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The review of literature pertaining to the study “In-vivo evaluation of protein supplement in the management of uremia” is explained under the following headings.

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A) Uremia - metabolic changes and effect on nutritional status

1. Uremia

   The term Uremia or Uraemia is called as ‘urea in the blood’. The primary component of urine is urea. Urea is compound derived from metabolic end product of amino acids and dietary protein along with the accumulation of creatinine. Urea and creatinine usually excreted in the urine under normal function of kidneys. Uremia or uremic syndrome is terminal clinical manifestation of kidney failure (Bishop *et al*, 2010). Azotemia also refers to uremia when the abnormality can be measured when there is no symptoms (Bishop *et al*, 2010). The accumulated compounds are called as uremic toxins or uremic solutes and interfere with normal physiological or biological functions. It is the indication of inadequate regulatory, endocrine and excretory functions of kidneys (Burtis *et al*, 2007).
1.1. **Uremic Toxicity**

Uremia affects virtually all tissues, cells and organs in the body with the consequences for most systems and body functions with metabolic, nutritional, immune and hormonal system derangements are therefore noticeable features of the uremic syndrome. The word uremia is derived from two ancient Greek words: ounon means urine and haima means blood; literally, uremia is urine in the blood. Thus, the in term uremia implies that accumulation of excretory products such as urea in the blood results in the toxic condition of uremia (Kopple, Massry and Kalanter-Zedah, 2013).

1.2. **Uremic symptoms and signs**

Uremia affects central nervous system with striking neurological signs and symptoms including mental changes, fatigue, muscle twitching, stupor, convulsions and coma, as well as symptomatic peripheral neuropathy (Krishnan and Kiernan, 2007).

The gastrointestinal systems such as anorexia and vomiting may be associated with stomatitis, glossitis, gastritis, pancreatitis and enterocolitis (Carrero, 2011).

Cardiovascular symptoms including hypertension (Sarnak et al 2003 and Van Buren and Toto, 2012), left ventricular hypertrophy (Stack and Saran, 2002 and Paoletti et al, 2012) and heart failure (US Renal Data System, 2010) (disorders connected with salt and water retension), pericarditis (US Renal Data System, 2010), cardiac arrhythmias (Herzog et al, 2008), cardiomyopathy (Cice et al, 2003), endothelial dysfunction and atherosclerosis (Tonelli et al., 2004) are also common features of the uremic syndrome.

Other typical changes in fluid and electrolyte balance are hyperkalemia (Krishnan and Kiernan, 2007), metabolic acidosis (Phisitkul et al, 2010), hyperphosphotemia (Craver et al, 2007) and disturbance in calcium homeostasis (Martin and Ritter et al, 2005). Decreased production and increased destruction of red cells result in anemia (Kopple, Massery and Kalander-Zadeh, 2013).
Impaired homeostasis leads to the bleeding from mucous membrane and in the skin (caused by defect in platelet aggregation) and fibrinolysis are inhibited as well (Zachee et al., 1993). Pruritis (Berger and Steinhoff, 2011 and Mettang and Weisshar, 2010) and hyperpigmentation (Kuypers, 2009) of the skin in chronic uremia are also frequently encountered.

2. Metabolic changes in uremia

Changes in acid base balance in the uremic condition are the predominant cause of other metabolic changes such as alteration in carbohydrate, protein and fat metabolism. Other typical metabolic changes pertaining to the endocrine functions such as renal anemia and bone mineral disease occurs in uremia (Chadban et al., 2010).

2.1. Acid-Base balance

Kidneys play a predominant role in maintaining acid base and electrolyte balance in the human body. Maintenance of acid-base balance is achieved by renal excretion of daily acid load (1 meq/kg per day) produced from synthesis of sulphuric acid during the metabolism of sulphur containing amino acids (Warnock, 1988 and Bailey, 2005).

As the nephrons declines in chronic kidney disease, excretion of acid is maintained by increase in the ammonium excreted by the nephrons until the Glomerular Filtration Rate (GFR) falls below 40-50 mL/min (Bailey, 2005). This is followed by the retention of hydrogen ions leading to metabolic acidosis (Uribarri and Douyon, 1995). The acid retained is buffered by bicarbonate present in the extracellular fluid in tissues and bones (Lemann, Litzow and Lennon, 1966). As the renal function worsens, progressive metabolic acidosis develops and become more prominent in advancing stages of Chronic Kidney Disease (CKD) (Eustace et al, 2004). The figure of acid base balance is shown in the figure 1 (Koeppen, 2009).
Role of kidneys in acid base balance

A sustained decrease in glomerular filtration rate is the hallmark of progressive kidney disease. As GFR decreases, solutes that are excreted by the kidney (creatinine and urea) accumulate in body fluids, and the concentration of solutes in the plasma increases. Other solutes also can accumulate in body fluids, including phosphates, sulfates, uric acid and hydrogen ions. The accumulation of hydrogen ions leads to the development of metabolic acidosis (Mitch, 2006).

2.2. Disorders of carbohydrate metabolism

Massry (2007) describes that the abnormal glucose metabolism of patients with renal insufficiency is characterized by fasting euglycemia, abnormal glucose tolerance, a delayed decrease in blood glucose in response to insulin, hyperinsulinemia and hyperglucagonemia. The factors leading to abnormal glucose metabolism in renal insufficiency are as follows

- Fasting blood glucose level is usually abnormal
- Possible spontaneous hypoglycemia (because of decreased gluconeogenesis and alanine deficiency)
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- Fasting hyperinsulinemia, increased plasma levels of proinsulin and peptide-C
- Increased plasma levels of immunoreactive glucagon and growth hormone
- Decreased glucose use in response to insulin in peripheral tissues (mainly in muscle)
- Impaired insulin secretion from pancreatic islets

2.3. Insulin resistance

The major mechanism underlying the development of glucose intolerance in uremia is resistance of peripheral tissues, particularly muscle, to insulin. Metabolic studies, both in vivo and in vitro have uncovered impaired insulin-mediated glucose uptake in muscle. A circulating factor might induce insulin resistance at the level of muscle, but increased levels of growth hormone also might contribute to the resistance of peripheral tissues to insulin and impaired insulin secretion (Mitch, 2009).

2.4. Metabolic acidosis

According to DeFronzo (1981) the metabolic acidosis of renal insufficiency can contribute to insulin resistance, leading to impaired glucose transport. Indeed, chronic acidosis, produced by ammonium chloride administration in healthy individuals leads to changes insulin dependent glucose transport similar to those seen in patients with renal insufficiency.

2.5. The role of β cells

The glucose tolerance of patients with renal insufficiency can be normal if the β cells of the pancreas secrete insulin approximately. If this condition occurs, patients have normal fasting serum glucose level, but at the expense of elevated levels of insulin in plasma. A deficiency of calcitriol (1,25-dihydroxycholecalciferol) can contribute to the resistance of peripheral tissues to insulin. Calcitriol apparently interacts with pancreatic islets to modulate the secretion of insulin (Cecchin, 1988).
2.6. Glucose transport in fat cells

According to Stumvoll (1996) fat cells from patients with Chronic Kidney Disease exhibit decreased glucose uptake in response to insulin when compared with adipocytes obtained from healthy subjects. Lipogenesis in response to insulin is also blunted, and decreased levels of insulin or resistance to its effect can decrease the activity of lipoprotein lipase, which has an important role in removing triglycerides.

2.7. Abnormal insulin release

A reduced release of insulin during initial response to hyperglycemia indicates β cells in the pancreas have reduced sensitivity to glucose. Parathormone (PTH) apparently by enhancing the movement of calcium into the β cells seems to impair insulin secretion from such cells. Thus, the secondary hyperparathyroidism in patients with renal insufficiency can compromise the ability of the islets to secrete insulin appropriately and maintain normal glucose homeostasis (Weisinger, 1988).

3) Effects of uremia on nutritional status

3.1. Carbohydrate metabolism in uremia

In patients with chronic kidney disease, abnormalities of carbohydrate metabolism are encountered at different levels of the insulin-glucose cascade. The two major defects that underlie glucose intolerance in Chronic Kidney Disease (CKD) are resistance to the peripheral action of insulin and impaired insulin secretion. When these two abnormalities are present glucose intolerance ensues (Massry and Smogorzewski, 2007).

According to DeFronzo et al (1973) patients with end-stage renal disease are almost always more or less resistant to the peripheral action of insulin. This is true although the half-life of insulin is prolonged as a consequence of delayed insulin removal by the damaged kidney as well as by extrarenal organs. Consequently plasma insulin concentrations tend to be higher at any given rate of insulin secretion.
As reported in detail by Westervelt and Schreneir (1962), using the forearm perfusion technique, peripheral glucose uptake is reduced in CKD. This observation was confirmed by DeFronzo (1980) using gold standard method, i.e. the euglycemic insulin clamp technique, which allows quantitating the amount of glucose metabolized per unit of insulin (DeFronzo and Alverstand, 1980). Such peripheral resistance to insulin is seen in early stages of CKD. Peripheral resistance to insulin is a clinically important parameter, because in CKD it is tightly correlated to increased cardiovascular risk (Shinohara et al, 2002) and to accelerated progression of CKD (Becker et al, 2005).

Insulin resistance is involved in the development of the catabolic state of uraemia. Muscle catabolism in inflammatory states is mediated by proinflammatory cytokines, e.g. interleukins 1 and 6 (IL-1, IL-6) and TNF-α (Hotamisligil et al, 1994; Hotamisligil, 2003 and Ikizler, 2008).

A number of studies showed that insulin secretion is impaired in CKD (Fadda et al, 1991 and Oh et al, 1994). One factor responsible for impaired insulin secretion in CKD is high plasma parathormone (PTH) concentrations. Insulin secretion, as assessed by the hyperglycemia clamp technique, is improved when the parathyroid gland is suppressed (Mak et al, 1985). Glucose-induced insulin secretion is impaired even in rats with normal renal function when they receive daily injections of PTH (Fadda et al, 1988 and Perna et al, 1990).

Apart from glucose, amino acids are stimuli triggering insulin secretions, the most potent being L-leucine (Milner, 1969) and an additional stimulus is K⁺ (Oberwetter, 1987).

The kidney plays a major role in the insulin metabolism in normal subjects (Adrogué, 1992). Molecular weight of insulin is 6000 and it can be freely filtered. Approximately 60 percent occurs by glomerular filtration and 40 percent by extraction from the peritubular vessels of total renal insulin clearance. Insulin in the tubular lumen enters proximal tubular cells by carrier-mediated endocytosis and is then transported into lysosomes, where it is metabolized to amino acids.
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(Carone and Peterson, 1980). Impaired tissue insulin sensitivity occurs in almost all uremic subjects and is largely responsible for the abnormal glucose metabolism seen in this setting (Alvestrand, 1997).

Madzarovova-Nohejlova (1969) determined the disaccharidase activities in small intestinal biopsies of uremic patients in which there was reduction in lactase and cellobiase activity. The activities of sucrase, trehalase and maltase were also found to be diminished in the structural changes of mucosa of uremic patients. Olsen and McNair (1974) examined the biopsy specimens of uremic patients observed diminished activity of maltase and sucrase along with lactase. Denneberg *et al* (1974) demonstrated the fall in activity of trehalase activity in renal insufficiency patients but in contrast Pointner *et al* (1974) observed the normal trehalase tolerance in chronic hemodialysis patients. Grimmel *et al* (1974) studied in 5/6 nephrectomised rats that there was the fall in maltase and sucrase activity whereas there is no change in cellobiase activity. Bloch *et al* (1973) investigated using segmental small intestinal perfusion of sixty three year old phenacetin induced nephropathy female patient with serum creatinine 7.6 mg/100 ml and serum urea of 130 mg/100 ml found glucose absorption of 50.87 mg/cm/h was found in the lower normal range.

In oral glucose tolerance test with a 30 g/m² loading dose in chronic hemodialysis subjects, it was ruled out that the rise in blood glucose was lesser than in healthy individuals (Denneberg *et al*, 1974), whereas Cerletty and Engbring (1967) studied higher mean blood glucose levels in uremic patients after receiving oral glucose of 100g. Olsen and McNair (1974) also found that glucose-galactose tolerance test by administration of 25g to each uremic subjects produced normal but delayed rise in blood glucose.

Mcvicar *et al* (1980) studied the effect of intestinal transport of glucose in chronic uremic rats demonstrated that there was significant less weight gain in uremic rats compared to normal control rats regardless of similar food intake signifying decreased energy efficiency in chronic uremic rats. The increased absorption of glucose was also noted in uremic rats due to altered glucose
metabolism in uremia (DeFronzo et al, 1973). This increased glucose absorption was taken up by the liver which increases the sensitivity of glucagon present in uremia (Habold et al, 2005).

3.2. **Overview of dietary protein and amino acid metabolism**

Each and every part of our human body is made up of proteins and these proteins have various functions signalling molecules and receptors, structural membranes, intracellular trafficking component, enzymes, hormones, ion pumps, carbon di oxide and oxygen transporters etc. Proteins are found in muscles, collagens, tendons, enzymes, hormones, bones etc. and they involve in various biochemical reactions. The most important function of protein is for growth and repair. Even though the body can synthesis proteins from amino acids, the important source of proteins is food. Since human beings are unable to synthesis the 20 amino acids, the proteins and amino acids required by the body should be supplied through diet only (May, 1987).

3.3. **Metabolic demand for amino acid**

Metabolism of protein, nitrogen and aminoacids together define the metabolic demand for protein in the human diet. The major purpose for metabolic demand for amino acids is to maintain suitable tissue level, to provide for all amino acid derived metabolites and to provide during addition needs during growth, pregnancy and lactation. The regulation is obtained from dietaryproteins, tissue proteins (after proteolysis), de novo synthesis (amino acids and ammonia from urea), bacterial metabolism at lower Gastro Intestinal tract (Fuller and Reeds, 1998).

Uauy et al, (1981) states that the metabolic demand for amino acids seems to involve essential and adaptive components. The requisite components for subjects at equilibrium (maintenance) comprises transformation of some individual amino acids into important metabolites that are further transformed into nitrogenous end products, mainly urea and other compounds in urine, faeces or sweat, as well as net synthesis of proteins lost from the body as skin, hair and any other secretions (Raguso, Perreira and Young, 1999). The extent of the
maintenance component is assumed empirically equal to the sum of all nitrogen losses from the body observed on a protein-free diet, after losses have stabilized at a low level, i.e. the obligatory nitrogen loss.

The adaptive component of the metabolic demand represents amino acid oxidation at a rate varying with the habitual protein intake, which occurs as a result of the increasing activities of the pathways of oxidation of amino acids that regulate free amino acid pool sizes (Millward, 2003).

Amino acid oxidation and urea synthesis are irreversible. The rate of urea synthesis is usually in excess of the rate of urea excretion, because some urea enters the lower gastrointestinal tract and is hydrolysed by bacteria. Most of this nitrogen is utilized by bacteria and since little is lost as faecal nitrogen, it is eventually returned to the systemic pool as ammonia and amino acids, including indispensable amino acids (Jackson, 1995).

3.4. Protein metabolism in uremia

Several studies suggest that high intake of protein by subjects with kidney disease contribute to the weakening of kidney function (Millward, 1999; Anderson and Brenner, 1986, Wiseman, 1987 and Rudman, 1988). Glomerular filtration rate also declines as aging in healthy subjects but it has no improvement when low protein intake is followed (Brenner, Meyer and Hotteller, 1982). Studies in rats shows that low protein intake during chronic renal failure prevent the prolongation of the disease (Brenner et al, 1982).

However, the mechanism would occur in humans, with the decline in kidney function through deterioration in filtration by non-sclerotic nephrons, rather than by glomerular sclerosis that occurs in rats (Walser, 1992). In another study, where the group of subjects consuming wide variety of dietary proteins where looked for protein intake and kidney function, it was found that there was relationship between glomerular filtration rate and protein intake, but there was no relation with indicator of renal function, the albumin excretion (Brandle, Sieberth and Hautmann, 1996).
Walser (1992) argued that symptomatic renal failure does not result from the physiological decrease in glomerular filtration rate that occurs with age, because symptoms do not occur until the glomerular filtration rate has decreased much more than occurs with ageing. Moreover, the protein restriction lowers glomerular filtration rate, suggesting that the decline in protein intake as age progresses and is unrelated to deterioration of renal function (Lew and Bosch, 1991). As concluded by Walser (1992), protein restriction on grounds of renal function is admissible and prudent only in subjects who develop kidney failure owing to hypertension, polycystic kidney disease or diabetes.

Metabolism of proteins and amino acids is altered in patients with chronic kidney disease and as renal function decreases, nitrogenous waste products of protein metabolism accumulate, eventually resulting in symptomatic uremia. During the evolution of progressive renal insufficiency, subtle alterations occur in the concentrations of plasma proteins and in the levels of amino acids in plasma and intracellular compartments (Kopple, Maasery and Kalender-Zadeh, 2013).

The loss of protein stores can be result of inadequate dietary intake or increased requirements caused by changes in intermediary metabolism that result from kidney failure. In certain situations, extravascular pools of albumin can be reduced, even though the serum albumin concentration remains normal. Although inadequate protein or energy intake frequently can be measured, the possibility that renal failure per se disturbs one or several steps in the complex process of protein synthesis and degradation has not received sufficient study. Recent research indicates that increased catabolism of proteins can indeed occur in the muscle of patients with only moderate renal insufficiency (Kopple, Maasery and Kalender-Zadeh, 2013).

3.5. Changes in amino acid profiles in renal insufficiency

Chronic kidney disease subjects have decreased ratio of essential to non-essential amino acids, a pattern that mimics that seen in protein-calorie malnutrition. The abnormalities in plasma amino acids in patients with renal insufficiency cannot be explained on the basis of malnutrition alone, because
these abnormalities also occur when protein intake is optimal suggesting that abnormal levels in plasma might be the result of changes in amino acid metabolism caused by renal insufficiency (Alvestrand et al., 1982).

Deferrari et al, (1988) states that the plasma levels of branched chain amino acids like valine, leucine, and isoleucine and their respective ketoacids are decreased with valine being reduced to a greater extent than the others. The potential mechanism responsible for the decreased concentration of branched chain amino acids might be related to oxidation of such amino acids in skeletal muscle as a consequence of metabolic acidosis.

Patients with renal insufficiency also exhibit decreases in plasma concentrations of threonine and lysine, as well as low serine levels. The low serine levels might be attributable to decreased production of this amino acid from glycine in the kidney. Tyrosine levels are usually decreased, and this decrease presumably is related to defective phenylalanine hydroxylation. The plasma levels of phenylalanine are usually normal, whereas total tryptophan is decreased in patients with uremia, although free tryptophan levels are normal (Young et al, 1975).

The explanation for this difference is that the binding of tryptophan by plasma proteins in subjects with uremia is reduced. Levels of certain amino acids like glycine, citrulline, cystine, aspartate, methionine and 1 and 3-methylhistidine are increased in plasma of patients with renal insufficiency (Suliman et al, 1997). The elevated plasma concentration of citrulline is caused by a decrease in conversion of this amino acid to arginine in the damaged kidney. Interestingly, the higher citrulline level in plasma seems to correct any arginine deficit in patients with advanced renal disease. Metabolites of sulphur-containing amino acids accumulate in the blood (Druml et al, 1994).

**B. Gentamicin nephrotoxicity in female wistar rats**

With increasing number of drugs and easy availability of over-the-counter medications like Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), the incidence of drug induced nephrotoxicity has become more prevalent. Other medications
like angiotensin converting enzyme inhibitors, antibiotics and NSAIDs play a pivotal role in producing kidney damage. Among Indians, drug-induced Acute Renal Injury contributes 20 per cent out of 40 per cent are caused by amino glycosides (Jha and Chug, 1995).

Approximately 20 percent of hospital-acquired and community based acute renal injury caused by drugs (Bellomo et al, 2006). The incidence of drug induced nephrotoxicity in older adults is around 66 percent (Kohli et al, 2000).

### Table I

**List of drugs associated with nephrotoxicity (Kim and Moon, 2012)**

<table>
<thead>
<tr>
<th>Drugs class/drug(s)</th>
<th>Pathophysiologic mechanism of renal injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesics</td>
<td>Chronic interstitial nephritis</td>
</tr>
<tr>
<td>Acetaminophen, aspirin</td>
<td>Acute interstitial nephritis, altered intraglomerular hemodynamics, chronic</td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory drugs</td>
<td>interstitial nephritis, glomerulonephritis</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Tubular cell toxicity</td>
</tr>
<tr>
<td>Antidepressents/mood stabilizers</td>
<td>Rhabomyolysis</td>
</tr>
<tr>
<td>Amitriptyline, doxepin, fluoxetine</td>
<td></td>
</tr>
<tr>
<td>Lithium</td>
<td>Chronic interstitial nephritis, glomerulonephritis, rhabdomyolysis</td>
</tr>
<tr>
<td>Antihistamines</td>
<td>Rhabomyolysis</td>
</tr>
<tr>
<td>Diphenhydramine (Benadryl), doxylamine (Unisom)</td>
<td></td>
</tr>
<tr>
<td>Antimicrobials</td>
<td>Acute interstitial nephritis, crystal nephropathy</td>
</tr>
<tr>
<td>Acyclovir (Zovirax)</td>
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</tbody>
</table>

Gentamicin, an amino glycoside antibiotic used to treat the infections caused by gram negative bacteria. Because of its nephrotoxic effects the use is limited in clinical sectors. Renal oxidative stress, acute tubular necrosis, glomerular damage and renal inflammation are some of the effects caused by gentamicin in creating nephrotoxicity in rats. The mechanism behind inducing nephrotoxicity is that it causes lysosomal phospholipidosis which interrupts the normal kidney function (Raheem, 2009).
Mechanism of gentamicin induced nephrotoxicity (Luft, 1984)

The mechanism behind gentamicin in inducing nephrotoxicity is explained:

The drug gets accumulated in the renal cortex and proximal tubular cells in the kidneys. From the cortical cells, the aminoglycosides bound to lysosomes forming myeloid bodies or secondary lysosomes. It was believed that the aminoglycosides release into the cytoplasm and interferes with phosphatidylinositol pathway.

The graphical representation of cytotoxicity effects of gentamicin (Pessoa et al., 2009) is shown in figure 2.

![Mechanisms and cell signaling pathways underlying the cytotoxic effect of gentamicin. ATP, adenosine triphosphate; CaSR, extracellular calcium-sensing receptor; Cyto c, cytochrome c; ER, endoplasmic reticulum; PPARα, peroxisome proliferator-activated receptor-α; ROS, reactive oxygen species; UPR, unfolded protein response.](image-url)

**Figure 2**

Cytotoxicity effects of gentamicin
Integrative pathophysiology of gentamicin nephrotoxicity (change the wordings)

Tubular dysfunction leads to the loss of fluid and electrolytes that swiftly fire the Tumorous Growth Factor (TGF) response, which reduces Renal Blood Flow (RBF) and Glomerular Filtration Rate (GFR) to the appropriate level. Because, under physiological circumstances approximately 99 per cent of water and electrolytes in the ultrafiltrate are reabsorbed along the tubule, a drastic reduction in GFR must be accomplished to compensate for a small reduction in tubular reabsorption, thus preventing the life-threatening loss of water and electrolytes. That is why even a mild injury to the tubular epithelium may bring about a pathological reduction in GFR and renal failure. However, TGF adapts within hours and its control over GFR is lost even in the presence of an increasing tubular incompetence.

Below Figure 3 shows the mechanisms leading to a reduced GFR. It can be observed that tubular malfunction leading to a defective reabsorption is the only mechanism that causes no GFR reduction directly, although it decreases GFR indirectly by activating the TGF mechanism, at least transitorily. Tubular obstruction increases progressively with tubular damage, as does its contribution to the reduced GFR. As such, it only partially explains the whole reduction in GFR, especially in the initial phase of acute kidney injury, which is the most relevant clinical situation. Contracting factors produced by mesangial, vascular, and tubular cells, including ROS, PAF, angiotensin-II, and endothelin-1 act in an autocrine and paracrine manner to induce contraction of glomerular vessels and mesangial cells, which reduce RBF and $K_r$, respectively, and lower GFR (Martínez-Salgado et al, 2007).

A question for the future is if a part of the reduction in GFR caused by gentamicin would still occur, should tubular alterations be completely and specifically prevented, or, whether most glomerular and vascular effects are, at least partially, independent of tubular damage. As explained above, gentamicin-induced mesangial activation and contraction have been documented in cultured, isolated mesangial cells, indicating that no tubular-derived stimulation is necessary for these effects (Martínez-Salgado et al, 2007).
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Integrative view of the mechanisms leading to gentamicin nephrotoxicity. It can be appreciated that, in the absence of a significant tubular obstruction, vascular and mesangial mechanisms are necessary to explain the reduction in glomerular filtration (GFR) and renal excretion, once the tubuloglomerular feedback adapts. ANG-II, angiotensin-II; ATP, adenosine triphosphate; ET-1, endothelin-1; GFR, glomerular filtration rate; $K_u$, ultrafiltration coefficient; $\Delta P$, net ultrafiltration pressure; PAF, platelet-activating factor; $P_t$, intratubular pressure; RBF, renal blood flow.

Figure 3

Integrative view of the mechanisms leading to gentamicin nephrotoxicity
In addition, reduced GFR and RBF may contribute to aggravating gentamicin-induced tubular damage, probably because they limit oxygen and nutrient availability to tubular cells and facilitate oxidative stress, as it has been demonstrated in the ischemic renal failure (Moran et al, 1992).

**C. FORMULATION OF DIETARY SUPPLEMENT**

Dietary Supplement Health and Education Act defines dietary supplement is a product (other than tobacco) that is intended to supplement the diet; contains one or more dietary ingredients (including vitamins, minerals, herbs or other botanicals; amino acids and other substance) or their constituents; is intended to be taken by mouth as pill, capsule, tablet or liquid and is labelled on the front panel as being a dietary supplement.

Huang et al (2013) reported in his study that low protein diet with keto acid supplementation obliterated the initiation of autophagy in skeletal muscle and decreased muscle loss in rats with type 2 diabetic nephropathy. Moreover, Shimomura et al (2014) revealed that dietary L-lysine supplementation prevents arterial calcification in adenine-induced uremic rats by modifying the key pathways that exacerbate vascular calcification.

Animal studies have shown that low protein diet decelerates the growth and decrease serum albumin levels and supplementation with keto acids may correct these aberrations in rats with chronic kidney disease (Huang et al, 2011). In addition, results of a clinical trial have recommended that protein restriction along with essential amino acids supplementation and ketoanalogues postpone the onset of end stage renal failure without worsening the nutritional status of patients with non-diabetic (Chauveau et al, 1999 and Aparicio et al, 2000) or diabetic nephropathy (Barsotti et al, 1987 and Barsotti et al, 1988).

**D. DIETARY PROTEIN QUALITY EVALUATION**

**4.1. Concept of requirement**

Protein requirement has historically been challenging to estimate, thus engendering wide debate (Millward and Jackson, 2004). The word ‘requirement’,...
may in itself, be bewildering as it can refer to: (1) Metabolic requirement i.e., biological demand for the quantity of nitrogen and Indispensable Amino Acid (IAA) consumed in various metabolic pathways. (2) Dietary requirement (or estimated average requirement) for the minimum amount of dietary nitrogen and indispensible amino acid that satisfies the biological demand for nitrogen and indispensible amino acid at the individual level.(3) Safe level of intake at the population level which takes into account individual variations in requirement (Reeds and Beckett, 1997; FAO/WHO/UNU, 2007).

Metabolic requirement includes needs for maintenance of body protein equilibrium, which represent the largest part of the requirement in the adult human, and needs for growth (protein deposition), plus extra needs for reproduction or lactation. Whereas the growth requirement can be defined from the amino acid composition of newly deposited protein and the efficacy with which bioavailable amino acid support protein deposition (Reeds and Garlick, 2003).

Determination of protein and amino acid requirement is very difficult since it lies on numerous factors like individual biology (genotype, reproductive/developmental stage), environment (infection or injury), lifestyle (level of physical activity) and nutrition (adequate energy intake).

Adaption of body’s metabolism to low protein diet is an unresolving issue (Nicol and Philips, 1976; Waterlow, 1990 and Millward, 2003). Millward (2003) and Millward and Jackson (2004) strongly sued that long term and slow adaptive amino acid oxidation illustrated in previous studies in which from 7 to 40 days are required to accomplish nitrogen balance after variations in the protein intake level. Conversely, authors like Young and Borgonha (2000) and Rand et al (2003) consider that metabolic adaption is not a main issue, as studies have reported that an adaption period of 4-5 days (usually used in nitrogen balance studies) is acceptable for the body to reach a fairly steady state of nitrogen balance. This is been authorized by international authorities (FAO/WHO/UNU, 2007).
4.2. Dietary protein requirement

In spite of many shortcomings in the accuracy of nitrogen balance method, it is the only valid method available for the evaluation of nitrogen requirement (FAO/WHO/UNU, 2007). The meta-analysis conducted by Rand et al (2003) on nitrogen balance studies gave inputs on estimation of current protein requirement (FAO/WHO/UNU, 2007). The determination of total requisite nitrogen losses, determined after feeding the subjects with a low-protein diet for approximately 5-6 days, is critical. But nitrogen losses in the urine and the faecal losses can be measured directly.

The criterion on which the nitrogen requirement definition is based has a question, as although it is presumed to reflect health maintenance, it might not emulate the optimal conditions of specific physiological functions like bone health, immune function or muscle mass (Millward, 1996). Till now there is no clear quantifiable data on nitrogen requirement (FAO/WHO/UNU, 2007).

In summary, the current dietary protein requirement has to be considered within the limited context of the nitrogen balance approach and has thus been defined recently as “the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity, plus, in children or in pregnant or lactating women, the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health” (FAO/WHO/UNU, 2007).

4.3. Indispensable amino acid requirements

There are two different types of amino acids and there exist a absolute need for each amino acids for which some amino acids are synthesised (dispensable) in the body and whereas some amino acids should be obtained from dietary sources since it cannot be synthesised (indispensable) in the body. The formation or synthesis of dispensable amino acids depends on appropriate nitrogen intake (FAO/WHO/UNU, 2007). The classification of amino acids (Reeds et al, 2000) is given in Table II.
Table II
Classification of amino acids (Reeds et al, 2000)

<table>
<thead>
<tr>
<th>Dispensable amino acid</th>
<th>Conditionally Indispensable amino acid (Essential under specific pathological conditions)</th>
<th>Indispensable amino acids (Essential under all circumstances)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic Acid, Glutamic Acid, Alanine, Serine, Asparagine</td>
<td>Cystine, Tyrosine, Taurine, Glycin, Arginine, Glutamine, Proline</td>
<td>Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine</td>
</tr>
</tbody>
</table>

The WHO/FAO/UNU (2007) expert consultation conducted a detained critical analysis of the reported amino acid requirement values for infants, children and adults and the methodologies are used in their derivation. The amino acid requirements for adult humans (FAO/WHO/UNU, 2007) are listed in Table III.

Table III
Amino acid requirements for adult humans

<table>
<thead>
<tr>
<th>Indispensable amino acids</th>
<th>Requirements (mg/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>15</td>
</tr>
<tr>
<td>Valine</td>
<td>26</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>20</td>
</tr>
<tr>
<td>Leucine</td>
<td>39</td>
</tr>
<tr>
<td>Phenylalanine+tyrosine</td>
<td>25</td>
</tr>
<tr>
<td>Lysine</td>
<td>30</td>
</tr>
<tr>
<td>Histidine</td>
<td>100</td>
</tr>
<tr>
<td>Methionine + cysteine</td>
<td>15</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>4</td>
</tr>
</tbody>
</table>

In-vivo evaluation of protein supplement in the management of uremia
4.4.  *In vivo* protein quality index

Routine determination of protein quality index has to be done. Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) were the older method to determine the protein quality in growing rats but these methods were criticized for difference in growth rates and requirement for amino acids for humans and rats particularly sulphur containing amino acids (National Research Council, 1974). Moreover, these methods does not employed for mixed protein source and hence other methods were proposed (Table IV).

**Table IV**

Amino acid scoring pattern across species

The below table shows the amino acid scoring pattern across species

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Adult human(^a) (mg/g protein)</th>
<th>Laboratory rat(^b) (mg/g protein)</th>
<th>Growing pig(^c) (mg/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>23</td>
<td>41</td>
<td>34</td>
</tr>
<tr>
<td>Valine</td>
<td>39</td>
<td>49</td>
<td>36</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>30</td>
<td>41</td>
<td>28</td>
</tr>
<tr>
<td>Leucine</td>
<td>59</td>
<td>71</td>
<td>50</td>
</tr>
<tr>
<td>Phenylalanine+tyrosine</td>
<td>38</td>
<td>68</td>
<td>48</td>
</tr>
<tr>
<td>Lysine</td>
<td>45</td>
<td>61</td>
<td>52</td>
</tr>
<tr>
<td>Methionine+cysteine</td>
<td>22</td>
<td>65</td>
<td>30</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>6</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>


4.4.1. Biological value

The “biological value” of a protein, as the term was applied originally by Karl Thomas, referred to the utilization by the body of the products of protein digestion. The biological value was expressed as the percentage of the absorbed nitrogen which was retained by the body for repair or the construction of nitrogenous tissue (Mitchell, 1952).
Biological value is the measure of protein quality by calculating the amount of nitrogen used for formation of tissues divided by the amount of nitrogen absorbed from the food and is multiplied by 100 and expressed as percentage of nitrogen utilized from the food. Biological value provides a measurement of how efficient the body utilizes the protein consumed in the diet. The biological value of the protein can be measured in different ways (Bender, 1953) under standardized conditions to determine the protein quality for the species and age of animal under investigation.

Osborne, Mendel and Ferry (1919) concisely expressed the view that “Economy of food can be affected only by supplying the young animal with as much as it will eat; below that at which the normal growth can be maintained”. Animal food sources are good protein and have higher biological value since it provides all the essential amino acids for the growth and development whereas protein sources from the vegetables lack certain essential amino acids and hence low biological value (Hoffman and Falvo, 2004). The biological value also measures a proteins maximal potential quality rather than requirement levels.

Biological value is also discussed in terms of demand of indispensible amino acids for protein synthesis. It is greatly influenced by relative amount of dispensable and indispensible amino acids and other nitrogen-containing compounds (Milliard and Rivers, 1986). Biological value of the yeast and brewer's yeast were determined by nitrogen retention method and growth method (AOAC, 2006).

4.4.2. Net post prandial protein utilization

After PDCAAS, Net Postprandial Protein Utilization (NPPU) method was developed fully and applied over years proposed by Humayun et al (2007) based on “Amino acid oxidation indicator”. This tool was very useful in the determination of the metabolic availability of amino acids in the dietary proteins. Net Post prandial Protein Utilization (NPPU) was determined by the classical nitrogen balance method. The net protein retention on daily weight basis was the major limitation in this method of estimation.
4.5. Chemical Score

Classical method of evaluating protein quality were bioassays by measuring the retention of nitrogen or growth in the young laboratory rats fed on diets containing varying kind and amount of protein. The initiation of methodology for amino acids estimation led to the idea of chemical score.

FAO/WHO defines the chemical score as protein relative to the amino acid composition of egg protein. The biological assays such as Protein Efficiency Ratio (PER), Biological Value (BV) and Net Protein Utilisation (NPU) involve animal feeding trials in which biological value and net protein utilisation requires the collection of biological matters such as urine and faecal for the purpose of nitrogen balance measurement and determines more work than other procedures.

Chemical score is determined by measuring the content of indispensable amino acids in the test protein and comparing the same with the reference protein. Egg albumin is considered as complete and nutrient dense. The test protein required purification before the hydrolysis. It is hydrolysed to constituent amino acids and subjected for analysis in the amino acid analyser. The amino acid in the test protein in the lowest level is called limiting amino acid and the value obtained in the percentage is called chemical score.

4.6. Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The Protein Digestibility Corrected Amino Acid Score (PDCAAS), a method to assess the protein quality is based on indispensable amino acid requirements, was recognized as a best method (FAO/WHO/UNU, 2007; FAO/WHO, 1991). The formula for calculating the PDCAAS based on protein digestibility and amino acid score is (Schaafsma, 2000)

\[
PDCAAS = \text{Digestibility} \times \frac{\text{mg of first limiting Amino acid in 1g of test protein}}{\text{mg of the same Amino acid in requirement pattern}}
\]
Here the digestibility refers to the fecal nitrogen digestibility assessed in growing rat. This method also was criticized by few authors (Darragh et al, 1998, Tome and Bos et al, 2002). The important factor under criticism is digestibility. First, the PDCCAS refers to overall digestibility of proteins, which does not concern about the digestibility of individual amino acid digestibility. Second factor under criticism is ileal digestibility should be considered rather than faecal digestibility of protein in the calculation of PDCAAS (Darragh and Hodgkinson, 2000) because ileal digestibility is appropriate for dietary protein digestibility. Thirdly estimation of digestibility in pigs rather than rats is best because pigs might be the better model for humans (Rowan et al, 1991; Darragh and Hodgkinson, 2000).

Another controversial matter relates on PDCAAS assumption is based on amino acid score in which amino acid score determines the biological value. This suggests that biological value is determined only by amino acid profile of the dietary protein (Millward, 2003).

It was projected that PDCCAS is an index to evaluate the protein quality of the diet on the whole and does not rely on specific protein, the PDCAAS value of above one were of no special interest. A “protein source quality index” for individual proteins with PDCAAS value higher than one could be established (FAO/WHO/UNU, 2007). Furthermore PDCAAS does not consider the potential anti nutritional factor present in the protein and thus overestimates the nutritional quality of the protein (Sarwar, 1997 and Gilani et al, 2005).

Gilani et al (2005) and Sarwar (1997) highlighted in the PDCAAS method of evaluation of protein quality, the anti-nutritional factors that are present in the proteins are not considered which leads to the overestimation of the nutritional quality of dietary protein under estimation.

Finally it was postulated that even though PDCAAS method is useful in determination of protein quality, revisions are still required. Particularly, it does not take in to account consequent metabolism of amino acid absorbed and thus hence the accuracy of biological value of the protein is affected (Gaudichon et al,
5. **Digestibility**

Protein digestibility is a key component in evaluation of protein quality evaluation as it indicates the extent to which dietary protein has been digested and absorbed as amino acid by the gastrointestinal tract, and thus provides a measure of bioavailability i.e., the proportion of dietary amino acid that are absorbed in a chemical form that renders them potentially suitable for protein metabolism (Fuller and Tome, 2005; Stein *et al.*, 2007; Moughan, 2003). Indirect measure of protein digestibility can be done by obtaining the difference between intake and gut losses. Hence the overall digestibility of protein is determined from the measure of nitrogen digestibility. In addition to it, digestibility of individual amino acid can also be determined.

**5.1. Faecal versus ileal digestibility**

In the past, the digestibility of the proteins is derived from faecal estimation. But concerns raised due to the prolific microbial nitrogen metabolism within the hindgut and because amino acid are absorbed predominantly in the small intestine (Krawielitzki *et al.*, 1990) and this method was considered inaccurate in estimation of absorbed dietary amino acid (Darragh and Hodgkinson, 2000; Moughan, 2003; Fuller and Tome, 2005). Colonic and caecal microbial metabolism includes degradation of undigested amino acid and peptides and the de novo synthesis of microbial amino acid yielding a faecal amino acid profile which is quantitatively and qualitatively different from the pattern of undigested amino acid (Fuller and Tome, 2005). Mosenthin (2002) and Moughan (2003) revealed that faecal digestibility coefficient differ from ileal coefficients, especially for individual amino acids.

Ileal and faecal digestibility in human was compared in few controlled studies and it was shown that ileal nitrogen digestibility was lower compared to faecal level (Rowan *et al.*, 1991). Greater difference were observed for amino acid digestibilities, where the ileal levels are much lower (2-17 per cent) compared to
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faecal level. Hence, in summary, ileal amino acid and nitrogen digestibility values can be obtained after correction of endogenous loss compared to faecal values for the estimation of protein quality of dietary amino acid and nitrogen that are absorbed from the intestine.

5.2. Apparent versus true digestibility

Apparent nitrogen digestibility is determined as the difference between the quantity of ingested nitrogen and the total nitrogen losses from the gut. A major disadvantage of apparent digestibility is the non-linear correlation between apparent digestibility and dietary protein level, which results in non-additivity of apparent nitrogen digestibility in mixtures of protein sources (Stein et al., 2007). This is due to the relative contribution of gut endogenous nitrogen to total nitrogen losses. Endogenous nitrogen losses are components basal or minimal losses that are lost by the body and are independent of dietary factors and specific losses that are influenced by dietary factors (Stein et al., 2007). When apparent nitrogen digestibility is corrected for total (basal and specific) endogenous nitrogen losses, true digestibility also referred as real digestibility (Mariotti et al., 1999) is determined.

True digestibility reflects the specific fate of dietary nitrogen within the gut and corrects for any variation of the endogenous fraction related to dietary factors. It is thus fundamental property of protein source itself (Mosenthin, 1999) and allows the metabolic costs associated with synthesis and recycling of gut endogenous amino acid losses (Stein et al., 2007). Protein digestibility when determined at the ileal level and corrected for endogenous losses appears to be a good predictor of dietary amino acid bioavailability compared to faecal digestibility (Hendriks et al., 2003). Heat treatments of some proteins can lead to chemical modification of amino acids such as lysine, which is commonly absorbed through the intestinal membranes but not utilized for protein synthesis (Moughan and Rutherford, 1996). The recycling of intestinal dietary nitrogen, possibly in microbial amino acid, contribute to the differences between digestibility and bioavailability (Fuller and Tome, 2005; Metges et al., 1999).
Studies conducted in growing rats on apparent digestibility shows that coefficient of apparent faecal digestibility overestimated by 14 per cent (Bodwell et al, 1980 and Low, 1989) and were poorly correlated with human coefficients, which means the values for true faecal digestibility were in better agreement between species with rat average values are 3 per cent higher (Table V).

Table V

**Apparent and true faecal digestibility of nitrogen as estimated in adult humans and growing rats within controlled studies**

<table>
<thead>
<tr>
<th>Protein source</th>
<th>Number</th>
<th>Apparent digestibility (per cent)</th>
<th>True digestibility (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Human</td>
<td>Rat</td>
</tr>
<tr>
<td>Canned tuna</td>
<td>4 6</td>
<td>75.2</td>
<td>93.7</td>
</tr>
<tr>
<td>Spray dried whole egg</td>
<td>5 6</td>
<td>77.8</td>
<td>91.4</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>5 6</td>
<td>84.9</td>
<td>94.2</td>
</tr>
<tr>
<td>Peanut flour</td>
<td>4 6</td>
<td>76.3</td>
<td>88.9</td>
</tr>
<tr>
<td>Soy isolate</td>
<td>5 6</td>
<td>81.0</td>
<td>91.7</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>4 6</td>
<td>81.7</td>
<td>93.3</td>
</tr>
<tr>
<td>Mixed protein sources(^2)</td>
<td>- 6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mixed protein sources(^3)</td>
<td>8 5</td>
<td>87.6</td>
<td>89.2</td>
</tr>
<tr>
<td>Mixed protein sources(^4)</td>
<td>7 5</td>
<td>88.4</td>
<td>90.8</td>
</tr>
</tbody>
</table>

Source: Bodwell et al, 1980

\(^1\)Corrected for constant estimate of faecal endogenous nitrogen losses

\(^2\)Average digestibility over 2 mixed diets based on vegetable or vegetable/animal protein

\(^3\)Vegetable and animal proteins. Average digestibility over 4 diets with similar protein content but different fiber content

\(^4\)Vegetable and animal proteins. Average over 3 diets with similar protein content but different fibre content

Table V shows the apparent and true faecal digestibility of nitrogen as estimated in adult humans and growing rats within controlled studies.

The greater difference between rats and humans for apparent digestibility than for true digestibility may result from a higher contribution of endogenous nitrogen losses to total nitrogen losses in humans than in rats, although the amino acid compositions of their endogenous protein losses seem to be similar.
E. FLOW OF NITROGEN ALONG THE DIGESTIVE TRACT

6. Endogenous flow of nitrogen

Endogenous secretion of nitrogen originates from oral cavity, stomach, bile, pancreas and small intestine. Nitrogen in the mouth originates from saliva which contains digestive enzymes like α-amylase, glycoproteins (mucins), urea, free amino acid, uric acid and creatinine (Buraczewska, 1980). Nitrogen in gastric juice originates from digestive enzymes (pepsins), mucins, desquamated cells and to a lesser extent, urea and ammonia (Just, 1981; Buraczewski, 1980).

The small intestine is functionally divided into duodenum, jejunum (two-fifths of the length of the small intestine) and finally ileum, structurally indistinct from the jejunum (Sanford, 1982). The small intestine can synthesize up to 400 g protein/day in pigs (Souffrant et al., 1985), which is one of the highest synthesis rate capacities in the body.

The nitrogen input from the small intestine has been reported to be higher than that produced from bile, pancreas and stomach together (Just and Jorgensen, 1982). Dietary factors have been reported to influence intestinal secretions. Intestinal secretions of nitrogen compounds were markedly decreased in pigs fed a protein-free diet (Buraczewska, 1979).

Intestinal endogenous nitrogen arises from mucins, epithelial enzymes (aminopeptidases, maltases, lactases etc), pancreatic enzymes, desquamated cells and to a lesser extent urea and some leaked plasma proteins (albumin, glutathione) (Fauconneau and Michel, 1970; Snook, 1973). Although not strictly endogenous, microbial proteins are usually included in the estimate of endogenous protein.

The large intestine, which comprised the caecum (for rats and pigs), colon and rectum, has been reported to secrete 1.6-4.5 g endogenous nitrogen per day (Krawielitzki et al., 1990). Microbial nitrogen was reported to contribute up to 60-80 per cent of total faecal nitrogen (Low and Zebrowska, 1989). Microbial activity is maximal in large intestine; it is also present to a lesser extent in the
duodenum and jejunum, and to an intermediate extent in the ileum (Moughan and Donkoh, 1991).

Bacteria can metabolize nitrogen from non-protein (ammonia) or protein sources from endogenous or dietary origin. Although this measure was undertaken at the ileal level, an increased dietary protein intake was shown to induce a higher intestinal flow of bacterial nitrogen, mostly due to a higher incorporation of dietary nitrogen into microbial proteins (Mason et al, 1976).

Intestinal microflora may synthesize de novo IAA. Synthesis and utilisation of microbial IAA have been demonstrated in various monogastric mammals (Belenguer et al, 2005, Metges, 1999 and Metges and Loh, 2003).

F. DETERMINATION OF ILEAL NITROGEN FLOW

7.1. Ileal digesta collection

7.1.1. Indigestible marker

When a total digesta collection cannot be undertaken, non-absorbable markers have to be used to allow the sample to be extrapolated to a dietary intake or a total intestinal effluent. Ideally, a marker should be neither digested nor absorbed through the gastrointestinal tract; it should be inert; it should have the same dissolving properties as the substance under study and should have a homogeneous distribution within the intestinal lumen; and the method for its analysis in samples should be sensitive and specific (Modigliani et al, 1973; Kozloski et al, 1998). Although in fact there are no such markers, substances meeting most of criteria have been used.

Chromic oxide (Cr$_2$O$_3$) and titanium dioxide (TiO$_2$) have been widely used as dietary markers in animal digestibility studies. Jagger et al (1992) demonstrated that Cr$_2$O$_3$ had a lower faecal recovery (quantity collected from a total collection of faeces expressed as a proportion of that ingested, an important indicator of the marker reliability) than TiO$_2$ and that TiO$_2$ induced lower standard errors for apparent ileal nitrogen digestibility than Cr$_2$O$_3$. An accurate and reproducible method for TiO$_2$ determination was proposed by Short et al (1996).
TiO2 has been proposed as a reliable marker that can be used in both animals and humans (Rowan et al., 1991; Kavanagh et al., 2001). However, the large amount of digesta sample (1.5-2 g) required for analytical determination is a limiting factor.

7.2. **Measurement of endogenous nitrogen and amino acid losses**

7.2.1. **Protein-free diet**

When a protein free-diet is fed to animals or humans for several days, all of the nitrogen and amino acid recovered in ileal digesta is of endogenous origin. The use of protein-free diets is known to induce lowered ileal endogenous protein flows compared with protein-containing diet (Darragh et al., 1990; Butts et al., 1993a; Moughan et al., 2005). Feeding a protein-free diet to animals or subjects creates a non physiological state (Low, 1980) by inducing a negative nitrogen balance, possibly leading to a decreased rate of body and possibly gut protein synthesis (Millward and Garlick, 1976).

**G. THE INFLUENCE OF KIDNEY DISEASE ON PROTEIN AND AMINO ACID METABOLISM**

Epidemic analyses reveal that chronic kidney disease is associated with defects in many metabolic processes. It should not be surprising therefore, that among the defects are abnormalities in protein and amino acid metabolism. Identification of specific abnormalities in protein and amino acid metabolism and interventions to stop or attenuate the loss of protein stores that occurs in subjects with Chronic Kidney Disorder should be done. Besides the intellectual satisfaction of learning how chronic kidney disease stimulates the body weight and influences the “intracellular milieu”, understanding the mechanisms that underlie metabolic abnormalities in protein and amino acids is the first step towards devising strategies to block or ameliorate such defects.

8.1. **Chronic kidney disease interrupts the components of protein metabolism**

Proteins in all tissues are continually “turning over” (i.e., being degraded and replaced by new synthesis). This concept was introduced as early as 1939
when Schoenheimer et al. developed methods for tracking the fate of individual proteins and amino acids labelled with “heavy” isotope of nitrogen ($^{15}$N). The selectivity of protein breakdown is not achieved by a method in which each protein is degraded by its specific protease. Instead, it appears that different conditions or stimuli result in activation of specific proteases to eliminate the substrate protein. The principle protease in all cells is Ubiquitin-Proteasome System (UPS). It is activated by different stimuli and it degrades a large variety of individual protein or sets of proteins (Kopple, Massry and Kalantar-Zadeh, 2013).

In response to kidney disease, there is an imbalance between protein synthesis and degradation resulting in net loss of protein stores, including that in the major store of protein the body, skeletal muscle. This loss of protein stores contributes to the excessive frequency of morbidity and mortality in patients with kidney disease. The epidemiologic and clinical reports document that muscle wasting increases the risk of morbidity and mortality in kidney disease as it does in other catabolic conditions including heart failure, cancer and aging (Kestanbaum et al 2005, Huang et al, 2010 and Kotler, 2000).

8.2. Changes in the concentration of certain amino acids in kidney disease

Fasting subjects with chronic kidney disease have many abnormalities of plasma amino acid concentrations. These include an increase in 3-methylhistidine and 1-methylhistidine, apparently caused by reduced renal clearance of these methylated amino acids. In fasting subjects with chronic kidney disease, plasma valine is usually low as are leucine and isoleucine but to more modest extent. These results indicate that chronic kidney disease changes the metabolism of Branched Chain Amino Acids (BCAAs). Garibatoo et al (1993) have reported that similar differences in BCAA concentrations occur after a meal (Alverstrand et al, 1982; Kopple and Swenseid, 1974; Garibatto et al 1993 and Young et al, 1975).

At least two mechanisms contribute to low level levels of BCAA in subjects with chronic kidney disease. A low protein intake can contribute to the plasma
levels while decreased gastrointestinal absorption plays a minor role (DeFerrari et al, 1988). Unfortunately, the contribution of diet to changes in plasma amino acids is unpredictable because the results from rats with experimental chronic kidney disease indicates that those fed an excess of protein had the most abnormal BCAA in blood (Meireles et al, 1999).

The second mechanism for low levels of BCAA is metabolic acidosis because it stimulates the acceleration of BCAA catabolism. All three BCAA are irreversibly decarboxylated by Branched-Chain Ketoacid Dehydrogenase (BCKAD) and several factors, including metabolic acidosis and glucocorticoids, stimulate its activity in skeletal muscle (Liao et al, 1996 and May et al, 1987).

8.3. Links between amino acids and protein metabolism

Certain examples suggest that the metabolism of amino acids and proteins in muscle are linked. In one situation, there are parallel changes in the catabolism of protein and amino acids in muscle that are initiated by common metabolic processes. The link between protein and amino acid metabolism is that systemic metabolic acidosis also accelerates the catabolism of BCAA in muscle in a parallel fashion. There is an increased activity of branched chain ketoacid dehydrogenase, the rate limiting enzyme in the breakdown of BCAA and there is increased transcription of genes which encode subunits of branched-chain ketoacid dehydrogenase. The result is the activation of the enzyme and catabolism of BCAA (May et al, 1987 and England et al., 1995).

Another metabolic response in protein metabolism that are linked to amino acids depends on an inverse relationship between changes in rates of protein synthesis and degradation and the metabolic responses that are initiated by leucine and its metabolites. In isolated muscles, it was shown that the rate of protein synthesis increased in muscles treated with leucine (Mitch and Clark, 1984).

Since CKD affects both protein and amino acid metabolism, it is important to understand how these links affect the pathophysiology of nutritional problems that prevails in chronic kidney disease.
H. Benefits of egg white protein in management of uremia

Egg white is an important source of dietary protein. Eggs are inexpensive and highly nutritious food providing 18 vitamins and minerals, the composition of which can be affected by several factors such as hen diet, age, strain as well as environmental factors (Fraeye et al, 2012 and Samman et al, 2009).

This is because of high bioavailability of egg white protein and high content of all essential amino acids. The egg white consists of 10.5 per cent protein and 85 per cent water and there are 40 different proteins found in the white. The egg white is built up by four layers with similar protein composition, except of higher content of ovomucin in the more viscous layers (Coultate, 2009).

Two major factors affect the digestibility of egg white protein like digestive health and components in food. The bioavailability of egg protein increases from 65 per cent in raw egg to 95 per cent in cooked egg protein (Seuss-Baum, 2007). Pressure induced egg white gels are more digestible than boiled egg white. It was revealed that essential amino acids stimulate skeletal muscle protein synthesis in animals and human models and the protein in egg has the highest biological value (Glynn et al, 2010).

Fifteen grams of egg white protein contains about 1300 mg of leucine (the third most common amino acid in egg, after glutamic and aspartic acids) and is also an abundant source of branched chain amino acids and aromatic amino acids. Recent data showed that leucine induces a maximal skeletal muscle protein anabolic response in young people which suggests that egg white protein intake might have an important effect on body mass accretion (Hida et al, 2012). Specifically leucine stimulates skeletal muscle synthesis independently of all other amino acids in animal models and is a potent stimulator of the cell hypertrophy mammalian target of rapamycin complex pathway. Additionally, leucine decreases muscle protein breakdown and breakdown-associated cellular signalling and mRNA expression (Glynn et al, 2010).

Giordano and Maggiore (1964) and their team advocated a low protein diet (2-4 g total nitrogen daily) of high biological value proteins either in the form of essential amino acids or egg whites shown reduced protein catabolism.