Endothelial vasoregulation and mechanosensitive ion channels in hypertension

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Introduction

Haemodynamic forces associated with blood flow play a crucial role in the acute regulation of vascular tone and long-term changes of vascular structure and remodelling [1,2]. Alterations in endothelial function appear to be involved in the disturbed vasoregulation in hypertension and in the development of atherosclerotic lesions of the vessel wall [2]. Located at the interface between blood and vessel wall, the endothelium is directly subjected to haemodynamic forces associated with blood flow and responds to haemodynamic stimulation with the secretion of vasoactive mediators. Increases in blood flow induce a vasodilation which depends on an intact endothelium and is mediated by endothelium-derived factors like nitric oxide (NO) or prostacyclin (PGL2). Flow-dependent vasodilatation is an important protective mechanism to avoid damage of the vessel wall by frictional wall shear stresses associated with flow.

The mechanism by which the endothelium senses flow and the associated haemodynamic forces and transforms them into biochemical signals ultimately leading to the secretion of endothelial mediators has been incompletely characterized so far. Activation of ion channels and changes in intracellular calcium homeostasis are involved in the acute response of the endothelium to mechanical stimulation [3]. This is demonstrated by the observation that the flow-induced endothelium-dependent vasodilatation in perfused arteries depends on the activity of K+ channels [4] and involves an increase in intracellular Ca2+ concentration [5]. It should be mentioned that in contrast to previous reports [6,7] the Ca2+-dependent phase of flow-induced NO production was recently reported to be abolished in perfused isolated conduit arteries of the rabbit after restoring the assumed in vivo length of the arteries [8].

Stretch-activated channels

Mechanosensitive, stretch-activated ion channels have been postulated to act as endothelial mechanosensors [9] and are involved in Ca2+ mobilization induced by mechanical stretch [10]. Stretch-activated ion channels were originally identified in chick skeletal myocytes by use of the patch-clamp technique. They are involved in the mechanotransduction of various tissues in vertebrates and non-vertebrates [11]. Pathophysiologically a dysfunction of mechanosensitive ion channels have been reported to be involved in stretch-induced arrhythmias [12] and in muscular dystrophy [13]. These specialized ion channels are sensitive to changes in membrane tension or stretch which can be induced under experimental conditions by suction applied to the rear of the patch pipette. The activation of the channels shows a graded increase of channel activity with respect to the degree of membrane stretch elicited by different strengths of pipette suction. The electrophysiological properties of the channel, i.e. ion selectivity and channel conductance, remain unaffected by membrane stretch. Endothelial stretch-activated channels have a channel conductance between 25 and 45 pS and are impermeable for anions and permeable for mono- and divalent cations [9,14,15]. The channel permeability for Ca2+ ions is 3–4 times lower than for monovalent cations e.g. K+ and Na+. However, indirect evidence showed that due to the large inwardly directed electrochemical gradient for Ca2+ ions an activation of the stretch-activated channels leads to a sufficient Ca2+ influx into the endothelial cell and increases the intracellular Ca2+ concentration [14,16]. Stretch-activated ion channels are blocked by gadolinium, a trivalent lanthanide, in concentrations of less than 30 μmol/l.

Furthermore, two K+-selective mechanosensitive ion channels have been observed in endothelial cells. An endothelial stretch-activated K+ channel (SACk) has been identified in freshly isolated rat aortic endothelial cells [17]. This channel had a mean channel conductance in cell-attached patches of 70 pS and was selectively permeable for K+ ions with a permeability ratio K+ : Na+ of 11 : 1. A shear-stress-activated K+ channel was observed in cultured bovine aortic endothelial cells [18]. The channel had a mean channel conductance of 31 pS and was inwardly rectifying. The activation of these K+ selective mechanosensitive channels led to an efflux of K+ ions and subsequently to a hyperpolariz-
Mechanosensitive ion channels in hypertension

In human and experimental hypertension an endothelial dysfunction has been described. The increased vascular tone in hypertension is in part due to an imbalance in the secretion of vasodilating and vasoconstricting mediators by the endothelium [23]. Also, the endothelial response to haemodynamic stimulation has been reported to be impaired in hypertension as indicated by a decreased flow-induced endothelium-dependent vasodilatation in spontaneously hypertensive rats compared to normotensive Wistar–Kyoto rats [24]. In adult spontaneously hypertensive rats and in rats with renovascular hypertension (2K1C) a more than twofold increase of the density of the SACK and the pressure-activated channel was found [22] proving that mechanosensitive channels are regulated in the presence of altered haemodynamic forces. Channel upregulation and associated hyperpolarization by SACK as well as the increased Ca^{2+} influx through the pressure-activated channel would lead to an enhanced vasodilatory response to haemodynamic stimulation. However, it cannot be excluded that coactivation of depolarizing non-selective cation channels by pressure-activated channels, as stated above, would impair...
endothelial function and contribute to the endothelial dysfunction in hypertension.

## Conclusion

Endothelium-dependent flow-induced vasodilatation is an important mechanism to protect the vessel wall from damage by increased shear stress associated with increases in blood flow. Mechanosensitive ion channels act as endothelial mechanosensors and mechanotransducers of haemodynamic forces. Upregulation of mechanosensitive ion channels in hypertension might represent an important adaptive mechanism for vessel-wall protection.

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**Amylin—its role in the kidney**

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## Introduction

Amylin, also known as islet amyloid polypeptide, is a 37 amino acid polypeptide co-secreted with insulin by the pancreatic beta cell [1]. Previous research has primarily evaluated the role of this peptide in carbohydrate metabolism with studies concentrating on its role in beta cell function and in the genesis of insulin resistance [1]. Amylin has also been reported to have effects on bone metabolism [2], which may ultimately be relevant in renal osteodystrophy, although an exploration of this aspect has not been reported.
However, this report will focus on recent findings by our group which indicate that amylin acts in the kidney probably via a specific G-protein coupled receptor [3]. These effects of amylin have potential implications for various pathological conditions including hypertension and the renal response to injury. These findings are of particular interest since the amylin analogue, pramlintide, is now in phase III clinical trials as an agent to control postprandial hyperglycaemia in diabetes [4].

**Amylin receptors**

High-affinity amylin binding sites have been detected in both the kidney [3] and certain regions of the central nervous system [5]. Using in vivo autoradiographic techniques, we have described high-affinity sites of amylin binding (Kd ~1 nM) in the rat renal cortex [3]. The pharmacological characteristics of these binding sites, as determined by the pattern of inhibition using peptide antagonists, was very similar to those previously described in the rat brain [5,6]. Amylin is a member of the same homologous group of peptides as calcitonin, adrenomedullin and calcitonin gene-related peptide [1]. Thus far, the calcitonin (CTR), adrenomedullin (ADR) and CGRP receptors have been cloned [7,8]. From the deduced sequences, these receptors are members of a subclass of receptors which traverse the membrane seven times and form an active ternary complex with G-proteins. These complexes represent the first step in the mechanism of signal amplification and activate or inhibit second-messenger systems such as adenylyl cyclase. We have found that the hydrolysis-resistant analogue of GTP, GTP<sub>S</sub>, inhibits amylin binding by 92% to the renal cortex, providing pharmacological evidence that the amylin receptor is itself a member of that same group of receptors [3].

Of importance in these experiments is the difference between binding affinities as determined using autoradiography of tissue sections (nM range), compared with the binding constant determined in membrane preparations of the nucleus accumbens of the basal forebrain, which is 28 pM [6]. This difference, which we believe will be reflected also in the binding characteristics of the renal cortical sites, suggests that receptors are subsaturated within the average physiological plasma concentration of amylin. This is relevant since it would suggest that functions in the kidney and brain would respond in the physiological and pathophysiological range of plasma amylin concentrations. Plasma amylin levels are elevated from an average concentration of less than 10 pM in normal individuals, to around 50–100 pM in hypertensive subjects [9] and up to 400 pM in certain animal models of obesity and hypertension [1].

**Amylin's actions in the kidney**

Besides the novel finding of high-affinity binding sites for amylin in the renal cortex, we have discovered several other important aspects of the renal biology of amylin in the rat. Firstly, in experiments employing in vitro labelling techniques with [125I] rat amylin, using high-resolution light-microscopy and emulsion autoradiography, we have demonstrated that amylin binds to proximal tubules rather than distal tubules, collecting ducts, or the interstitium [10]. Secondly, in micropuncture experiments, amylin, when introduced from the peritubular but not the luminal side of the proximal tubules, significantly stimulated sodium/water reabsorption by 29%. This effect was blocked by the competitive inhibitor of the sodium/hydrogen exchanger, ethyl isopropyl amiloride [10]. Amylin, in these experiments, was demonstrated to have similar efficacy to powerful pressor agents such as angiotensin II and endothelin. Furthermore, when the amylin receptor antagonist, the peptide analogue AC187, was introduced intravenously, sodium/water reabsorption was decreased by 22%, suggesting inhibition of the effects of endogenous amylin and therefore a role for endogenous amylin in salt homeostasis. We have reported that amylin introduced into rats [3] and human volunteers [11] stimulate an acute increase in plasma renin activity. The exact mechanism by which amylin stimulates plasma renin activity is not resolved and may involve both direct and indirect pathways, including the activation of adenylyl cyclase, a known stimulator of renin secretion.

**Amylin as a mitogenic factor**

A significant and related finding is that amylin stimulates the proliferation of primary cultures of epithelial cells isolated from proximal tubules [10]. It is as potent as epidermal growth factor in this regard. This proliferative effect is inhibited by the same peptide antagonists that inhibit binding [10]. Thus this effect is most probably mediated by the amylin receptor located on the peritubular, basolateral side of the proximal epithelial cells, and involves the activation of the sodium/hydrogen antiporter oriented on the luminal surface. Amylin has no significant effect on vascular smooth-muscle cell proliferation. However, the mitogenic effects of amylin have been reported in other cell types such as human endothelial cells of umbilical origin and osteoblasts [12,13].

**Amylin and hypertension**

In the insulin resistance syndrome (syndrome X) there is a constellation of clinical and biochemical features including hypertension, glucose intolerance, obesity, and dyslipidaemia [14]. Although hyperinsulinaemia is commonly observed in this syndrome, the underlying pathogenetic mechanism to explain the link between hypertension and glucose intolerance remains unknown. It has been postulated that amylin, which
influences both kidney functions and carbohydrate metabolism, could be the elusive link [14,15].

To further explore this issue, we have evaluated the role of amylin and its receptors on blood pressure and in various renal models of hypertension. Preliminary results indicate that low concentrations of amylin administered intravenously may have acute pressor effects of ~10 mmHg in the rat, which can be blocked by peptide antagonists and inhibitors of the renin–angiotensin system (Haynes, Hodgson and Cooper, unpublished data, 1996). However, no such acute effects of amylin on blood pressure have been reported after subcutaneous infusions of amylin. Furthermore, the long-term effects of chronic exogenous amylin administration on blood pressure are unknown. Nevertheless, the recent report that amylin antagonism retards the development of hypertension in an animal model of insulin resistance [16] provides further evidence linking amylin to the genesis of certain forms of hypertension. In the animal model of renal ablation (5/6th nephrectomy), which is associated with the development of hypertension, there was a positive correlation between systolic blood pressure and the density of amylin binding in proximal tubules [17]. However, exactly how hyperamylinemia and/or activation of its receptors in the proximal tubules may be involved in the development of hypertension is not clear at this stage.

**Conclusion**

The actions of this hormone relevant to the kidney include activation of plasma renin, stimulation of sodium/water reabsorption, actions as a mitogen on proximal tubular cells, and the ability to elevate blood pressure acutely. It appears that many of these responses are mediated by receptors localized in the proximal tubules and each of these effects could be involved in the genesis and development of hypertension. The advent of amylin agonists and antagonists will provide us and others with the opportunity to explore the role of amylin in renal physiology and ultimately in various pathophysiological states, including hypertension.

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Phosphatonin—a new phosphaturetic hormone? (Lessons from tumour-induced osteomalacia and X-linked hypophosphataemia)

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Phosphorus plays a critical role in cellular metabolism [1]. Phosphorus is an important component of the cell membrane phospholipids; it plays an exceptionally important role in intermediary and energy metabolism; the role of phosphorus in signal transduction and its role in the activation or deactivation of various molecules, usually proteins, by phosphorylation and dephosphorylation is well known; finally, phosphorus is an important component of both RNA and DNA. Clearly, perturbations that alter phosphorus concentrations in the body will have significant biochemical and clinical consequences. Some of the clinical problems associated with hypophosphataemia include osteomalacia and rickets, rhabdomyolysis, and cardiomyopathy. High phosphorus concentrations, such as occur in renal failure, induce secondary hyperparathyroidism and attendant complications.

A normal human ingests about 1–1.5 g of phosphorus in the diet per day [1]. Approximately 75% of what is ingested is absorbed in the intestine, enters the extracellular fluid pool, and equilibrates between bone and serum. About 85% of total body phosphorus is present in bone as hydroxyapatite. Serum inorganic phosphorus is filtered by the glomerulus, and about 80% of the filtered load is reabsorbed, mostly along the proximal nephron [2]. Thus, the kidney plays an exceptionally important role in regulating phosphorus balance.

Normally, shortly after the ingestion of a meal, there are large, influxes of calcium and phosphorus into the body. The resulting elevation of the calcium-phosphate product favours the precipitation of calcium and phosphate as salts in tissues. One of the reasons why calcium and phosphate do not precipitate in the extracellular fluid and soft tissue is that the kidney efficiently excretes excess phosphate by hormonally regulated processes. Several hormones and factors such as parathyroid hormone, 1,25-dihydroxyvitamin D, growth hormone, insulin, and insulin-like growth factors alter renal phosphate reabsorption. However, these hormones and factors, are not solely involved in the regulation of phosphorus concentrations. For example, parathyroid hormone and 1,25-dihydroxyvitamin D are also responsible for the control of serum calcium concentrations. The effects of growth hormone, insulin, and IGF are on other metabolic processes such as anabolism, glucose homeostasis, or cellular growth. It would be biologically advantageous for an organism to possess a factor or a hormone that would specifically respond to alterations in serum or extracellular fluid phosphate concentrations without responding to changes, in calcium or other signals.

There are several lines of evidence suggesting that a factor which specifically regulates phosphorus concentrations might exist in vivo. This notion comes from the study of diseases such as tumour-induced osteomalacia, the epidermal nevus syndrome and X-linked hypophosphataemic rickets (and the Hyp mouse homologue of the latter condition). Patients with these diseases develop hypophosphataemia and osteomalacia [3–9]. Previous work had shown that epidermal nevi and certain tumours might elaborate a factor(s) capable of inhibiting renal phosphate transport [7,8].

Recently, Cai et al. studied a patient with tumour-associated osteomalacia due to a haemangiopericytoma [3], and showed that a heretofore unrecognized factor, 'phosphatonin', is elaborated by haemangiopericytoma cells. The patient had a recurrent soft-tissue tumour associated with hypophosphataemia, a reduced TMP/GFR, low 1,25-dihydroxyvitamin D concentrations, normal parathyroid hormone (PTH) and parathyroid-hormone-related peptide (PTH-RP) concentrations, and osteomalacia. Removal of the tumour on two occasions resulted in a complete resolution of biochemical and clinical defects. The tumour, which on histology was a haemangiopericytoma, was removed and cells were cultured to determine if they were secreting a novel substance capable of inhibiting renal phosphate transport.

Medium conditioned by tumour cells inhibited the sodium-dependent uptake of phosphate in opossum kidney (OK) cells (a model of cultured proximal tubular cells). Control RPMI medium, RPMI medium that had been exposed to Chinese hamster ovary (CHO) cells, did not inhibit phosphate uptake. Parathyroid hormone, as expected, inhibited phosphate transport. The effect of the tumour cell conditioned medium was specific for phosphate, inasmuch that only phosphate transport was inhibited in OK cells, whereas glucose transport and amino acid transport were not. The tumour cell conditioned medium inhib-
ited phosphate transport in OK cells without altering cAMP concentrations within the cell. This suggested that the factor present in tumour conditioned media was not parathyroid hormone. Parathyroid hormone inhibited phosphate uptake, and as expected increased cAMP content in OK cells. The tumour factor was between 8000 and 25 000 Daltons in size and was heat sensitive, suggesting that it might be a protein.

Parathyroid-hormone-like material was detected in the supernatants of the tumour cells with a two-site immunoradiometric assay, RPMI medium, or RPMI medium exposed to CHO cells, had some immunoreactivity, whereas the tumour cell conditioned medium contained twice as much PTH immunoreactivity. The material present in tumour cell supernatants was not parathyroid hormone because, on serial dilution, the decrease in immunoreactivity did not parallel that observed with authentic PTH. To test this further, the effects of a PTH receptor antagonist on the biological properties of the tumour factor or PTH were examined. The antagonist blocked the effect of PTH on phosphate transport, but it did not block the effect of the tumour factor, suggesting once again that this molecule is not parathyroid hormone. PTH-RP was not detected in tumour cell supernatants. The tumour itself stained with anti-PTH antibodies. Since we found PTH immunoreactivity, we thought that the phosphate inhibitor might, in some way, be related to parathyroid hormone in structure.

A tumour cDNA library was screened with PTH antibodies [4]. A clone (Hem 1) which, in its longest open reading frame encoded a peptide that was 381 amino acids long, with a predicted molecular weight of 42 000 Daltons, was isolated. The primary sequence of this peptide did not resemble PTH. The peptide did, however, contain a protein kinase C activation domain similar to that of PTH. This is of some interest, as PTH activates cellular processes by stimulating protein kinase A and protein kinase C. It may be that the PKC activation domain of this particular peptide is responsible for inhibiting phosphate transport. Expression of Hem 1 has not, however, resulted in the synthesis of a phosphate transport inhibitory peptide. Some of the original tumour was implanted in nude mice, athymic mice, and serum phosphorus concentrations in these mice were monitored periodically over a period of several months. Serum phosphorus concentrations decreased in the mice in whom the tumour was implanted.

Because patients with fixed hyperphosphataemia might elaborate a similar renal phosphate transport inhibitor, we sought the presence of such a factor in dialysates obtained from patients dialysed with polysulphone membranes, which have a 30 000–40 000, Dalton cutoff, and would allow such a molecule to pass through [4]. We have characterized such a factor in these dialysates. It appears to differ from that in tumour cells in that it is of somewhat larger molecular weight and inhibits glucose and alanine transport in addition to inhibiting phosphate transport.

Information from the Hyp mouse and from patients with X-linked hypophosphataemic rickets suggests that tumour-induced osteomalacia and the former conditions may be related [5,6]. Meyer et al. showed with parabiosis experiments that Hyp mice with hypophosphataemia and renal phosphate wasting, produce a substance that causes hypophosphataemia in the normal mouse of a normal–Hyp parabiotic pair [5,6]. Reversing the parabiosis normalizes serum phosphorus in the normal mouse. The factor is not PTH, as parathyroidectomizing Hyp mice does not alter their ability to produce hypophosphataemia in normal animals of a parabiotic pair. Nesbitt and others showed that a normal kidney transplanted into a Hyp mouse, exhibits a reduced tubular maximum for phosphate [6]. Conversely, a Hyp mouse kidney transplanted in normal mouse ceases to waste phosphate in the urine.

Recently a mutant gene (PEX) was isolated by the Hyp Consortium from patients with X-linked hypophosphataemic rickets [9]. The abnormal protein was not a phosphate transporter but was related to a family of endopeptidases. It is very likely that the product encoded by this gene does not directly influence phosphate transport. It may, however, break down a normal circulating factor that causes hypophosphataemia. Thus the mutated gene might be responsible for the hypophosphataemia seen in these patients by allowing an excess of this hypophosphataemic factor to circulate.

In conclusion, there is persuasive evidence that a new factor, ‘phosphatonin’, may be responsible for the alteration in phosphate transport seen in various diseases such as tumour-induced osteomalacia, X-linked hypophosphataemia, and hypophosphataemia in the Hyp mouse and the gyroscopic mouse. ‘Phosphatonin’ may be abnormally low in patients with tumoural calcinosis, an unusual disease associated with elevated serum phosphorus, and 1,25-dihydroxyvitamin D concentrations, and ectopic deposition of calcium and phosphorus. ‘Phosphatonin’ may be appropriately elevated in patients with renal failure who have phosphate retention. The study of inhibitors of renal sodium phosphate transport and the characterization of these factors will yield insights into the control of phosphate transport and balance.

References

Protein degradation by proteasomes: molecular mechanisms of muscle catabolism

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Introduction

The turnover of protein in normal adults is enormous, averaging 280 g/day [1], about 90% being intracellular as mammalian cells continually degrade and replace protein. The degradative rates of cellular proteins vary widely: some cytosolic enzymes have half-lives as short as 20 min while others last for days, and the majority of rat hepatocyte proteins are replaced in days but in muscle or brain, the process takes weeks. Clearly, the cell’s proteolytic mechanisms must be highly selective and tightly regulated. If this were not true, uncontrolled destruction of essential proteins or failure to degrade short-lived regulatory proteins would drastically alter cell function. Dramatic increases in our understanding of the mechanisms and regulation of protein degradation have implications for treating human diseases.

Controlled destruction of cell proteins serves important homeostatic functions [1]: (1) rapid removal of rate-limiting enzymes and regulatory proteins is critical in controlling growth and metabolism (e.g. the programmed destruction of cyclins, the cell cycle regulatory proteins); (2) proteolysis allows cells to adapt to physiological conditions (e.g. during fasting, hepatic enzymes for glucose storage disappear while gluconeogenic enzymes accumulate, but within hours of refeeding this pattern reverses); (3) selective elimination of damaged or improperly folded proteins (e.g. after oxygen radical damage or the mutated transmembrane regulator protein in cystic fibrosis); (4) proteolysis is required by the immune system in its surveillance for cancer or virus-infected cells [2]; (5) an inadequate diet or catabolic diseases stimulate the degradation of cell proteins to provide amino acids for gluconeogenesis and protein synthesis. In all these cases, the ATP–ubiquitin–proteasome pathway plays a critical role.

The ATP–ubiquitin–proteasome proteolytic pathway

The ATP–ubiquitin–proteasome pathway degrades proteins by a novel mechanism and functions in the nucleus and cytoplasm of all cells. Proteins degraded by this pathway are first ‘marked’ by covalent conjugation to the small protein cofactor, ubiquitin, in a multistep process requiring three enzymes and ATP (Figure 1) [3]. After a chain of ubiquitin molecules is added, the protein can be recognized by the large 26S proteasome complex in another ATP-dependent process which releases ubiquitin for recycling. The proteolytic core of this complex is the 20S particle consisting of two inner rings, each consisting of 7 beta-type subunits positioned between two outer rings, each consisting of 7 alpha-type subunits [3]. After another ATP-dependent reaction, which releases ubiquitin for recycling, the protein is unfolded and degraded by a novel proteolytic mechanism utilizing threonine at the amino terminus of each beta-type subunit to catalyse cleavage of peptide bonds. This mechanism digests the entire protein to small peptides (i.e. a processive mechanism) which are rapidly degraded to amino acids by cytoplasmic peptidases [3].

How is the cell protected from the disaster of non-specific proteolysis by this pathway? Selectivity depends on the ubiquitin conjugation process because cells contain a variety of ubiquitination enzymes that are specific for different types of proteins (e.g. specific E2 and E3 enzymes combine to catalyse ubiquitination of the individual cyclins to regulate mitosis) [1,3]. Second, the active sites of the 26S proteasome are isolated within its central chamber and only unfolded proteins can enter the narrow opening at the ends of the proteasome complex; unfolding requires recognition of the ubiquitin chain. These characteristics and ATP consumption at multiple steps provide a remarkable degree of selectivity, efficiency, and control of protein degradation.
In catabolic illnesses, the loss of muscle results largely from accelerated breakdown of the long-lived, myofibrillar proteins (actin and myosin) which comprise 60–70% of muscle protein. In response to acidosis, infection, or certain tumors, skeletal muscle protein is lost preferentially while visceral organs (e.g. the kidney and liver) lose little or no protein, and the brain is unaffected. Studies of rat muscles during fasting, acidosis, or denervation atrophy demonstrated that the loss of muscle is primarily due to activation of the ubiquitin–proteasome pathway [4,5]. When atrophying muscles were incubated in vitro with agents that blocked the activity of lysosomal or calcium-activated proteases (calpains), the accelerated proteolysis persisted. However, inhibitors of ATP production reduced muscle protein degradation to levels in control muscles.

Increased levels of ubiquitin-conjugated proteins in muscles coincident with maximal protein degradation provided more definitive evidence for activation of the ubiquitin-proteasome pathway [6]. There also were increased levels of the mRNAs encoding ubiquitin and subunits of the proteasome during fasting, acidosis, and denervation atrophy despite a lower RNA content of muscles [4]. In rats with acidosis from acute or chronic uraemia (CRF), there is increased muscle protein degradation and mRNAs encoding components of the ubiquitin–proteasome pathway [1,7]. In CRF, correcting acidosis blocks the accelerated proteolysis and prevents the rise in mRNAs of ubiquitin and proteasome subunits [7]. These findings point to coordinated adaptations that enhance the proteolytic capacity of the ubiquitin-proteasome pathway and favour muscle wasting.

In experimental models that mimic muscle-wasting conditions, there is consistent activation of the ATP-dependent proteolytic pathway causing muscle protein breakdown [1]. Presumably this response evolved to provide the injured or infected organism with amino acids for energy and synthesis of new proteins (e.g. acute-phase reactant proteins) but if prolonged, muscle wasting occurs. For example, within hours of injecting endotoxin, or live bacteria, or puncture of the caecum, the degradation of myofibrillar proteins by an ATP-dependent process rises sharply [1,8]. After thermal injury, protein catabolism increases markedly in muscles distant from the injury and ATP-dependent proteolysis rises concurrently with ubiquitin mRNA
The ubiquitin–proteasome pathway appears to be responsible for muscle wasting in trauma patients, since mRNAs encoding components of the pathway are high in muscle biopsies [10]. Muscle wasting in cancer cannot be explained simply by anorexia, since tumour-bearing rats show greater muscle loss and higher rates of proteolysis compared to rats fed identical amounts of food [11]. Again, cancer stimulates activity of the ATP-dependent proteolytic pathway and increases ubiquitin mRNA in muscle.

The increased levels of mRNAs encoding ubiquitin and proteasome subunits in catabolic states suggest activation of a common genetic programme to enhance the expression of pathway components, and hence the capacity of this degradative pathway. Recently nuclei were isolated from muscles of CRF or insulinopenic rats and it was found that both conditions stimulate transcription of genes for polyubiquitin and proteasome subunits despite a generalized reduction in RNA in the same muscles [7,12]. Interestingly, expression of enzymes metabolically linked to protein breakdown appears to increase, since acidosis stimulates the oxidation of branched-chain amino acids in muscle and increases the mRNAs encoding subunits of the rate-limiting enzyme in this process, branched-chain ketoacid dehydrogenase [13].

Circulating factors that activate muscle proteolysis

In denervated muscles, cellular events must signal catabolism, whereas in fasting, acidosis, sepsis, etc., muscles respond to circulating factors. One essential factor is glucocorticoids because adrenalectomized rats do not express increased muscle proteolysis or mRNAs encoding components of the ubiquitin–proteasome pathway in response to acidosis or fasting [1]. In cultured myocytes as well, glucocorticoids play a permissive role in stimulating muscle proteolysis and gene expression in response to acidification [14]. Other potential factors include TNF because activated macrophages release TNF and other cytokines in sepsis, certain cancers, and burns (these conditions also release glucocorticoids) [15]. Although TNF does not directly increase muscle proteolysis, it causes anorexia and release of other cytokines that could be signals for accelerated muscle proteolysis in sepsis or cancer [1].

Therapeutic prospects

Greater understanding of the mechanisms and regulation of proteolysis should suggest ways of limiting the excessive proteolysis in cachectic patients. One approach that could be clinically relevant would be to use pharmacological inhibitors of the ubiquitin–proteasome pathway [7]. Complete inhibition would block essential cellular processes but partial suppression of muscle proteolysis in cachectic patients could be beneficial. For example, inhibitors can block the proteasome-dependent activation of the critical transcription factor, NF-κB and can exhibit potent anti-inflammatory effects in models of diseases [1]. Another therapeutic strategy would be to limit production or actions of factors initiating accelerated proteolysis or to stimulate cellular programmes that conserve body proteins. Clearly, knowledge of the regulation and mechanisms of protein breakdown will provide insights into disease processes.

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References

How important is vitamin D deficiency in uraemia?

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Introduction

The identification of 1,25-dihydroxyvitamin D₃ (calcitriol) as the principal hormonal form of vitamin D, and also the development of methods for the reliable measurement of vitamin D metabolites [1] and of parathyroid hormone (PTH), led to a rapid increase in our understanding of the pathophysiology of bone and mineral metabolism in patients with renal disease. Inevitably the progress has also posed new questions and dilemmas. In the earlier years of renal replacement therapy, hyperparathyroidism dominated the clinical picture, but now there is concern that our improving ability to control hyperparathyroidism effectively has led to the emergence of increasing numbers of patients with abnormally low bone turnover—adynamic or aplastic bone disease. Because nutritional vitamin D deficiency is also associated with low bone turnover, it is pertinent to re-examine the question of vitamin D deficiency in uraemia. Although it has been widely assumed that replacement of the deficient hormone by means of alfacalcidol or calcitriol therapy is an appropriate substitute, the possibility that other vitamin D metabolites may also have an important role in the maintenance of normal bone metabolism must be considered.

Renal and non-renal production of calcitriol

In the untreated chronic renal failure (CRF) patient, relative or absolute reduction of circulating calcitriol concentration is invariable. The calcium homeostatic hormone, 1,25-dihydroxyvitamin D₃ (calcitriol), is formed in the kidney by 1α-hydroxylation of circulating 25-hydroxyvitamin D₃. This hydroxylation is tightly regulated by PTH, phosphate, ionized calcium and calcitriol itself. 1α-hydroxylation of 25-hydroxyvitamin D₃ also occurs in a relatively unregulated manner at a number of extrarenal sites [2]. These include sarcoid tissue, pulmonary alveolar macrophages, and placenta. In conjunction with other paracrine factors, these tissues may under normal circumstances produce small quantities of calcitriol necessary for local events.

Work in the early 1980s using embryonic bone cells suggested the possibility that these cells could produce calcitriol from 25-hydroxyvitamin D₃ but this work has never been confirmed and there is no clear evidence for the presence of 1α-hydroxylase in human bone cells, and no physiological role for such an extrarenal enzyme has been demonstrated. This question, however, remains unresolved and awaits the cloning of the 1α-hydroxylase when Northern or Western analysis will be available. Normal peripheral macrophages derived from monocytes synthesize calcitriol from 25-hydroxyvitamin D₃ and in uraemia these macrophages show enhanced calcitriol production and decreased catabolism [2]. A further non-renal 1α-hydroxylase has also been described in pig liver which has a relatively high Kₘ for 25-hydroxyvitamin D₃ (17 nM as compared to 890 nM for the renal enzyme. The Kₘ of this liver enzyme in uraemic rats is increased twofold, indicating decreased substrate affinity. In renal disease the reduction of total 25-hydroxyvitamin D₃-1α-hydroxylase activity in the kidney increases the proportionate contribution of extrarenal calcitriol generation to total calcitriol production.

Actions of 25-hydroxyvitamin D₃ and calcitriol in uraemia

Decreased calcitriol concentration leads to downregulation of the vitamin D receptor (VDR) in the parathyroid gland [3] and in other target tissues such as duodenum and there is evidence suggesting that this is a translational or post-translational effect [4]. Downregulation of VDR would be expected to lead to decreased expression of calcitriol responsive genes; for example 24-hydroxylase activity is decreased by uraemia. The overall effect of these changes is to reduce the catabolism of 25-hydroxyvitamin D₃. A number of low-molecular-weight components of uraemic serum have been shown to decrease VDR synthesis, decrease uptake of ligand bound VDR to the nucleus, reduce binding of ligand-bound VDR to DNA, and decrease binding of ligand-bound VDR to the osteocalcin vitamin D response element (VDRE), and it is now clear that uraemia per se will lead to decreased expression of calcitriol-responsive genes [5] as well as inhibiting renal 1α-hydroxylase. In uraemia, ‘normal’ calcitriol levels may, therefore, still be insufficient to produce appropriate levels of calcitriol responsive gene products. This state of affairs would worsen the consequences of lack of 25-hydroxyvitamin D₃ and may...
in part be the explanation for end-organ resistance in CRF [5].

Both calcitriol and 25-hydroxyvitamin D$_3$ are carried in the plasma bound to vitamin D binding protein (DBP). This protein is never saturated and the affinity of calcitriol for it is approximately 350 times less than that of 25-hydroxyvitamin D$_3$ [6]. It is thought that, like other steroids, it is the free unbound fraction of calcitriol which is physiologically active—this correlates positively with GFR, although the proportion of free calcitriol as a percentage of the total remains constant.

The relative activity of vitamin D, its metabolites and analogues, is usually assessed by measuring their affinity for the vitamin D receptor (VDR). In such assays 25-hydroxyvitamin D$_3$ has been demonstrated to have approximately 2400-fold less affinity for the VDR than calcitriol [6]. 25-Hydroxyvitamin D$_3$ circulates in human plasma at approximately 1000 times the concentration of calcitriol and it might therefore be expected that only very high levels of 25-hydroxyvitamin D$_3$ in plasma would contribute significantly to the overall ‘calcitriol effect’. However, our own studies using transcriptional activation have indicated that 25-hydroxyvitamin D$_3$ is only 500 times less active than calcitriol in a whole cell system [6]. It is likely, therefore, that 25-hydroxyvitamin D$_3$ contributes a significant part of the total ‘vitamin D effect’, and that this contribution is likely to be proportionately greater in uraemia, when calcitriol concentration is low.

How common is vitamin D deficiency in uraemia?

This question presumes that we can first define vitamin D deficiency. In statistical terms it could be, and often is, defined as a plasma concentration of 25-hydroxyvitamin D that falls below the 95% confidence limit (assuming a normal distribution of plasma 25-hydroxyvitamin D concentration) in normal healthy subjects. Published data suggest concentrations of 90±11 nmol/l, implying a lower limit of normal at 68 nmol/l. More relevant biologically, although not adequately assessed, would be the concentration of 25-hydroxyvitamin D below which the first signs of compensation (e.g. secondary hyperparathyroidism, increasing calcitriol concentration or skeletal change) develop. This is likely to be substantially below 68 nmol/l in normals, and is unquantifiable in these terms in uraemia where PTH is profoundly disturbed for other reasons.

A number of studies have suggested that reduction of 25-hydroxyvitamin D$_3$ is common in patients with uraemia. 25-hydroxyvitamin D$_3$ was measured in 22 patients with varying degrees of CRF (in some cases very advanced), and in seven of them concentrations comparable with those in non-CRF patients with documented vitamin D deficiency osteomalacia were found [7]. The same group in 1979 reported low 25-hydroxyvitamin D$_3$ concentrations in patients with terminal renal failure and during the first year of haemodialysis treatment, with normal concentrations found in patients who had been on haemodialysis for more than 1 year or with functioning transplants [8]. Similar findings have been reported by other workers [9–11] who found low-normal, or frankly subnormal, 25-hydroxyvitamin D$_3$ concentration in CRF patients. It is not clear whether ethnic factors are significant determinants of vitamin D status in uraemia. Asians from the Indian subcontinent and East Africa who migrate to Northern Europe have a greatly increased incidence of nutritional rickets and osteomalacia. Furthermore, uraemics show the same seasonal changes in 25-hydroxyvitamin D concentration as do normals [8], suggesting similar dependence of vitamin D sufficiency on sun exposure. It seems likely that ethnic Asians living in temperate climates are at increased risk of vitamin D deficiency, whether or not they have renal disease.

There is, therefore, evidence that measured 25-hydroxyvitamin D$_3$ concentrations are generally lower in CRF patients than in normals and that in subsets of CRF patients, 25-hydroxyvitamin D$_3$ levels may fall in a range that in non-CRF patients is associated with osteomalacic bone lesions. Much less clear is the true prevalence of clinically relevant vitamin D deficiency. This difficulty centres around the definition of vitamin D deficiency and the confounding influence of uraemia on diagnostic criteria (secondary hyperparathyroidism with elevated PTH and/or elevated alkaline phosphatase and/or typical bone lesions) that would apply in non-uraemic subjects. In addition, plasma 25-hydroxyvitamin D$_3$ concentration as a defining parameter of vitamin D deficiency is flawed by questionable assay methodology in many of the early studies and by the necessity for arbitrary definitions of ‘normality’. In the UK, the National External Quality Assurance Scheme has demonstrated wide variations between the results obtained from different laboratories. It is important, therefore, that any claim that 25-hydroxyvitamin D$_3$ levels are lower than normal must be assessed against the normal reported range for the individual laboratory.

It is not entirely clear why 25-hydroxyvitamin D$_3$ levels should be lower in renal disease except that lifestyle alteration and intercurrent illnesses may lead to lack of exposure to sunlight and decreased skin production of the 25-hydroxyvitamin D$_3$ precursor. In patients with significant protein loss (nephrotic syndrome or those on CAPD) a negative correlation has been demonstrated between serum 25-hydroxyvitamin D$_3$ and DBP in the dialysate and the degree of proteinuria [12].

Consequences of vitamin D deficiency and response to treatment

Central to this issue is the possibility of biological actions specific to the various vitamin D metabolites. The presumption that all biological functions of vitamin D are subserved by calcitriol would, if correct, render irrelevant concerns of vitamin D deficiency—calcitriol therapy given for standard indications should
adequately cover those patients with vitamin D deficiency as well. A number of studies have suggested that 25-hydroxyvitamin D₃ and possibly 24,25-dihydroxyvitamin D₃ also, may have actions that differ qualitatively as well as quantitatively from calcitriol. For example in vitamin-D-deficient non-uraemic rats 25-hydroxyvitamin D₃, but not calcitriol, increased muscle phosphate content [13]. This metabolite also improved muscle strength of non-dialysed osteomalacic CRF patients with parallel improvement in mineralization in all the reported patients [14].

Treatment with 24,25-dihydroxyvitamin D₃ has been claimed to attenuate the calcemic effect of calcitriol [15] and 24,25-dihydroxyvitamin D₃ has been reported to have effects in uraemic patients that were qualitatively different from those of calcitriol [15,16]. Despite these findings, however, the notion of a biological role for 24,25-dihydroxyvitamin D₃ remains controversial.

At the level of the skeleton there is little doubt that treatment of CRF patients with 25-hydroxyvitamin D₃ can improve most of the indices of disordered mineral metabolism. Thus 25-hydroxyvitamin D₃ treatment has been shown to increase intestinal calcium absorption and serum calcium concentration and also to decrease elevated PTH and to reduce the severity of osteitis fibrosa [14,17,18]. 25-hydroxyvitamin D₃ has also been shown to be of benefit in patients with osteomalacic bone lesions [14,18]. Comparisons between 25-hydroxyvitamin D₃ and calcitriol are few. Fournier et al. [19] treated matched groups of haemodialysis patients with 25-hydroxyvitamin D₃ or alfalcacidol, and found potentially important differences in the effects on bone. While alfalcacidol was more effective in increasing Ca x Pi product, reducing alkaline phosphatase and improving hyperparathyroid bone lesions, 25-hydroxyvitamin D₃ was more effective in improving bone formation.

The mechanisms underlying these differences remain obscure. It is very likely that 25-hydroxyvitamin D₃ is acting directly on the vitamin D receptor, albeit only when given in pharmacological doses, as occurs in health to a limited degree [6]. It is also likely that the large doses of 25-hydroxyvitamin D₃ that have been used increase residual renal and/or extrarenal generation of calcitriol [2], perhaps at specific local sites.

Practical considerations

The scale of the clinical problem relating to vitamin D deficiency in uraemia is difficult to assess. Nevertheless, the inclusion of modest vitamin D supplementation in the overall package of dialysis therapy has potential benefits and few, if any, drawbacks if given at a dose of 1000–2000 units/day. This would be sufficient to induce ‘normal’ plasma concentrations of 25-hydroxyvitamin D₃ in virtually all subjects. Such supplementation should remove any remaining concern of vitamin D deficiency in uraemia. The routine administration of pharmacological doses of 25-hydroxyvitamin D₃ as a substitute for alfalcacidol or calcitriol is not convincingly supported by available data and would increase the likelihood of more prolonged periods of hypercalcaemia in the event of overdosage.

References

Should hyperlipidaemia in dialysis patients be treated?

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The pattern of lipid abnormality in dialysis patients

The abnormalities of lipoprotein metabolism that are associated with renal failure are not corrected by renal replacement therapy and may be manifested as hyperlipidaemia in the dialysis population [1]. Elevated plasma triglyceride levels are found in between 30 and 70% of individuals undergoing maintenance dialysis whilst hypercholesterolaemia, although less commonly observed, is more likely to be present in patients receiving CAPD [2]. Global measurement of plasma lipids tends to underestimate the size and scope of the problem since even when concentrations of total triglyceride and cholesterol levels fall within conventional normal ranges, assessment of lipoprotein profiles reveals accumulation of partially metabolized, triglyceride-enriched very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) particles. In addition there may be a reduction in high-density lipoprotein (HDL) and an increase in lipoprotein (a). Low-density lipoprotein (LDL) levels are normal or only modestly increased, although the composition and size of these particles may be altered.

The precise pathogenesis of these abnormalities is not fully understood, although impaired function of certain key enzymes including lipoprotein and hepatic triglyceride lipases appears to be important [1]. Longitudinal studies suggest that any changes associated with peritoneal dialysis occur early in the course of treatment and that lipoprotein abnormalities remain otherwise stable over time on dialysis [3].

Cardiovascular risk in dialysis patients

With the availability of a number of new lipid-lowering drugs, nephrologists may now be in a position to offer safe and effective therapy to correct the lipoprotein disturbances associated with uraemia [4]. However, many will need to be convinced that such intervention is appropriate and that any benefits outweigh potential risks. The most compelling argument in favour of treatment is that patients receiving dialysis appear to be at particularly high risk of cardiovascular disease. This observation, originally made over 20 years ago, is supported by recent registry data demonstrating that such individuals experience a 16–19-fold increased risk of myocardial ischaemia and infarction when compared to appropriate age- and sex-matched populations without renal failure [5]. Similar results were obtained in both Southern and Northern European countries, despite underlying differences in the susceptibility to cardiovascular disease in the indigenous populations of these regions. Furthermore, patients with an underlying diagnosis of diabetic nephropathy appear to be at 2 to 3 times greater risk than those with non-diabetic renal disease.

With the success of dialysis and renal transplantation, cardiovascular diseases including myocardial infarction, sudden death, cardiac failure, and stroke have become the leading cause of mortality in patients with chronic renal failure, collectively accounting for approximately 50% of deaths in those who would have naturally succumbed to uraemia. Further efforts to prolong the lives of these individuals will need to take into account the risk factors that may contribute to both vascular and myocardial disease. In addition to hyperlipidaemia, these may include hypertension, glucose intolerance, hyperfibrinogenemia, and elevated plasma homocysteine levels [6].

Lipid abnormalities and cardiovascular risk

To date there have been no controlled clinical trials demonstrating that correction of deranged lipid metabolism in individuals receiving renal replacement therapy protects against cardiovascular disease. However, studies in the general population have established that lipid-lowering therapy leads to a reduction in both ischaemic cardiac events and mortality. Some nephrologists may be content to simply extrapolate these data to the high-risk group of patients under their care who arguably may benefit most from such treatment. Others may correctly point out that studies in non-renal populations have concentrated on the consequences and management of hypercholesterolaemia resulting from elevated LDL levels, an abnormality which is not a major feature of lipid disturbances complicating uraemia. Hypertriglyceridaemia, in contrast, is not considered to be an independent risk factor and as such, has not become a primary target for intervention. This approach may be misguided according to a recent study which suggested that elevated plasma triglyceride levels are a powerful predictor of coronary disease risk if coincident with a high ratio of LDL to HDL cholesterol [7]. These observations imply that the pattern of lipoprotein disturbance present in dialysis patients would be expected to enhance cardiovascular risk and argue strongly in favour of offering

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such patients therapy aimed at lowering triglycerides and LDL whilst restoring HDL levels to normal.

In an attempt to strengthen the case for treatment, a number of studies have examined the relationship between various lipid parameters and the presence of clinically apparent atherosclerosis in individuals receiving renal replacement therapy. Unfortunately many of these trials have been cross-sectional in nature, including only small numbers of patients, and have failed to distinguish atherosclerosis-related events from other forms of cardiovascular disease. In addition, many have failed to control for other risk factors and have assessed only total plasma lipid levels rather than the lipoprotein disturbances which characterize the uraemic dyslipidaemia. It is therefore not surprising that the conclusions have been conflicting [6]. In the largest and longest study to date, 419 dialysis patients were followed prospectively over a 21-year period during which time 49% died of cardiovascular disease and 23% experienced fatal or non-fatal ischaemic events. Smoking, hypertension, and hypertriglyceridaemia were identified as independent risk factors for ischaemic cardiovascular disease in this population [8]. In contrast, several smaller cross-sectional studies have failed to find an association between elevated triglyceride levels and complications resulting from vascular disease [6]. Another recent prospective trial has identified elevated levels of lipoprotein (a) as an independent predictor of clinical events attributable to atherosclerotic cardiovascular disease in patients receiving haemodialysis [9]. Furthermore, in a group of 196 uraemic diabetics receiving haemodialysis, elevated cholesterol levels with high LDL to HDL ratios were associated with an increased risk of cardiac death during a 45-month follow-up period. In this study, hypercholesterolaemia was more common in the diabetics than in matched non-diabetic dialysed controls [10].

**Lipid lowering therapy in dialysis patients**

Nephrologists who choose to treat lipid abnormalities in their dialysis patients may be reassured by a few studies demonstrating the efficacy and safety of lipid-lowering drugs [4]. Such data should dispel early fears that therapy would be associated with an unacceptably high incidence of side-effects in this population. Whilst dietary changes should not be neglected and optimal regimens still need to be established, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors and fibric acid derivatives are proving to be the most promising agents in the dialysis population. Fibric acid derivatives are a logical choice because they enhance the activity of lipase enzymes that facilitate VLDL metabolism and can result in reduction in VLDL triglycerides of up to 50% and correction of low HDL levels [1]. Unlike clofibrate, newer drugs in this class do not accumulate in renal failure although low starting doses are generally recommended. HMG-CoA reductase inhibitors lower cholesterol by enhancing receptor-mediated clearance of LDL, particularly by the liver. Because elevated levels of this lipoprotein are not a prominent feature of the uraemic dyslipidaemia, the choice of this class of agent would appear less logical. However, HMG-CoA reductase inhibitors have been reported to improve clearance of partially metabolised VLDL ‘remnant’ particles. Whilst these agents may be particularly useful in the rarer patients who develop hypercholesterolaemia, looking beyond reductions in plasma lipids as the principal therapeutic goal, they may well have an important impact on the lipoprotein disturbances present in dialysis patients. Indeed, the precise targets of lipid-lowering therapy in such individuals still need to be clearly defined. For example, the benefits of cholesterol lowering may not be confined to patients with elevated baseline values in this high risk population.

**Conclusion**

Ideally, a controlled trial of lipid-lowering therapy in dialysis patients is needed. Such a study will require a large population randomized to receive either treatment or placebo and long-term follow-up. Risk factors other than lipid abnormalities will need to be controlled for and clearly defined cardiac end-points assessed. Such a trial will become increasing difficult to conduct as nephrologists become uneasy about withholding lipid-lowering therapy from their patients and logically conclude that abnormalities of lipid metabolism in the dialysis population represent a serious risk factor for progressive atherosclerosis.

**References**

Metabolic abnormalities in renal transplant recipients. Risk factors and predictors of chronic graft dysfunction?

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Introduction

Renal transplant patients are known to be at increased risk of cardiovascular disease. Since cardiovascular mortality is the leading cause of death in transplant recipients after the first year, it represents a major threat for the long-term results of transplantation [1].

The search for risk factors for atherosclerotic cardiovascular disease has challenged many investigators during the past few decades. Numerous of these risk factors are closely related to each other. Insulin resistance seems to be a key feature of the most frequently observed disease entity associated with increased cardiovascular morbidity in the industrialized world [2]. This syndrome, described originally by Reaven as syndrome X [3] is called by many names, such as the ‘syndrome of insulin resistance’, the ‘metabolic risk factor syndrome’, the ‘metabolic syndrome’, the ‘cardiovascular metabolic risk factor syndrome’ etc. We prefer the name ‘metabolic risk factor syndrome’.

The classical features of this syndrome include obesity, in particular a central one, insulin resistance and hyperinsulinaemia, with normal or impaired glucose tolerance and eventually manifest diabetes. Hypertension and lipid abnormalities, in particular elevation of the very-low-density lipoproteins (VLDL) and low levels of HDL cholesterol are other characteristics of this syndrome. As recently revealed, elevated levels of the major inhibitor of fibrinolysis, plasminogen activator inhibitor type 1 (PAI-1) is another important part of the metabolic risk factor syndrome [4]. Recent evidence suggests an important role of genetic factors in the development of this syndrome [5,6].

The lipid abnormality

Hypercholesterolaemia, probably through the effect of oxidatively modified LDL, and elevation of Lp(a) are other established risk factors for atherosclerotic vascular disease [7–9]. Even though these features do not represent a part of the metabolic risk factor syndrome, the induction of PAI-1 gene expression by oxidized LDL or Lp(a) and the observation that features of the metabolic syndrome are present in subjects with small, dense, cholesteryl ester depleted LDL particles [6], known to be more susceptible for oxidative modification [10] point towards a possible link between these ‘independent’ risk factors and the metabolic risk factor syndrome.

Abnormalities in lipid and carbohydrate metabolism occur frequently in patients with chronic renal disease [11,12]. After renal transplantation, these abnormalities are even more pronounced. The currently used immunosuppressive therapy with cyclosporin and corticosteroids is generally considered to have an important contributory role in this regard [11]. However, pretransplant lipid levels seem to have a strong correlation with post-transplant hyperlipidaemia [13]. Two years following transplantation, an increase in total cholesterol, due to elevation of LDL and HDL cholesterol but a decrease in VLDL cholesterol, and unchanged total and VLDL triglycerides with a decrease in LDL triglycerides have been reported in patients on triple immunosuppression [14]. Insulin resistance is a feature frequently observed in patients with end-stage renal disease [12]. Both prednisolone

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and cyclosporin are considered to be diabetogenic, and at present more than 10% of cyclosporin/prednisolone-treated transplant recipients will develop post-transplant diabetes mellitus (PTDM). A positive family history of diabetes is more frequent in these patients, pointing towards the role of associated genetic factors [15]. Similarly to patients with NIDDM, insulin resistance with hyperinsulinaemia and subsequent development of insulin deficiency are observed in patients with PTDM [16].

Abnormalities of the fibrinolytic system

Abnormalities of the fibrinolytic system are associated with increased cardiovascular morbidity [17]. An impaired fibrinolysis is present in two of three patients at 1 year following renal transplantation, possibly due to prednisolone therapy [18]. The defective fibrinolysis, in the majority of the patients, is a consequence of elevated levels of plasminogen activator inhibitor (PAI-1), the specific inhibitor of the fibrinolytic system. In vitro, insulin as well as incubation with VLDL were shown to augment endothelial cell synthesis of PAI-1. Hyperinsulinaemia as well as hypertriglyceridaemia are associated with elevated PAI-1 levels, and this may well be the link between defective fibrinolysis and the metabolic risk factor syndrome [4].

The metabolic syndrome and chronic graft rejection

In addition to the increased risk of cardiovascular disease, metabolic abnormalities were recently suggested to be involved in the pathogenesis and progression of chronic rejection, a major reason for late graft losses [19,20]. The histopathological hallmark of chronic vascular rejection (CVR), vascular intimal hyperplasia, resembles early atherosclerotic lesions, which is the reason why this process is also called transplant arteriosclerosis. The pathogenetic mechanisms of CVR in renal transplants have been suggested to be similar to that of ‘naturally’ occurring atherosclerosis [20].

Hyperlipidaemia and chronic graft rejection

Evidence from both experimental and clinical studies indicate a role of lipid abnormalities as important contributors to the development and progression of CVR. In renal transplant patients with manifest CVR, hyperlipidaemia is more pronounced with a more atherogenic pattern compared with recipients with a stable graft function [19]. Moreover, as it was recently shown in a prospective investigation, the development of histopathological changes compatible with CVR at 6 months after transplantation, which in turn is an excellent predictor of subsequent late graft losses [21,22], is associated with more pronounced lipid abnormalities already prior to transplantation. Conversely such histopathological changes are also more frequent in patients with pretransplant hypercholesterolaemia [23]. Hypercholesterolaemia both before [14] and after [22] renal transplantation were found to predict graft loss due to chronic rejection, while other studies could only identify hypertriglyceridaemia as an independent risk factor for chronic rejection [24].

Patients who developed PTDM were found to have an inferior graft survival at 5 years compared to patients without diabetes. Furthermore, extreme obesity was also found to be associated with an unfavourable graft outcome [25].

Fibrinolysis inhibitors and chronic graft rejection

In a recent prospective study elevated PAI-1 levels were found in one-third of renal transplant recipients before transplantation. Patients with elevated PAI-1 levels before transplantation were not different from patients with normal PAI-1 levels with regard to age, BMI, lipid variables, original disease, or the time on dialysis. However, at 2 years follow-up, they were more

<table>
<thead>
<tr>
<th>Table 1. Features of the metabolic risk factor syndrome. Prevalence before and after renal transplantation</th>
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<tr>
<td><strong>Features</strong></td>
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<tr>
<td>Obesity</td>
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<td>Hypertension</td>
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<td>Hypertriglyceridaemia</td>
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<td>Hypercholesterolaemia</td>
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<tr>
<td>PTDM</td>
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<td>Low insulin response</td>
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<td>Hyperinsulinaemia</td>
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<tr>
<td>Impaired fibrinolysis</td>
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<td>Elevated PAI-1 activity</td>
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</table>

PTDM, post-transplant diabetes mellitus.
Table 2. Metabolic risk factors 6 months after renal transplantation. Graft function at 6 months and outcome after 2 and 3 years

<table>
<thead>
<tr>
<th>Metabolic risk factors at 6 months</th>
<th>All (n = 12)</th>
<th>None (n = 10)</th>
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<tbody>
<tr>
<td>Graft function and morphology at 6 months</td>
<td></td>
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<tr>
<td>S-creatinine$^{2,3}$</td>
<td>194 ± 52</td>
<td>123 ± 40</td>
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<tr>
<td>C-cr$^{2,4}$</td>
<td>37 ± 21</td>
<td>60 ± 24</td>
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<tr>
<td>U-albumin excretion$^{2,5}$</td>
<td>0.32 ± 0.23</td>
<td>0.06 ± 0.06</td>
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<tr>
<td>CGD score$^{3}$</td>
<td>6.90 ± 2.85</td>
<td>3.17 ± 1.72</td>
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<tr>
<td>Graft outcome after 2 and 3 years</td>
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<tr>
<td>Graft loss 6 months–2 years</td>
<td>2</td>
<td>0</td>
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<tr>
<td>Graft function at 2 years (n = 10)</td>
<td>233 ± 93</td>
<td>120 ± 30</td>
</tr>
<tr>
<td>S-creatinine$^{2,3}$</td>
<td>30 ± 18</td>
<td>57 ± 16</td>
</tr>
<tr>
<td>Cr-EDTA clearance$^{2,4}$</td>
<td>0.40 ± 0.74</td>
<td>0.25 ± 0.66</td>
</tr>
<tr>
<td>U-albumin excretion$^{2,5}$</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Graft outcome 6 mths–3 years</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Graft function at 3 years (n = 7)</td>
<td>191 ± 55</td>
<td>113 ± 25</td>
</tr>
</tbody>
</table>

$^{1}$mmol/l; $^{2}$values are mean ± SD; $^{3}$ml/min; $^{4}$g/24 h. C-cr, endogenous creatinine clearance; CGD, chronic graft damage score in histological specimen.

obese, had lower HDL cholesterol levels and an inferior glucose tolerance in addition to higher PAI-1 levels (unpublished observations).

Elevated pretransplant PAI-1 activity was found to have prognostic value in the sense that it predicted an unfavourable outcome with regard to early and late graft and patient loss. During a 3-year follow-up, a more than fivefold increase in the risk of graft loss was observed when PAI-1 activity was elevated prior to transplantation. In those patients with a functioning graft at 6 months, histopathological features compatible with CVR were also more pronounced.

**The whole spectrum of the metabolic risk factor syndrome and chronic graft rejection**

Patients presenting at 6 months after transplantation with the whole spectrum of the metabolic risk factor syndrome, including obesity, dyslipidaemia, impaired glucose tolerance or manifest NIDDM and/or hyperinsulinaemia with elevated PAI-1 levels and/or impaired fibrinolytic capacity were older and more overweight and had more lipid abnormalities and higher PAI-1 levels before transplantation, compared to patients without any of these metabolic risk factors at 6 months. Graft function at 6 months was worse in patients with the whole spectrum of the metabolic risk factor syndrome, and they also had histopathological changes compatible with CVR to a higher degree based on a higher CGD score on transplant biopsy. Graft outcome at 3 years post-transplant was different, with 5/12 grafts lost among these patients, while no graft losses occurred in patients without metabolic risk factors at 6 months (unpublished observations).

**Pre-existing vs acquired risk factors**

It could be argued, that impaired renal allograft function contributes to the metabolic disturbances observed. However, patients with all features of the syndrome X present at 6 months post-transplant were different from patients without metabolic abnormalities before transplantation, i.e. during the phase of end-stage renal failure. Thus it seems reasonable to assume that the extent of metabolic abnormalities at 6 months was not only a consequence of impaired graft function, and that pre-existing metabolic abnormalities could have contributed to a worse graft function at 6 months.

**Summary**

In summary, abnormalities in lipid and carbohydrate metabolism, including features of the metabolic risk factor syndrome, are frequently present in patients both before and after renal transplantation. Risk factors of atherosclerosis may not only contribute to increased cardiovascular morbidity and mortality in this patient population, but can also be assumed to contribute to the development and progression of CVR and chronic graft dysfunction.

For preventing both early graft losses and the development of graft damage leading to late graft dysfunction and graft loss, it appears to be essential to identify patients at risk early, prior to transplantation. Intervention aiming to reduce overweight, diet and exercise may be of benefit. The role of $\omega-3$ unsaturated fatty acid supplementation remains controversial. Pharmacological intervention by antioxidants or agents to reduce lipids and/or decrease PAI-1 synthesis may prove to be beneficial. Early identification of patients at risk and intervention in due time may improve the results of renal transplantation.

**References**


