vascular tone without changing P_s . A pervasive assumption, arising from the notion that P_s (or L_p) is a constant and only changes in the pathophysiological state of inflammation, is that agents used to vasodilate an organ during measures of CFC or PS to maximize S do not, themselves, alter L_p or P_s .

2.6.2 Changes vs. basal P_s and L_p

In the heart^{21,22} and SKM,^{23–25} not only does the vasodilator adenosine (ADO) alter P_s , but sex modifies the direction and magnitude of changes in P_s (Table 4). The sex dependence changes in adaptation to

endurance exercise training.²⁶ Sexual dimorphism in P_s responses to ADO in juvenile rat SKM reveals a genomic component; in adults, the patterns of change reveal involvement of the sex hormones E2, P4, and T.^{24,25} Juvenile sexual dimorphism differs from adults and is displayed for albumin clearances from rat mesentery on exposure to insulin.²⁷ Similarly, data from two laboratories using identical methods on adult rats of a single sex demonstrate that platelet activating factor (PAF) elicits a greater change in L_p for males^{28,29} relative to females^{30,31} (Table 4).

Few comparisons of basal permeability (P_s or L_p) exist for simultaneous studies of both sexes. To date, sex-linked differences in P_s (Table 4) have been seen only for adult rat SKM venules²⁵ and coronary venules of endurance-exercise-trained pigs.²⁶ Attempting to compare separate studies of males and of females is frustrated by the small sample sizes and lack of statistical power.

2.6.3 The status of exchange from measures of blood volume, plasma volume, and volume handling

Absolute plasma volume (PV) and blood volume (BV) of conscious male rats exceed that of age-matched females (Table 3). When normalized to body weight, female volumes are greater than males. Once sex differences in haematocrit are accounted for, PV and BV (per 100 g) are 18 and 14% higher, respectively, in females.³²

Renal function, which obviously influences fluid balance, displays remarkable sex differences in the Munich–Wistar rat (MWF/Ztm), a strain studied extensively for the higher number of superficial glomeruli. Protein excretion rates in MWF/Ztm males at 7 weeks are double than that of females (note: 7 week males are juveniles; females mature), by 21 weeks the difference is ~10-fold.³³ Insulin clearance and single nephron glomerular filtration rate are elevated in males in the face of comparable mean glomerular capillary pressure and transcapillary pressure gradient. Despite sex-independence in

Table 4 Single vessel permeability and responses

Parameter	Stimulus	Age		Vessel	Values	Organ	Species	Reference
Permeability to albumin (cm s^{-1})	Control	Adult	M = F	Arteriole	M $8 \pm 1 \times 10^{-7}$; F 7.5×10^{-7}	SKM	Rat	24,25
		Juvenile	M = F		JM $7 \pm 1 \times 10^{-7}$; JF $9.9 \pm 0.4 \times 10^{-7}$			
	Control	Adult	M > F	Venule	M $26 \pm 2.5 \times 10^{-7}$; F $12 \pm 1.5 \times 10^{-7}$	SKM	Rat	24
		Juvenile	M = F		JM $8 \pm 2 \times 10^{-7}$; JF $10.7 \pm 2 \times 10^{-7}$			
	Control/Sedentary	Adult	M = F	Arteriole	M $4.5 \pm 1 \times 10^{-7}$; F $6.5 \pm 1 \times 10^{-7}$	Heart	Pig	22,23
			M = F	Venule	$22.2 \pm 3.5 \times 10^{-7}$			
	Control/Sedentary + Adenosine	Adult	M = F	Arteriole	M $3.8 \pm .5 \times 10^{-7}$; F $5.7 \pm .7 \times 10^{-7}$			
			M	Venule	$17.6 \pm 0.7 \times 10^{-7}$			
			F		$29.4 \pm 4.5 \times 10^{-7}$			
	Exercise Training	Adult	M = F	Arteriole	M $5 \pm 1 \times 10^{-7}$; F $4.6 \pm 0.4 \times 10^{-7}$	Heart	Pig	26
			M < F	Venule	M $8.1 \pm 31 \times 10^{-7}$; F $21.6 \pm 61 \times 10^{-7}$			
Hydraulic conductivity ($\text{cm s}^{-1} \text{ cm H}_2\text{O}^{-1}$)	Control	Adult	M	Venule	M $0.62 \pm 0.05 \times 10^{-7}$	Mesentery	Rat	28
					$1.6 \pm 0.2 \times 10^{-7}$			
					$1.6 \pm 0.2 \times 10^{-7}$			
					$1.3 \pm 0.2 \times 10^{-7}$			
					$16.9 \pm 0.21 \times 10^{-7}$			
	10 nM PAF peak response	Adult	M	Venule	$24.3 \pm 1.7 \times 10^{-7}$	Mesentery	Rat	28
					$7.5 \pm 0.9 \times 10^{-7}$			
					$7.4 \pm 1.1 \times 10^{-7}$			

number or size of glomeruli, CFC is higher in males than females³³ (Table 3). Only in male rats does chronic volume overload (via infra-renal aorto-caval fistula) result in 10-fold greater mortality, greater increases in left and right ventricular mass, increased lung weight and development of heart failure.³⁴ This illustrates a case where an identical stimulus, volume overload, elicits a sex-specific response.

3. Mechanisms responsible for the sexual dimorphisms

3.1 Genomics vs. sex hormones: juveniles vs. adults

Studies of sexual dimorphism of vascular function focus mainly on adults. There has been recent evidence for differences existing in pre-pubertal children and starting *in utero* via foetal programming. For example, EC-dependent and EC-independent responses examined in children (11 years) of both sexes a decade after they had been on diets restricting saturated fats were compared with age-matched children. Boys of the diet-intervention group had increased EC-dependent dilatation responses relative to their age-matched controls (Table 3); diet was without effect in the girls.³⁵ These differences in the control of vascular tone have potential impact on the regulation of exchange via control of S and pressure as discussed previously.

3.2 Epigenetics

Another mechanism that can induce sex differences is epigenetics—those gene activities not involving alterations to genetic code, *per se*, but modifying phenotype and able to influence passage of genetic information to at least one successive generation. These patterns of gene expression are governed by cellular material, the epigenome, in close proximity to (just outside—hence the prefix *epi*) the genome. These epigenetic ‘marks’, via the addition of a methyl group to the epigenome (DNA methylation), provide on/off and amplitude of gene expression. Factors shown to influence these epigenetic marks include environmental toxins, diet, and stress.

Nutritional status and stresses during pregnancy can also alter CV responses and disease susceptibility of the mother and offspring. Moderate protein restriction during gestation in rats results in remarkable increases in BP and decreases in nephron number in male, not female, offspring³⁶ (Table 3). Rodford *et al.*³⁷ demonstrated that same prenatal intervention induces sex differences in EC function. The most striking are reduced resistance artery responsiveness to ACH, increased arterial eNOS mRNA expression, and decreased HO-1 mRNA expression in the liver of male, not female, offspring.³⁷ Why female offspring should be resistant to maternal nutritional status during pregnancy is the focus of current study. To date, the mechanisms are not known.

3.3 Sex hormones

The role of the reproductive sex hormones is not simple. On their own, sex hormones can alter VSM tone (Table 3) and multiple functions involved in exchange appear to change with the female reproductive cycle.³⁸ E2, particularly through non-genomic actions, influences EC NO production.¹⁷ With respect to basal SKM and coronary and mesenteric microvessel P_s^{albumin} , the reproductive cycle *per se* appears to be without influence.^{21–24} This does not mean that some of the responses could not be secondary to sex hormones.

3.3.1 Androgens

Androgens, including testosterone, T, have been shown to up-regulate volume regulatory hormone expression, including arginine vasopressin receptor mRNA only in male hamster brain.³⁹ Relative to males, macrophages from female donors express lower levels of androgen receptor.⁴⁰ Androgens up-regulate VSM thromboxane receptors⁴¹ and macrophage foam cell formation exclusively in males.⁴² Male macrophages express >4-fold higher androgen receptor mRNA and up-regulate >25 genes mediating lipoprotein processing, cells surface adhesion, extracellular signalling, coagulation and fibrinolysis, and transport proteins. The macrophages illustrate sexual dimorphism of atherosclerotic potential, a process associated with altered exchange barrier function, exclusive to healthy males compared with age-matched females.⁴²

3.3.2 Female menstrual cycle: oestrogen (E2) and progesterone (P4)

Changes in PV of females do not correlate uniformly with the menstrual cycle phase (Table 5). A recent study of healthy young males and females before and after exercise was designed to measure haemodynamics and sex hormones with sufficient statistical power to compare between sexes.⁴³ It was presumed that E2 and P4 would influence arterial BP and extracellular fluid volume regulation by increasing PV when E2 rose and by contracting PV when P4 rose during the menstrual cycle. Consistent with previous reports,² SBP of men > women at all phases of the cycle; DBP was sex-independent. Cardiac output was higher in men than women in early follicular (EF) and ovulatory (OV) phases, but not midluteal (ML) phase. Stroke volume in men was higher than women at all phases. No M/F differences in femoral blood flow or femoral, thigh, or forearm cutaneous vascular conductance existed. PV and BV were higher in the OV compared with EF or ML. Thus while endogenous E2 and P4 levels changed with menstrual cycle and differed with sex and PV fluctuated, the changes were not linked to plasma hormone levels.⁴³

No direct measures of P_s or J_v have been made as a function of the menstrual cycle. The dose-response relationship of E2 and mesenteric microvessel L_p reveals a multi-phasic relationship with increases in L_p when E2 was at levels grossly comparable to ovulation and pregnancy.⁴⁴ Limited studies exist of volume status and fluid distribution in normally cycling females. In one interstitial hydrostatic pressure, P_i , and π were measured using the ‘wick-in-needle’ and the ‘wick’

Table 5 Menstrual cycle and fluid balance in age-matched, young adults

Parameter	Males vs. females in early follicular (EF), ovulatory (OV), and mid-luteal (ML) phases	Reference
Systolic blood pressure	$M > [F_{EF} = F_{OV} = F_{ML}]$	3,43
Stroke volume	$M > [F_{EF} = F_{OV} = F_{ML}]$	43
Cardiac output	$[M = F_{ML}] > [F_{EF} = F_{OV}]$	
Plasma volume	$M < [F_{EF} = F_{ML}] < F_{OV}$	
Blood volume	$M < [F_{EF} = F_{ML}] < F_{OV}$	
Vascular conductance (skin, thigh, femoral)	$M = F_{EF} = F_{OV} = F_{ML}$	

methods, respectively.⁴⁵ From the follicular to luteal phase of the menstrual cycle a slight weight gain was accompanied by significant drops in plasma and interstitial π (~ 2 mmHg) in the absence of changes in P_i , albumin, haemoglobin, or haematocrit.⁴⁵

3.4 Exchange barrier responses to non-reproductive hormones and non-hormonal mediators

In addition to E2 and P4, hormones influencing volume exchange include the natriuretic peptides (ANP, BNP, and CNP), angiotensin II (AngII), and Aldo. These hormones exert effects in the kidney, resistance and exchange microvasculature, and brain to affect fluid exchange. The brain, an organ mentioned only superficially for the sake of brevity, has been studied most extensively with respect to sexual dimorphism and where foetal programming and pre-pubertal sex differences were first identified.

3.4.1 Renin–angiotensin–aldosterone system

It is notable that E2 and its receptor ER mediate protection from HTN induced by effector components of the renin–angiotensin–aldosterone system. Low-dose ANG II or ALDO brain infusion result in markedly greater HTN in male compared with female rodents^{46,47} (Table 3) and the response is reversed by E2.⁴⁷

3.4.2 Natriuretic peptides

Levels of the natriuretic peptides (NP) vary with sex in health and disease.⁴⁸ The NP will induce volume redistribution via renal actions of diuresis and natriuresis. In resistance vessels, NP can dilate VSM to increase functional exchange surface area and elevate pressure.⁴⁸ NP also acts directly in selected organs to increase L_p and P_s resulting in a fluid shift from the vascular to the interstitial space to reduce PV.⁴⁸ Plasma levels of N-terminal pro-A-type NP (Nt-ANP), N-terminal pro-B-type NP (Nt-BNP), and BNP⁴⁹ (Table 2) increase with age and are higher in women than men. Higher NP levels are associated with lower free-T levels⁵⁰ and there is significant extracardiac transcription of NP in the ovaries and uterus, even after menopause. Women also have lower plasma levels of renin, known to be inversely associated with NP levels.⁵⁰ A diagnostic marker for congestive heart failure is a rise in plasma levels of NP; in this disease state sex differences in NP are lost⁵¹ (Table 2). It is not known whether the ability of the NP to alter permeability²⁶ differs with sex in health or disease, or with menstrual cycle, or during pregnancy when volume expansion occurs.

3.4.3 Non-hormonal markers

A study of non-hormonal plasma markers associated with CV disease in >2000 individuals demonstrated higher levels of 29 and lower levels of 6 markers in females.⁵² One set of markers (acute phase reactants including C-reactive protein, ICAM, RAGE) related to inflammation would influence exchange directly. The others, relating more indirectly, influence lipid profile (HDL-component ApoA-I, VLDL-component ApoC-III, Lp(a), ApoE) and metabolism with elevated adipokines associated with both truncal obesity and glucose intolerance (leptin and resistin) offset by the insulin-sensitizing and cardio-protective adiponectin. There are suggestions that adipokines mediate elevations in microvessels permeability in the vicinity of fat cells facilitating lipid removal and storage.

3.4.4 Inflammatory mediators

In healthy subjects, no sex differences exist for TNF- α and Interleukin-6 (IL-6), two inflammatory mediators known to alter EC barrier function. In response to stimuli, sex differences appear (Table 3). Postprandially, a high-fat meal depresses TNF- α and elevates IL-6. The magnitude of these transients being greater in women than men with comparable C-reactive protein levels.⁵³ In response to sleep deprivation, females have a greater immune response with higher levels of TNF- α and IL-6 that remain elevated 12–18 h.⁵⁴ Finally, endotoxemic challenges result in higher TNF- α levels and greater loss of cardiac function in male than female mice that were abrogated by E2 treatment.⁵⁵ The cell signalling components correlating with these changes were higher Rac1 and NADPH oxidase activity, ERK1/2, and p38 MAPK phosphorylation, and toll-like receptor-4 (TLR-4) expression up-regulation.⁵⁵

A common approach in the early treatment of diabetes mellitus is the use of peroxisome proliferator activated receptor gamma (PPAR-gamma) antagonists. In coronary artery disease (CAD) patients, monocyte PPAR-gamma gene expression is >50-fold higher than healthy donors that released spontaneously higher amounts of pro-inflammatory cytokines. PPAR-gamma protein expressed from monocytes of CAD females greater than males and have the lowest basal TNF- α release inferring sex differences in response to PPAR-gamma agonist therapy.⁵⁶ Of interest, a side effect of thiazolidinedione therapy is oedema formation. Recent studies demonstrate that VEGF levels rise and remain elevated following treatment with at least two thiazolidinedione compounds.⁵⁷ Taken together, these studies point to possible sex-linked differences in response to PPAR-gamma agonists that have not been explored.

3.5 Foetal programming

Although a role for sex hormones mediating CV structure and function has been documented, little is known of the association between vascular sexual dimorphism and genes encoded on sex chromosomes. Increasing evidence, alluded to above, demonstrates sex differences in vascular function including BP, flow, and EC-dependent vascular reactivity that are either programmed *in utero* or observed in pre-pubertal children.

In humans, T production by the foetal testis is detectable at 9 weeks, peaks between 14 and 17 weeks and then falls sharply, so that by late pregnancy serum T concentrations of male and female fetuses overlap.⁵⁸ Ovarian endocrine activity begins shortly after birth; E2 levels are significantly higher in pre-pubertal girls than boys⁹ (Table 1). Given the low and non-cycling reproductive hormone levels of children in the face of multiple sex-specific differences, the importance of genotype-sex interactions requires additional research attention.

3.6 Sex chromosomes

3.6.1 Y chromosome

The Y chromosome, unique to genetic males, imparts male-specific phenotypes. In a set of elegant studies using consomic rats, the Y chromosome from normotensive WKY was replaced with that from spontaneous hypertensive rats (SHR) and vice versa.⁵⁹ The SHR background rats with the WKY Y chromosome had reduced systolic BP. WKY rats with the SHR Y chromosome had BP higher than WKY controls. Among six divergent loci of SRY, a gene encoding a transcription factor responsible for testes development, SRY1, was identified to be responsible for the increased systemic BP in SHR rats.⁶⁰

Studies using these consomic techniques to focus on exchange could provide insight into the mechanisms regulating exchange in juveniles and adults of both sexes and in several disease states.

3.6.2 X chromosome and X chromosome inactivation

Genes on one X chromosome are silenced randomly through X chromosome inactivation. Some genes, though, 'escape' X chromosome inactivation and are expressed by both copies of the chromosome, the process referred to as 'differential X chromosome-linked gene dosage'. This process contributes to sexual-dimorphism and contrasts with the dogma that dosage compensation for X-linked gene ensures equal X chromosome expression between males (XY) and females (XX) by transcriptional silencing of one X chromosome in XX female embryo.⁶¹ Females with Turner's syndrome, possessing 45 monosomic X-chromosomes, have a variety of developmental and functional disorders including abnormal growth, metabolic dysfunction, high BP, vascular architecture anomalies, and foetal lymphoedema.^{62,63} This genetic abnormality illustrates the importance of genes escaping X-inactivation on the second X chromosome for females to develop and function normally and to contribute to sexually dimorphism in health and disease.

3.7 Sexual dimorphism at the cellular level

Given that each cell retains sex and that sex differences in cell signalling exist is interesting. Selected examples demonstrate the levels at which sexual dimorphism exists.

3.7.1 Ion handling

Divalent ion handling has been shown to differ fundamentally between males and females. In both VSM and EC of intact vessels, Mg^{2+} plays an important role in Ca^{2+} homeostasis. In females, regulation of VSM contractile function (posited to involve E2 modulation of EC, probably via Na^+-Ca^{2+} exchange) involves Mg^{2+} -regulated internal Na^+ -dependent Ca^{2+} entry.⁶³ This study posited that this mechanism could account for protection of females, despite Mg^{2+} deficiency, from ischaemic heart and cerebrovascular disease until menopause.⁶⁴

3.7.2 Cell signalling mechanisms

Phosphodiesterases (PDE) hydrolyze second messenger cyclic nucleotides, cAMP and cGMP, shown to play critical and antagonistic roles in the regulation of the microvascular barrier function. The vascular barrier is tightened by cAMP accumulation while activation of cGMP-dependent pathways increase vascular leak. Of the 11 identified PDE superfamilies⁶⁵ PDE1–5 are expressed in primary cultured EC from microvessels isolated from SKM of male and female rats.⁶⁶ Expression levels of PDE1A (male > female) and PDE3B (male < female) mRNA are sex-dependent, while PDE2A, PDE4D, and PDE5A are sex-independent (Table 4). These findings suggest that cells retain intrinsic and inheritable sexually dimorphic genes in culture. Notably, male EC expressed the SRY gene even in culture. PDE2A, 3B, and 4D hydrolyze cAMP. Application of the PDE3 inhibitor, cilostazol, to microvascular EC monolayers results in reduced $P_s^{albumin}$ solely in male, not female, cells from the comparable sex-independent basal P_s . These pharmacological data demonstrate that sexually dimorphic genes have profound influence on cellular and tissue structure and function.

3.7.3 Aquaporins

The aquaporin family of proteins provides additional insights in the molecular mechanisms regulating transcellular water transport⁶⁷ and sex differences therein. Its first member, aquaporin (AQP)-1 (or CHIP28), a 28 kDa channel-forming integral membrane protein was identified initially in erythrocytes and later in the kidney.⁶⁸ AQP-1 has been identified in the apical and basolateral membranes of EC lining capillaries of several tissues.⁶⁸ Functional rat studies⁶⁸ and data from genetically modified mice⁶⁹ suggest that AQP-1 is the molecular counterpart of the ultra-small pores predicted by fluid transport theory.⁷⁰ The majority of peritoneal membrane J_v during peritoneal dialysis is thought to occur through the tetrameric AQP-1 structures that assemble in plasma membrane.⁷¹

Sexual dimorphism with respect to AQP-1 was found during the development of a C57BL/6J mouse model of peritoneal dialysis (Table 3). Females, relative to males, while possessing a similar peritoneal transport rate for small solutes, had lower sodium sieving that correlated directly with lower peritoneum AQP-1 mRNA and protein expression.⁷¹ Studies in humans and rodents demonstrate specific localization of AQP-1 in capillary, venular, and lymphatic EC but not in small-size arteries,⁷² consistent with the notion that water movement occurs primarily across the walls of low-pressure microvessels. It is not known how lower AQP-1 expression influences L_p and/or the pressure dependence of J_s . AQP-1 expression and contribution to transcellular fluid flux can be up-regulated by corticosteroids.⁷³ Given the well-characterized sex differences in adrenal cortical secretion,⁷⁴ the untested inference is that 'stressed states' would be accompanied by an increase in L_p .

3.7.4 Receptor distribution

Receptor distributions, including those for the gonadal hormones, can play a role in manifestation of sex differences. In cutaneous vessels, the endothelin-B receptor mediates tonic vasoconstriction in males and vasodilatation in females.⁷⁵ The functional sexual dimorphism with respect to P_s observed in adult and juvenile rat SKM and adult porcine coronary vessels could not be accounted for by differences in ADO receptors.^{23–25} These examples point to sex differences in cell signalling mechanisms elicited in response to receptor occupation and the potential for sexually dimorphic responses to vasoactive drugs.

4. Summary

Close inspection of the components regulating fluid and solute flux reveals multiple features that vary by sex. These features range from differences in the magnitude of the driving gradients, to the vascular structures, cell signalling mechanisms, inflammatory mediators, and circulating hormones. As both sexes are in volume homeostasis, the means by which this state is achieved must result from the combination of elements particular to each sex. Reproductive hormones contribute to sex differences through genomic and non-genomic interactions. Sex-specific phenotypes also arise prior to sexual maturity and are maintained by cells in culture in the absence of reproductive hormones implicating sex-specific gene expression. Evidence was presented demonstrating sex differences only following stimulation or perturbations to the system.

Why should we care? Because manifestation of CV disease with respect to exchange can differ by sex with trivial to profound consequences—failure to recognize the differences can result in morbidity and mortality. While men and women can regulate these functions

differently, the extent of the sex-sensitive differences, the mechanisms, and how they interact remain to be elucidated fully.

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