INTRODUCTION
Severe sepsis and trauma are associated with protein catabolism, negative nitrogen balance and immunosuppression and represent a major threat to survival. Nutritional support in these circumstances maintains nitrogen balance and reduces both mortality and morbidity. However catabolism associated with sepsis is not reversed by standard hyperalimentation formulations. There is evidence that individual amino acids, used in pharmacological doses, may modify metabolism and modulate immune function during sepsis. Examples of such amino acids include, leucine, isoleucine and valine (branched chain amino acids) which inhibit muscle protein catabolism, glutamine which improves gut immune function and arginine which enhances systemic immune function. This review will concentrate on the metabolic effects of L-arginine, its significance as a substrate for nitric oxide synthesis and its potential as a pharmacological agent.

ARGININE IN HEALTH
Nutrition – Arginine is a dibasic nitrogen-rich molecule and although it can be synthesized endogenously in many mammals including humans, via the Krebs cycle, most is obtained from the diet. It shares an active transport system (y+) with other basic amino acids (lysine, ornithine and cystine) for absorption in the small intestine and there is also an active uptake mechanism within the kidney preventing excretion. Liver and kidney are both capable of synthesizing arginine from citrulline and ornithine via the urea cycle and by the same mechanism supply of arginine can control the synthesis of other amino acids (Figure 1). The liver has a relatively high concentration of the enzyme arginase.
(which degrades arginine into urea and ornithine), whereas renal arginase activity is low. The kidney therefore is the primary organ for conserving arginine.10

Figure 1 Krebs-Henseleit urea cycle

In health dietary arginine is not required for maintenance of nitrogen balance, but during growth or in illness and stress endogenous synthesis from citrulline is insufficient to meet body demands.11,12 Thus arginine has been described as a semi-essential amino acid. In contrast, in vitro, arginine is an essential amino acid for cell culture systems13 and is an essential precursor for polyamine, histone and nucleic acid synthesis which in turn are required for mitosis and manufacture of cellular proteins.

Secretagogue effects – Arginine is the most potent amino acid stimulator of insulin secretion when given either orally or intravenously.14 There is however only a minor fall in plasma glucose concentrations due to simultaneous stimulation of growth hormone production.15 Arginine also increases secretion of prolactin,16 glucagon, pancreatic pancreozymin,17 pancreatic polypeptide and adrenal catecholamine18 but the importance of these secretory pathways is not fully understood.

Immunological effects – In vivo – Supplemental dietary arginine increases thymocyte production and thymic weight in healthy animals.19 Thymocytes from these animals show increased proliferative responses to the mitogens phytohaemaglutinin and conconavalin A.20 This effect is not noted in surgically or medically hypophysectomised animals suggesting that the hypothalamic/pituitary axis may be responsible for some if not all of the in vivo immune effects

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of arginine. In athymic nude mice arginine improves T cell function (as assessed by delayed type hypersensitivity responses) indicating that it can exert its effects on peripheral lymphocytes and not just those within the thymus.\textsuperscript{21} Arginine by improving host immunity enhances rejection of skin allografts in mic.\textsuperscript{22} \textit{In vitro} – T lymphocytes taken from healthy animals and humans given pharmacological doses of arginine show increased blastogenic responses to mitogens\textsuperscript{23-25} and also demonstrate an increase in the T helper to T suppressor cell ratio.\textsuperscript{26} Arginine has also been shown to be essential for maximal generation and activation of cytotoxic T cells and natural killer (NK) cells.\textsuperscript{27} Neutrophils and macrophages utilise arginine specifically for production of nitric oxide and stimulation of both cell types increases nitric oxide synthesis. Depletion of arginine from cell culture medium decreases the cytolytic activity of activated macrophages and similarly the microbistatic and tumouristatic effects of macrophages are dependent on nitric oxide as the effector molecule.\textsuperscript{28,29}

\[ \text{L-arginine} \xrightarrow{\text{synthase}} \text{Nitric oxide} + \text{L-Citrulline} \]

\[ \text{NO}_2^- + \text{NO}_3^- \]

Figure 2 – Arginine /nitric oxide pathway – showing production of nitric oxide from L-arginine with L-citrulline as the major co-product and nitrite (NO\textsubscript{2}^-) and (NO\textsubscript{3}^-) as by-products.

\textit{Nitric oxide (NO)} – This labile bioregulatory molecule is synthesized by many cell types from L-arginine with L-citrulline as the major co-product (Figure 2). It is highly lipophilic and therefore rapidly traverses cell membranes making it an effective intra- and inter-cellular messenger. It has a very short half-life (3-9 seconds) and must be produced in large quantities or over long periods to have prolonged biological effects.\textsuperscript{30} When produced by cells such as macrophages it can rapidly enter microorganisms and tumour cells and exert cytostatic and cytotoxic effects by increasing cyclic-GMP synthesis and inhibiting host mitochondrial electron transport and DNA replication.\textsuperscript{31,32} Induction of nitric oxide synthesis has also been demonstrated in neutrophils\textsuperscript{33} and cloned T lymphocytes\textsuperscript{34} but its role in cellular immunological activity is uncertain.

\textbf{ARGININE IN DISEASE}

Following major surgery, trauma and sepsis normal intra- and extracellular amino acid homeostasis is deranged, with consequent impairment of metabolic and physiological responses. There is decreased intestinal absorption of arginine during sepsis\textsuperscript{35} and following resuscitation from haemorrhagic shock.\textsuperscript{36} Plasma concentrations of arginine are low in sepsis and show a strong negative correlation with survival.\textsuperscript{37} In children with severe burns serum arginine concentrations are decreased and are associated with depressed immune function.\textsuperscript{8}
Effects of arginine in experimental models—In various animal models intervention with arginine supplementation results in improved biological and immune parameters and increased survival.

Sepsis—parenteral administration of arginine both before and after caecal ligation and puncture results in increased survival compared with controls. Oral supplementation in established acute peritonitis does not confer increased survival which may be due to sepsis-induced gastrointestinal malabsorption.

Trauma and burns—a diet supplemented with 1% arginine improves nitrogen balance and enhances wound healing in rats assessed by increased wound breaking strength and collagen content. There is an associated reduction in thymolysis which is dependent on an intact hypothalamic/pituitary axis. Arginine reduces nitrogen loss and abrogates thymolysis and T cell dysfunction following unilateral or bilateral femoral fractures. Supplemental arginine increases delayed type hypersensitivity reactions and bacterial containment in guinea pigs with 30% full thickness burns. In this burn model there is also increased resting metabolic expenditure on diets supplemented with 1% and 2% arginine.

Jaundice—obstructive jaundice results in impaired immune function with increased susceptibility to infection. Dietary supplementation with arginine following ligation of the common bile duct resulted in improved T cell function assessed by delayed type hypersensitivity reactions and decreased mortality following subsequent caecal ligation and puncture.

Malignancy—supplemental dietary arginine has inhibitory effects on many types of transplanted or chemically induced experimental tumours. In vivo arginine reduces the incidence, increases latency, decreases growth and metastatic spread, and improves survival in animals with tumours. These effects of arginine are associated with increased macrophage phagocytosis and some if not all of the effects may be dependent on the production of nitric oxide by immune cells. Tumours in rodents supplemented nutritionally with arginine are more susceptible to chemotherapy than those in protein depleted animals. These arginine effects are however dependent on the type of tumour present, as protein depleted animals with poorly immunogenic tumours do not respond as well as those with an obvious immune response.

Inflammatory bowel disease—the study of malnutrition, and the immune system dysfunction in inflammatory bowel disease has stimulated interest in the therapeutic effects of dietary supplementation using various amino acids including arginine, glutamine and the branched-chain amino acids. Recent evidence suggests that there is increased mucosal nitric oxide synthase activity in inflammatory bowel disease but the pathophysiological role of this remains uncertain. Using an animal model with many of the local and systemic features of Crohn’s colitis, and with increased nitric oxide synthase activity, the involvement of nitric oxide in the disease process has been investigated. Supplemental 2% arginine in drinking water increases the severity of colitis with subsequent weight loss, thymolysis and splenolysis. This proinflammatory effect of arginine on the colonic mucosa is blocked by addition of the nitric oxide synthase inhibitor L-NAME (100mg/l) to the drinking water. Similar research using a model of ileitis has demonstrated a reduction in inflammation and mucosal nitrite production following inhibition of nitric oxide synthesis.
would suggest that nitric oxide is a major mediator in the mucosal inflammatory process and that its proinflammatory effects are increased by additional arginine in the diet.

**CLINICAL APPLICATIONS OF L-ARGININE**

The antica>tabolic and immunostimulatory effects of arginine in cell culture systems and animal models have suggested that arginine has potential as a therapeutic agent in various clinical situations. Disadvantages of oral arginine treatment include its distinctive bitter taste, and increased gastrointestinal water excretion with resultant diarrhoea.

*Healthy Volunteers* – in healthy humans, as in animal studies, oral arginine supplementation increases peripheral blood lymphocyte mitogenesis, decreases the number of T suppressor/cytotoxic cells and increases the T helper to T suppressor cell ratio. The delayed type hypersensitivity response is increased as is the number of circulating NK and lymphokine-activated killer cells.

*Sepsis and Trauma* – parenteral administration of arginine (500mg/kg/day) improves nitrogen balance and reduces protein catabolism secondary to surgery and sepsis. In these situations arginine also helps maintain the urea cycle with a resultant reduction in ammonia toxicity. In patients with the acquired immune deficiency syndrome arginine improves T cell mitogenesis and the T helper/suppressor ratio, but so far this has not been shown to confer increased protection against the effects of the viral infection. In addition to reducing catabolism, supplemental arginine prevents reduction in lymphocytic immune response and decrease in T helper cell occurring in patients undergoing major abdominal surgery. Arginine therapy also improves wound healing and reduces the length of stay in hospital following major cancer surgery.

*Malignancy* – in women with breast cancer supplemental arginine increases the quantity and cytotoxic capability of circulating NK and lymphokine activated cells. However in apparent contrast to animal and in vitro studies arginine appears to stimulate cell growth by stimulating protein synthesis within the tumour substance. This paradox may be therapeutically beneficial and may be due to an increased lymphocytic infiltrate in such tumours. Furthermore this enhanced tumour growth might suggest increased cell sensitivity to chemo or radio-therapy.

*Combined therapies* – Recently clinical studies have included arginine as one component of enteral dietary supplementation. The combination of arginine, ribonucleic acid and short chain fatty acids improves blastogenic responses in peripheral blood lymphocytes from intensive care patients and those with gastrointestinal malignancies. In these studies postoperative infection was reduced, wound healing was improved and there was a significant decrease in the duration of stay in hospital.

**CONCLUSIONS**

Arginine is a semi-essential amino acid in health but becomes essential during periods of growth, illness or stress. In addition arginine given in pharmacological doses enhances hormone secretion and is a potent modulator of immune system function. By enhancing T lymphocyte function it improves wound

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healing and the immune response to sepsis in experimental animals and humans. In animal models and in cell culture systems arginine increases macrophage activity against microorganisms and tumour cells. Therefore supplemental arginine may be of value in patients undergoing major surgery or following trauma and sepsis. Although more information is required arginine treatment may enhance adjuvant chemo therapy and radio therapy in malignant disease.

An arginine/nitric oxide pathway has been demonstrated in most cells of the immune system, and nitric oxide may be the bioactive molecule by which arginine has its immune effects. Nitric oxide has important homeostatic roles, but increased production by immune cells may result in pathophysiological changes in conditions such as inflammatory bowel disease. In these conditions where increased production of nitric oxide is detrimental and stimulates the inflammatory process, systemic and topical administration of nitric oxide inhibitors may have therapeutic potential.

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