CKJ REVIEW

Albumin is an interface between blood plasma and cell membrane, and not just a sponge

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ABSTRACT

Albumin is the most abundant protein in blood plasma and acts as a carrier for many circulating molecules. Hypoalbuminaemia, mostly caused by either renal or liver disease or malnutrition, can perturb vascular homeostasis and is involved in the development of multiple diseases. Here we review four functions of albumin and the consequences of hypoalbuminaemia on vascular homeostasis. (i) Albumin is the main determinant of plasma colloid osmotic pressure. Hypoalbuminaemia was therefore thought to be the main mechanism for oedema in nephrotic syndrome (NS), however, experimental studies showed that intrarenal mechanisms rather than hypoalbuminaemia determine formation and, in particular, maintenance of oedema. (ii) Albumin functions as an interface between lysophosphatidylcholine (LPC) and circulating factors (lipoproteins and erythrocytes) and the endothelium. Consequently, hypoalbuminaemia results in higher LPC levels in lipoproteins and erythrocyte membrane, thereby increasing atherosclerotic properties of low-density lipoprotein and blood viscosity, respectively. Furthermore, albumin dose-dependently restores LPC-induced inhibition of vasodilation. (iii) Hypoalbuminaemia impacts on vascular nitric oxide (NO) signalling by directly increasing NO production in endothelial cells, leading to reduced NO sensitivity of vascular smooth muscle cells. (iv) Lastly, albumin binds free fatty acids (FFAs). FFAs can induce vascular smooth muscle cell apoptosis, uncouple endothelial NO synthase and decrease endothelium-dependent vasodilation. Unbound FFAs can increase the formation of reactive oxygen species by mitochondrial uncoupling in multiple cell types and induce hypertriglyceridemia in NS. In conclusion, albumin acts as an interface in the circulation and hypoalbuminaemia impairs multiple aspects of vascular function that may underlie the association of hypoalbuminaemia with adverse outcomes. However, hypoalbuminaemia is not a key to oedema in NS. These insights have therapeutic implications.

Keywords: albumin, free fatty acids, lysophosphatidylcholine, nephrotic syndrome, oxidative stress

INTRODUCTION

Albumin is the most abundant protein in the human circulation. Albumin has a turnover time in humans of ∼25 days being produced at a rate of 10.5 g/day and being cleared renally (∼6%), gastrointestinally (∼10%) and catabolized (∼84%) [1]. Changes in serum albumin can be the consequence of reduced production (hepatic disease, reduced protein intake or due to a negative acute-phase response in inflammation [2]) or increased loss of albumin [nephrotic syndrome (NS), protein-losing enteropathy or catabolism during severe disease]. Albumin is synthesized in the liver, in hepatocytes it is translated from a single gene as preproalbumin [3]. The preproalbumin protein is transported to the endoplasmic reticulum where the *N*-terminal

prepropeptide is cleaved by a serine protease. Afterwards, it is transported to the Golgi system before it is secreted into the circulation as a simple protein containing only amino acids without additives or prosthetic groups [1, 4]. Therefore it is one of the few plasma proteins without a carbohydrate additive, making albumin a non-glycoprotein. More recently, it has been suggested that albumin can undergo post-translational modification under specific situations, modifying its interaction with other molecules such as exogenous drugs [3]. One example of such a modification is glycation of albumin, which is decreased in NS but increased in diabetes mellitus [5].

Albumin consists of eight and one-half double loops formed by disulphide bonds involving adjacent half-cysteine residues, resulting in a molecular mass of 65–66 kDa. Albumin consists of 35 cysteine amino acids that form Cys-Cys pairs, except for Cys-34 having a sulfhydryl additive. It can be further divided into three homologous helical domains, numbered I, II and III, formed by two longer loops separated by a shorter loop [4]. This structure is well-preserved across species, having the same distribution of half-cysteines. Although the three domains are homologous, there ligand-binding functions are different. Within each domain the first two loops (loops 1–2, 4–5 and 7–8) are grouped together as subdomains IA, IIA, IIIA, respectively [4]. Additionally, loop 3, 6 and 9 are grouped as IB, IIB, IIIB, responsible for the three-dimensional structure and function of albumin. The main function of serum albumin is to bind and transport small molecules in the circulation. Due to its three-domain design, it has multiple binding sites and thus is capable of binding a wide variety of molecules. Hydrophobic organic anions of 100–600 Da, including long-chain free fatty acids (FFAs), haematin and bilirubin, bind the strongest to albumin. Smaller molecules such as tryptophan, ascorbate and 25-hydroxyvitamin D_3 are bound highly specifically but less strongly to albumin [4, 6]. For an extensive overview of the different molecules that can be bound to albumin, we refer to Peters *et al.* [4]. Through the binding of all these compounds, albumin can function as a depot, extending the available quantities beyond their solubility. In addition, albumin can bind circulating toxins, rendering them harmless until they can be cleared by the liver and/or kidneys. Importantly, several drugs are bound by albumin and thus the loss of circulating albumin can disrupt normal pharmacokinetics, thereby affecting half-life and plasma unbound drug concentration and ultimately altering normal metabolism and the function of drugs [7].

ALBUMIN, NS AND OEDEMA

In NS, defects in glomerular barrier function result in leakage of proteins into the pre-urine, surpassing the protein reuptake capacity of the proximal tubule epithelial cells [8]. This cascade can result in heavy proteinuria (>3.5 g/day) with subsequent hypoalbuminaemia, which, together with sodium retention, water retention and generalized oedema, is one of the hallmarks of NS [9]. Historically, hypoalbuminaemia was thought to be the main mechanism of oedema formation associated with NS (Figure 1). This so-called underfill hypothesis was based on the findings of Frank Starling, who formulated Starling's Principle of how plasma and tissue colloid osmotic and hydrostatic pressure are responsible for maintaining plasma–interstitial fluid equilibrium [10] (see Figure 1 for the full Starling's Principle). Interstitial albumin concentrations vary between tissues [11, 12]. It is dependent on plasma concentration of albumin [12], together with the local transcapillary escape rate and the passage of albumin from the interstitial to intravascular compartment via the
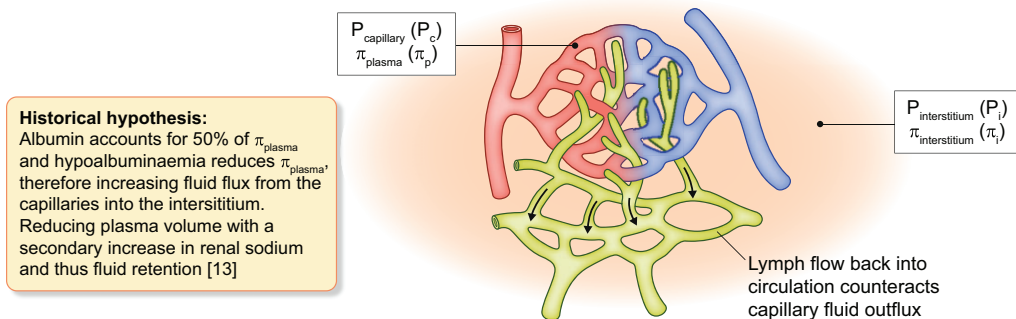
lymph or diffusion [13]. Interestingly, the transcapillary escape rate has been shown to be increased in common diseases inducing vascular leakage, such as diabetes [14]. Since albumin is one of the strongest determinants (~50%) of the plasma colloid osmotic pressure, it was hypothesized that the low plasma colloid osmotic pressure resulted in fluid flux into the interstitium, thus inducing generalized oedema and a hypovolemic state [13]. The kidneys try to prevent volume depletion by activation of the renin–angiotensin–aldosterone system (RAAS) and subsequently increase sodium and water retention, inducing a vicious circle [13].

Multiple observations from clinical and experimental studies investigating NS shifted this paradigm. The hypothesis implied that patients with NS are hypovolaemic, but data from clinical studies demonstrated that only a small portion actually are hypovolaemic and that the majority of patients are euvolaemic or hypervolaemic [15–18]. Plasma oncotic pressure was reduced in children with NS, while hypovolemic symptoms were only present in ~27% of the children with NS [19]. Hypovolaemia was accompanied with an activation of the RAAS; however, in a large cohort of NS patients, RAAS activation was not associated with blood volume [20]. Additionally, increased plasma renin activity was only increased in NS patients with hypovolemic symptoms [19]. It is suggested that minimal change disease represents a unique NS phenotype with increased neurohumoral activity, independent of blood volume [21]. In children with NS due to minimal change disease, sodium retention occurs before hypoproteinaemia and while renal blood flow is high [22]. After steroid-induced remission of NS is achieved, natriuresis is promoted while plasma protein content and blood volume are still low [23]. In contrast to all this evidence against the hypovolaemia hypothesis, there is one study showing that fractional sodium excretion is only reduced in NS patients with symptoms of hypovolaemia [19].

There are several oedema-preventing mechanisms that help maintain plasma and interstitial fluid equilibrium [13]. Mobilization of interstitial protein storage into the circulation is the main mechanism in hypoalbuminaemia-induced reduction in plasma oncotic pressure [13]. Only when the interstitial oncotic pressure cannot be further reduced will oedema occur. Interestingly, it has been shown that NS patients have oedema at higher levels of plasma oncotic pressure [13]. Joles *et al.* [24] demonstrated that plasma oncotic pressure due to hypoproteinaemia must be below ~8 mmHg for sodium to be actively retained, which is lower than in a significant portion of the NS patients [20]. In patients with NS, transcapillary oncotic gradient is maintained both at diagnosis and during (complete or partial) recovery [25]. This was also observed in Nagase analbuminaemic rats, which have a decrease in plasma colloid osmotic pressure but a maintained capillary-interstitial oncotic gradient due to a parallel decrease in interstitial colloid osmotic pressure [26]. Similar findings have been observed in a different rat model of hypoproteinaemia induced by starvation [27]. Rapid induction of hypoproteinaemia has been achieved by repeated plasmapheresis in dogs. In this model of hypoproteinaemia, a compensatory decrease in tissue oncotic pressure has been demonstrated and even an increased fluid recovery from haemorrhage [28]. Interestingly, studies conducted in this model have also demonstrated that moderate hypoproteinaemia does increase extra-cellular volume fraction, but does not alter blood volume or neurohumoral activation [24, 29, 30]. Strikingly, sodium balance was achieved when protein levels were still low at the onset of recovery of plasmapheresis [24, 30]. In line with these findings, in a unilateral nephrotic rat model, only the diseased kidney retains sodium [31]. Ultimately

Historical hypothesis: hypoalbuminaemia results in oedema in nephrotic syndrome (NS)

Starling's principle [10]: $J_v = L_p S[(P_c - P_i) - \sigma(\pi_p - \pi_i)]$

$P_{capillary}$ (P_c)
π_{plasma} (π_p)

$P_{interstitium}$ (P_i)
$\pi_{interstitium}$ (π_i)

Historical hypothesis:
Albumin accounts for 50% of π_{plasma} and hypoalbuminaemia reduces π_{plasma}, therefore increasing fluid flux from the capillaries into the intersititium. Reducing plasma volume with a secondary increase in renal sodium and thus fluid retention [13]

Lymph flow back into circulation counteracts capillary fluid outflux

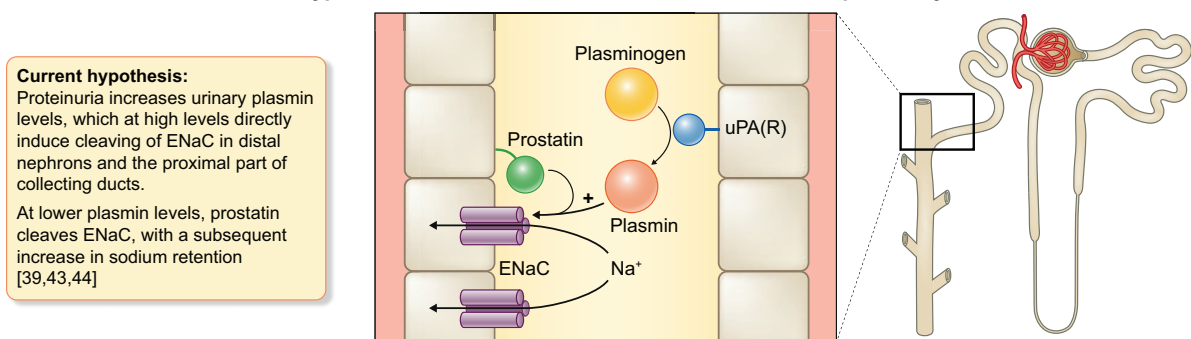**Paradigm shift from clinical and experimental observations**

Clinical observations:
- NS patients had maintained capillary–interstitial oncotic gradient [25]
- Most oedematous NS patients had normal to expanded intravascular volume but still had decreased fractional sodium excretion [15–18]
- Blood volume did not correlate to increased RAS activity and ACE inhibition did not improve volume status [20,21]
- Albumin infusion increased oncotic pressure but does not improve oedema in NS patients [111,112]
- Steroid-induced remission in NS patients resolved oedema more rapidly than albumin levels [23]

Experimental observations:
- In hypoalbuminaenic dogs, induced by repeated plasmapheresis, recovery from plasmapheresis increased sodium excretion and sodium balance was achieved while hypoalbuminaemia was maintained [24]
- In hypoalbuminaemic dogs, low plasma oncotic pressure resulted in a compensatory low interstitial oncotic pressure with maintained transcapillary fluid flux and even increased blood volume recovery after haemorrhage [28]
- Nagase analbuminaemic rats have a maintained transcapillary oncotic gradient [26]
- In unilaterally-induced massive albuminuria in rats, only the affected kidney retained sodium. This was not mediated by increased RAS- or SNS-activity [31]

Current hypothesis: intrarenal defect results in oedema in nephrotic syndrome

Current hypothesis:
Proteinuria increases urinary plasmin levels, which at high levels directly induce cleaving of ENaC in distal nephrons and the proximal part of collecting ducts.

At lower plasmin levels, prostatin cleaves ENaC, with a subsequent increase in sodium retention [39,43,44]

Plasminogen
Prostatin
uPA(R)
Plasmin
ENaC
Na+

FIGURE 1: Paradigm shift of the pathophysiological mechanisms of oedema formation in NS. Historically Starling's Principle was thought to be the main mechanism, but due to observations from clinical and experimental studies this was rejected. More recent studies show a role for urinary protein-activated plasmin promoting ENaC activation in the cortical collecting duct.

J_v, trans-endothelial filtration volume; L_p, hydraulic conductivity of the membrane; S, surface area; P, hydrostatic pressure; π, oncotic pressure; ACE, angiotensin-converting enzyme; uPA(R), urokinase (receptor).

43% of patients with congenital analbuminaemia do not experience oedema formation and 38% only experience mild ankle oedema [32]. Altogether, these findings suggest that generalized oedema in NS is not merely due to hypoalbuminaemia, as volume regulation even in the absence of albumin is mostly maintained, but that an intrarenal mechanism is responsible, the so-called overflow hypothesis.

In the overflow hypothesis for oedema formation in NS, the proteolytic activation of epithelial sodium channels (ENaCs) has been suggested to be the driving factor of sodium and subsequent fluid retention (Figure 1) [33]. Activation of ENaCs is the result of cleavage of the γ-subunit by urinary plasmin, a serine protease [34, 35]. ENaC activation by cleavage of the γ-subunit by proteases has been suggested to be a physiological process

[33]. However, glomerular defects in NS result in increased protein and proteases in the urine. In the urinary tract, plasminogen is predominantly converted to biologically active plasmin by urokinase-type plasminogen activator (uPA) [36]. Indeed, in the urine of NS patients, plasmin and plasminogen levels were increased [35, 37]. In a large cohort of NS patients, the urinary plasminogen–plasmin:creatinine ratio was an independent predictor of oedema formation, and a decrease after treatment was associated with oedema remission [38]. Furthermore, urine of patients with NS induced an increase in sodium current *in vitro*, which could be inhibited by amiloride and anti-plasmin [35]. In experimental NS, the serine protease inhibitor aprotinin prevented proteolytic activation of ENaCs [39]. This notion is further supported by findings in several patient case reports wherein inhibition of ENaCs has been shown to resolve resistant oedema in NS [40–42]. However, there are some indications that uPA-produced plasmin is not the sole contributor to NS-associated sodium retention [33]. Bohnert *et al.* [43] demonstrated that uPA does promote plasminogen–plasmin conversion but that it is not essential for oedema formation in experimental NS. This is in line with findings in other studies that demonstrate an inhibitory effect on sodium retention in experimental NS by uPA inhibition [44]. In conclusion, although the specific mechanisms are not completely unravelled, there seems to be a role for proteolytic activation of ENaCs in NS.

ALBUMIN AND LYSOPHOSPHATIDYLCHOLINE (LPC)

LPC is a phospholipid that is present as a minor component of the cell membrane and is present in circulating plasma. In contrast, phosphatidylcholines (PCs) are the major phospholipid component of cell membranes as well as lipoproteins. LPC is produced by the hydrolysis of PCs by phospholipase A2 (PLA$_2$), removing one of the fatty acid groups on the *sn*-2 position (Figure 2) or as a product by the lecithin cholesterol acyltransferase reaction (LCAT). In the circulation, LPC has a short half-life due to rapid degradation, which prevents impairment of a variety of vascular functions. Albumin is one of the most important transporters; normally 80% of LPC in the circulation is bound to albumin [45, 46]. A single molecule of albumin can bind up to five LPC molecules, suggesting multiple binding sites [47]. Albumin acts as an interface that binds LPC and transports it back to different tissues, but mainly the liver. In the liver, LPC can be formed back to PCs by lysophosphatidylcholine acyltransferase (LPCAT) in the presence of acyl-CoA. Subsequently, PCs are incorporated in the surface of very-low-density lipoproteins (VLDLs) and high-density lipoproteins (HDLs) and so released back into the circulation (the so-called Lands' cycle).

Hypoalbuminaemia can therefore limit the LPC return capacity to the liver, which can be detrimental to vascular homeostasis. Indeed, the total concentration of LPC in the blood increases in hypoalbuminaemia due to proteinuria [46]. However, LPC concentrations in lipoprotein-deficient plasma were decreased, showing that there was a shift of LPC binding to VLDL, intermediate-density lipoprotein and LDL, independent of the lipidaemic status of the patient [46]. Importantly, this phenomenon is observed not only in severe hypoalbuminaemia, but also in subnormal albumin (29 g/L) [46]. Furthermore, LPC has been shown to be increased about five times in oxidized LDL and about seven times in oxidized lipoprotein A [48]. This shift prolongs the half-life of LPC in the circulation and thereby impairs vascular function. For instance, it is well known that oxidized
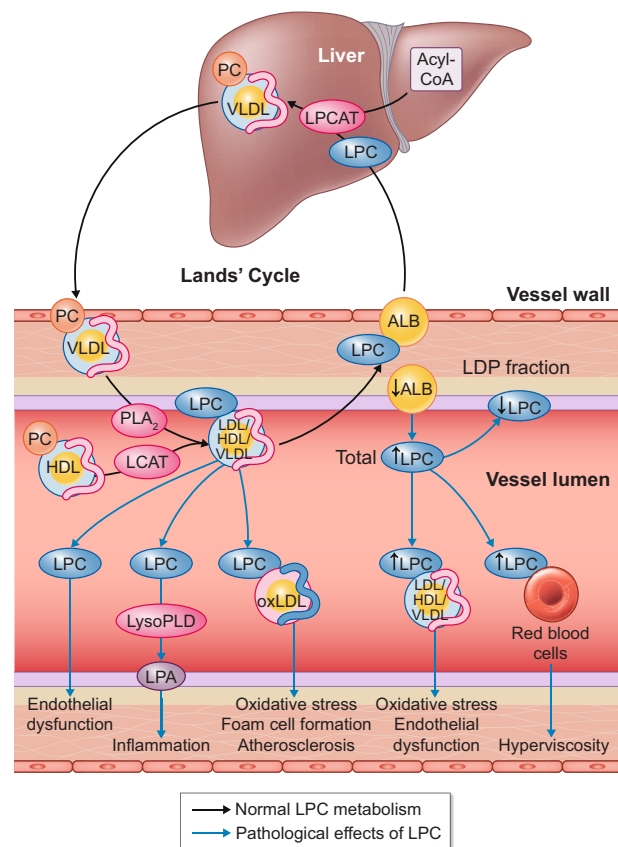

FIGURE 2: Normal and pathophysiological LPC metabolism and the role of albumin.

The Lands' cycle is responsible for rapid alterations in phospholipid composition of lipoproteins. These phospholipids are mainly PC. Both PLA$_2$ and LCAT are capable of hydrolysis of PC to LPC in the circulation. LPC is mostly bound to albumin (ALB), rendering its detrimental effects ineffective. Albumin-bound LPC is transported to the liver and LPCAT catalyses the conversion back to PC. The accumulation of non-albumin-bound LPC has multiple detrimental effects on vascular function.

LDP, lipoprotein-deficient plasma; LysoPLD, lysophospholipase D; LPA, lysophosphatidic acid; oxLDL, oxidized low-density lipoprotein.

LDL plays a pivotal role in atherosclerosis development through multiple pathogenic mechanisms, and it is thought that this could be attributed in part to LPC [49]. Inhibition of endothelial cell migration is often observed in atherosclerosis in the presence of oxidized LDL [50] or LPC specifically [51, 52]. Increased oxidative stress is a known mediator of atherosclerosis and, in particular, oxidative stress due to LPC has been shown to induce endothelial cell apoptosis. Interestingly, adding albumin might reduce cytotoxicity in a dose-dependent manner in cultured Jurkat T cells treated with 20 µmol of LPC [47]. Furthermore, LPC is associated with endothelial dysfunction, which is both associated with atherosclerosis development and with the loss of endothelial-dependent vasodilation [48, 49, 53]. Loss of endothelial-dependent, but not endothelial-independent, vasodilation has been demonstrated in aortic rings incubated with LPC [54]. In Nagase analbuminaemic rats, renal LPC concentrations and renal blood flow were normal, but intrarenal infusion of LPC induced more pronounced vasoconstriction and doubled the LPC concentration, while it had no effect in control rats [55].

Notably, in isolated rat aortic rings [54] and *in vivo* in Nagase analbuminaemic rats [55], albumin restored LPC-induced vaso-constrictor effects. This demonstrates that LPC can induce vascular dysfunction by impairing multiple vascular cell types and that albumin is able to prevent or restore these impairments.

Besides the shift of LPC binding to lipoproteins in hypoalbuminaemia, experiments in Nagase analbuminaemic and nephrotic rats have shown that LPC increasingly binds to red blood cells in hypoalbuminaemia [56]. Even though hypoalbuminaemia reduces plasma viscosity, binding of LPC to red blood cells resulted in reduced red blood cell deformability and consequently in increased whole blood viscosity [56]. Moreover, this phenomenon was still present when red blood cells were suspended in serum, excluding fibrinogen as the culprit. Albumin supplementation or suspending red cells from analbuminaemic rats in normal rat plasma reduced binding of LPC from red blood cell membranes, increased LPC plasma levels and restored blood viscosity to normal [56]. Furthermore, LPC might also bind to various classes of leucocytes and thereby modulate inflammatory responses. However, whether LPC induces a pro- or anti-inflammatory response seems to be cell specific. For example, exogenous LPC promotes the expression of adhesion molecules in endothelial cells, induces the release of TNF-α and inter-leukins 1β and 6 from adipocytes, activates macrophages and B cells and increases interferon-γ release from peripheral leuco-cytes [45]. In contrast, the effects of neutrophil activation [57] as well as eosinophil activation and migration are inhibited by LPC, suggesting an anti-inflammatory effect of LPC in specific conditions such as allergic reactions [58].

ALBUMIN AND NITRIC OXIDE SIGNALLING

We previously described the detrimental effect of hypoal-buminaemia and subsequent increased circulating LPC on endothelium-dependent vasodilation. However, it has been suggested that albumin also plays a role in regulating endothelium-dependent vasodilation directly. Nitric oxide (NO) is considered one of the most important endothelium-dependent vasodilator mechanisms in most vascular beds also has multiple paracrine effects [59]. NO can be produced from the substrate L-arginine by three different isoforms of the NO synthase protein [59]. The isoform, endothelial nitric oxide synthase (eNOS), is considered the most important isoform contributing to vasomotor control. Endothelium-derived NO diffuses to vascular smooth muscle cells (VSMCs) surrounding the endothelial cells and stimulates soluble guanylate cyclase (sGC), catalysing the conversion of guanosine triphosphate to cyclic guanosine monophosphate (cGMP). Subsequent activation of cGMP-dependent protein kinases results in a reduction in intracellular Ca^{2+}, hyperpolarization and thus vasodilation [59]. NO is a highly reactive molecule and previous research has shown that NO plays an important role in redox reactions [59–61]. The main molecules that can react with NO are metals, thiols, oxygen (O_2) and superoxide (O_2^-) [62], particularly the reaction with O_2^- resulting in peroxynitrite ($ONOO^-$) and O_2 resulting in NO_2 (nitrogen dioxide), both considered powerful reactive nitrogen species [62]. Storage of gaseous NO is tricky due to its reactive nature, therefore it can be stored in different forms. Storage of NO as the metabolites NO_2^- (nitrite) and NO_3^- (nitrate) in the circulation is most common [63]. Additionally, red blood cells are known to be able to store, as an S-nitroso (SNO) adduct or as iron nitrosyl to haemoglobin, and release NO in the case of hypoxia or increased metabolic demand [63, 64]. A similar phenomenon is observed in albumin, as the single free cysteine, Cys-34, is particularly capable of

binding NO as an SNO adduct [60, 61, 64]. This makes NO more stable and makes albumin-SNO a circulating reservoir for NO [61, 65]. Indeed, the concentration of unbound NO in the circulation is about 3 nM, while 7 µM of S-nitrosothiols are present, of which 96% are protein-bound, of which 82% is bound to albumin [61].

Besides the evidenced capacity of albumin to bind NO and form albumin-SNO, it has been shown that albumin-SNO has a physiological role in vascular homeostasis and can influence vasomotor tone. In rabbits, inhibition of eNOS resulted in an increase in mean arterial pressure associated with an inverse decrease in albumin-SNO [61]. In mongrel dogs, an intravenous injection of SNO–bovine serum albumin (BSA) markedly reduced mean arterial pressure, produced epicardial vasodilation, increased coronary blood flow and inhibited platelet adhesion in a dose-dependent manner [66]. Although it was less potent than nitroglycerin and S-nitroso-cysteine, it has a longer duration, reflecting an increased half-life [66]. Another study nicely demonstrated that this effect is endothelium independent, as endothelium-denuded aortic arteries showed dose-dependent vasodilation to SNO-BSA [60].

With these findings on the physiological function of albumin-SNO, the question arises if changes in albumin can also alter NO production or signalling. In end-stage renal disease (ESRD) patients on haemodialysis, lower plasma albumin was observed and was associated with impaired vascular function [67]. From clinical studies performed in NS patients, it is known that peripheral vascular function is impaired [68–70]. This is mainly due to the blunting of endothelium-dependent vasodilation or a loss of NO-mediated vasodilation specifically, while endothelium-independent vasodilation is preserved [68–70]. It is unclear if this is mainly the effect of NS-associated dyslipidaemia or hypoalbuminaemia [68, 69]. In Nagase analbuminaemic rats, infusion with an NO donor produced an similar reduction in mean arterial pressure compared with healthy rats. However, the recovery time of the Nagase analbuminaemic rats was significantly reduced, suggesting the lack of 'long-lived' NO-producing prolonged vasodilation [65]. These alterations were associated with a 300% increase in plasma S-nitrosothiols 30 min after NO-donor administration in healthy rats, while no changes in Nagase analbuminaemic rats were observed [65]. Bevers *et al.* [71] demonstrated that NO metabolites were increased in the plasma of Nagase anal-buminaemic rats. Interestingly, aortic eNOS protein content was similar between Nagase analbuminaemic and healthy rats. However, they went on to show that hypoalbuminaemia increased NO production (measured by two independent methods: 4,5-diaminofluorescein diacetate and electron paramagnetic resonance) as well as eNOS activity of cultured endothelial (bEnd.3) cells *in vitro* [71, 72]. Moreover, this corresponded with a concomitant increase in eNOS-produced NO metabolites (Griess colorimetry), representing 'short-life' NO [71]. This suggests that albumin binding of NO, presumably at the endothelial surface, can limit diffusion of NO to VSMCs. Hypoalbuminaemia results in an increased flux of NO to the vascular smooth muscle, presumably necessitating downregulation of guanylate cyclase [73].

Indeed, endothelium-independent vasodilation to exogenous NO-donor sodium nitroprusside was markedly blunted in aortic rings of Nagase analbuminaemic rats versus normal control rats (median effective dose increased by nearly two orders of magnitude). Thus even in isolation, the downregulation of endothelium-independent vasodilation was maintained. Endothelium-dependent vasodilation to acetylcholine was slightly, but significantly, stronger in Nagase analbuminaemic
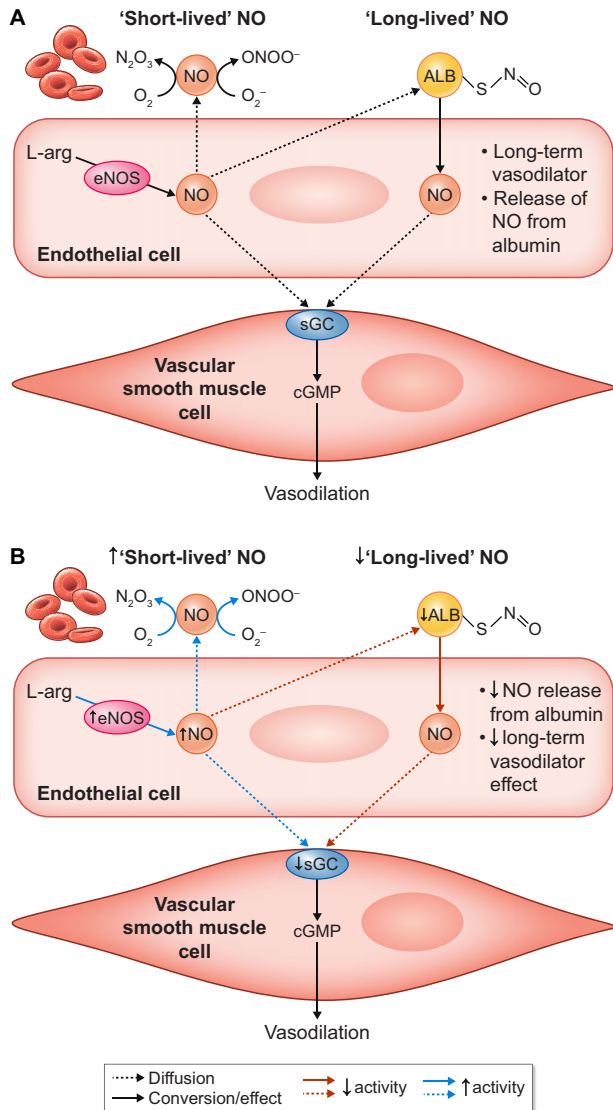
FIGURE 3: The role of albumin and hypoalbuminaemia in vascular NO signalling. NO is a potent vasodilator produced by eNOS. It induces vasodilation by stimulating soluble guanylyl cyclase (sGC) that increases cGMP concentrations in VSMCs. NO in the circulation is short-lived, as it is highly reactive. Circulating albumin (ALB) can bind NO as an S-nitrosothiol (-S-N=O), thereby prolonging its half-life. This figure shows the role of NO and albumin in (**A**) normoalbuminaemia and (**B**) hypoalbuminaemia.
N_2O_3, dinitrogen trioxide; O_2, oxygen; $ONOO^-$, peroxynitrite; O_2^-, superoxide radical; L-arg, L-arginine.

rats compared with healthy rats, but only at the high doses [71]. Overall, these demonstrate that hypoalbuminaemia results in a shift from 'long-life' NO to 'short-life' NO, resulting in an increase in NO production with subsequent NO-mediated desensitization as a negative feedback mechanism to maintain vascular homeostasis (Figure 3).

ALBUMIN AND FFAS

The inhibitory effects on albumin of NO production and synthesis by endothelial cells described above were actually a serendip-

itous finding. The primary aim of that study was to expose bEnd.3 cells to the FFAs oleate and palmitate to test the hypothesis that fatty acid inhibits endothelial NO production. However, we could only do this by adding these fatty acids to an albumin solution. Thus we also compared fatty acid–free albumin in the medium to albumin-free medium and discovered a marked depression of pNOroduction even without fatty acids [71, 72]. Normally, FFAs are bound to albumin in a 0.7:1 FFA albumin molar ratio in the circulation, and this ratio can increase to up to 8:1 in diseases such as diabetes mellitus [74] and NS [75]. Other studies also showed the pro-oxidative effect of oleate albumin complexes on endothelial cells and demonstrated a reduction in eNOS function; however, they did not investigate the sole effect of albumin or oleate [76]. Besides the effect on NO production by endothelial cells, albumin-bound palmitate was capable of inducing apoptosis in cultured VSMCs due to increased oxidative stress compared with albumin alone [77]. Interestingly, albumin-bound oleate did not induce this increase in oxidative stress and apoptosis, and even attenuated palmitate-induced oxidative stress and apoptosis [77]. Additionally, FFAs have a lipotoxic effect on pancreatic β-cells, contributing to impaired insulin production in obese patients [78, 79]. Moreover, albumin polymorphisms reduced β-cell cytotoxicity by increasing affinity of palmitate and oleate binding [78].

For a long time it was unsure how proteinuria resulted in hypertriglyceridemia, as observed in NS [80]. Experimental studies conducted in rats demonstrated that both hypoalbuminaemia and proteinuria contribute separately to the development of hypertriglyceridemia in NS [81, 82]. It was postulated that hypertriglyceridemia in NS was not the result of increased production, but rather decreased clearance [80, 82, 83]. An elaborate study from Clement et al [84]. went on to demonstrate that circulating angiopoietin-like 4 (ANGPTL4) plays a pivotal role in the development of hypertriglyceridemia, proteinuria, low circulating albumin and renal and systemic feedback mechanisms (Figure 4). Briefly, urinary loss of albumin results in hypoalbuminaemia and increased FFA content bound to albumin. Subsequently, the increased FFA:albumin ratio in plasma and tissue stimulates upregulation of ANGPTL4 in skeletal muscle, heart and adipose tissue [84, 85]. NGPTL4 inhibits endothelial-bound lipoprotein lipase activity, which inhibits FFA generation from triglycerides and thus induces hypertriglyceridemia. This comprises the local feedback loop, as reduced FFA results in a decrease in the FFA:albumin ratio [84, 85]. The systemic feedback loop reduces proteinuria by binding of ANGPTL4 to $\alpha v\beta_5$ integrins that are present at the basement membrane of glomerular endothelial cells. These integrins bind to different extracellular matrix proteins, e.g. vitronectin, and regulate vascular barrier integrity [86], subsequently lowering the leakage of protein into the urine [84, 85]. Albumin that is filtered by the glomeruli is reabsorbed by the proximal tubule epithelial cells, as discussed above [87–89]. Normally albumin is taken up in the proximal tubules in the kidney by receptor-mediated endocytosis, preventing urinary loss of albumin [87–89]. It should be noted, however, that this hypothesis might not yet be fully understood, as some studies show ANGPTL4 to be unaltered in NS and proteinuria [90]. It has been shown that excess albumin in the epithelial tubular cells activates protein kinase C, which subsequently increases NAD(P)H oxidase activity and thus reactive oxygen species production [91]. However, it has been suggested that this is not solely the effect of albumin, but also the effect of albumin-bound FFAs [92, 93]. Indeed, albumin-bound FFAs are capable of inducing higher levels of oxidative stress in proximal tubules than free albumin,
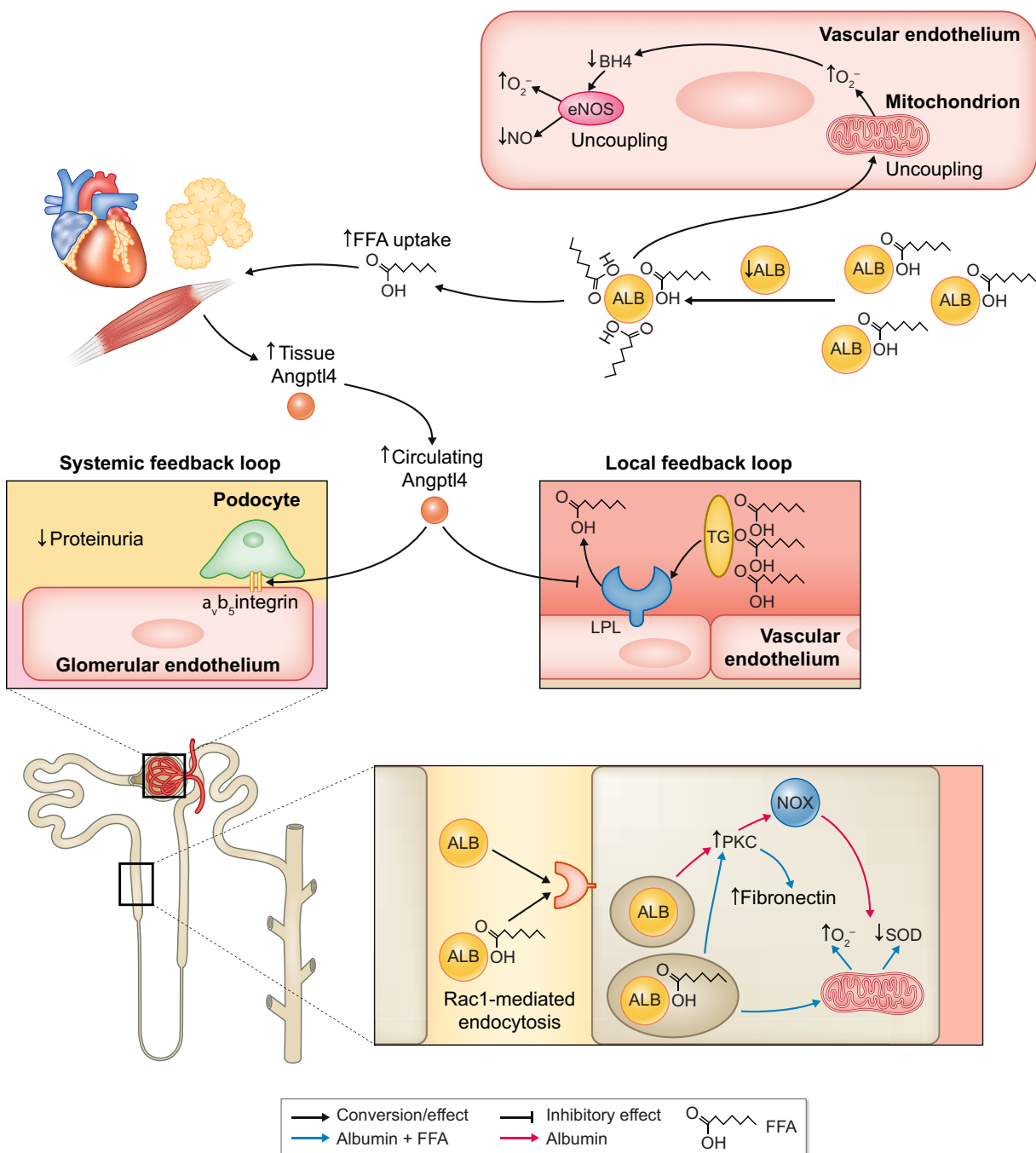
FIGURE 4: The role of albumin (ALB) in free FFA metabolism in vascular and tubular homeostasis.

ALB is capable of binding FFAs and hypoalbuminaemia increases the FFA:ALB ratio. Increased circulating FFAs bound to ALB uncouples endothelial mitochondria that produce superoxide radicals (O_2^-) and in turn uncouple eNOS, further inducing vascular dysfunction. Peripheral tissues increase their uptake of FFAs and subsequently release ANGPTL4, which in a local feedback loop reduces production of FFAs from triglycerides (TG) by lipoprotein lipase (LPL). In a systemic feedback, loop proteinuria is reduced by ANGPTL4 binding to αvβ5 integrins at the surface of the glomerular endothelial cell. ALB can induce oxidative stress and a fibrogenic response in proximal tubule epithelial cells, which seems to be aggravated when FFAs are bound to ALB, resulting in tubulotoxicity.

BH4, tetrahydrobiopterin; Rac1, Ras-related C3 botulinum toxin substrate 1; PKC, protein kinase C; NOX, NADPH-oxidase; SOD, superoxide dismutase.

by mitochondrial production of superoxide and downregulation of antioxidants [92, 94]. In a similar study, albumin had a proliferative effect on proximal tubular cells, while albumin–linoleate complexes were tubulotoxic [93]. Furthermore, oleate-bound albumin exerted a fibrogenic effect through protein kinase C and fibronectin signalling [93]. This study demonstrates that albumin-bound FFAs are capable of influencing and aggravating the detrimental effects of albumin on proximal tubular cells.

ALBUMIN IN THE CLINICAL SETTING

Reduced albumin plasma levels are a strong predictor of morbidity and mortality regardless of the implicated disease [7]. Specifically, the association of hypoalbuminaemia and worse outcome in ESRD has long been established and is mostly due to cardiovascular complications [95, 96]. Interestingly, this phenomenon is independently associated to both protein malnutrition and hypoalbuminaemia, but only hypoalbuminaemia is associated to the prevalence of vascular disease in patients on dialysis [97]. In this study, a 1 g/L decrease in albumin was associated with a 10% increase in mortality. This adds to the hypothesis that hypoalbuminaemia is not merely due to malnutrition in patients with ESRD. Other mechanisms linking hypoalbuminaemia and worse outcome in ESRD, such as inflammation, have been suggested and investigated over the years. Indeed C-reactive protein has been shown to be a strong predictor of death in haemodialysis patients and the predictive value of albumin was dependent on C-reactive protein [98]. Inflammation reduces the synthesis of albumin by inhibiting its transcription. This is mediated by interleukin-6/1β and tissue necrosis factor-α [7, 96]. The hypothesis of hypoalbuminaemia as an acute phase protein that is reduced in response to inflammation is strengthened by the fact that hypoalbuminaemia is also commonly present in severely ill patients such as those in the intensive care unit. In a meta-analysis, hypoalbuminaemia has proven to be a strong and dose-dependent predictor of poor outcome in such patients [99].

Hypoalbuminaemia in various diseases might be an epiphenomenon and therefore albumin might be a suitable marker but not a therapeutic target. Indeed, multiple studies have aimed to investigate the supplementation of albumin in the clinical setting. Theoretically, albumin 4.5% is about four times as effective in volume expansion as crystalloids [100]. However, in hypovolaemia and hypoalbuminaemia, human albumin is not superior in reducing mortality as compared with the much cheaper alternatives such as other colloid and crystalloid solutions [101]. It has been hypothesized that in a highly selected subgroup of patients, human albumin supplementation might be indicated. For example, a subgroup analysis of the Saline versus Albumin Fluid Evaluation trial [102] and a meta-analysis [103] demonstrated that albumin infusion tended to improve outcome in sepsis and septic shock patients. In contrast, a more recent meta-analysis [104] extracting new information from a large randomized controlled trial [105] showed no benefit of adding albumin supplementation of crystalloids in severe sepsis. Therefore albumin supplementation is recommended only in hypovolaemia as a second choice when treatment with crystalloids does not suffice [106]. This is also in line with the recommendation of the European Rare Kidney Disease Reference Network–European Society for Pediatric Nephrology Working Group to supplement albumin in infants with congenital NS, mainly to increase intravascular volume and not serum albumin concentration *per se* [107]. Intradialytic hypotension can result in organ hypoperfusion and therefore must be treated. In those patients, albumin infusion for volume expansion has been investigated in small trials and showed a beneficial haemodynamic effect with hyperoncotic but not iso-oncotic albumin solutions [108]. In patients on chronic haemodialysis treatment, hypoalbuminaemia is common partly due to loss of albumin by current dialysis techniques [109]. Whereas smaller membrane pores prevent albumin loss, they also limit the removal of middle molecules to the dialysate [109]. Medium cut-off membranes were developed to increase middle molecule clearance, but might cause more albumin loss compared with high-flux membranes [109, 110]. Larger trials comparing these two dialysis modalities are needed to further evaluate this. The therapeutic value of albumin supplementation in NS patients overall remains a knowledge gap, mainly due to the lack of well-performed randomized controlled trials [111], but small trials do not show any benefit [112].

CONCLUSION

This review provides an overview of the role of albumin in physiological and pathophysiological conditions associated with hypoalbuminaemia. Indeed, as we have presented above, albumin plays a pivotal role in binding of multiple small and large molecules in the circulation. Although hypoalbuminaemia is most pronounced in NS, it can also be the consequence of inflammation, underfeeding (catabolism) and hepatic diseases. Moreover, a reduction of albumin can aggravate multiple common cardiovascular diseases and therefore should be considered and evaluated in patients at risk for developing cardiovascular disease. Additionally, it has proven to be an excellent prognostic marker in severe illness [113, 114]. However, increasing albumin levels by albumin infusion has not been proven beneficial in all critically ill intensive care patients [113, 115] or in hypotensive patients on kidney replacement therapy [115] but possibly has a beneficial effect in limited subgroups [113]. Mechanistic studies further investigating the interaction between hypoalbuminaemia and inflammation might help us understand the lack of improved prognosis [2]. The beneficial effect of albumin infusion for the treatment of oedema in NS patients remains controversial [112], warranting better studies [111]. The detrimental effects of hypoalbuminaemia can be easily overlooked in both (preclinical) research and clinical practice. Therefore we feel that albumin as a marker and effector of multiple pro-inflammatory and pro-oxidative processes should be included in the analysis of studies performed within the field of nephrology, cardiovascular and inflammatory disease. We can conclude that albumin is definitely not a mere sponge but functions as an important interface between tissues and circulating factors in order to maintain homeostasis in multiple systems and tissues.

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The authors declare that there are no conflicts of interests regarding the publication of this article.

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