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Sir David Cuthbertson Medal Lecture

Impaired energy metabolism during neonatal sepsis: the effects of glutamine

Simon Eaton

Surgery Unit and Biochemistry, Endocrinology and Metabolism Unit, Institute of Child Health (University College London), 30 Guilford Street, London WC1N 1EH UK

Neonatal sepsis is an important cause of morbidity and mortality as a result of multiple organ system failure, particularly in neonates requiring total parenteral nutrition. Suitable therapies and support are needed both to prevent sepsis and to prevent multiple organ failure. After bacterial infection, pro-inflammatory cytokines trigger the antimicrobial activity of macrophages and neutrophils, resulting in production of reactive species such as H_2O_2, NO, superoxide and peroxynitrite. However, excess production can lead to host tissue damage. Incubation of either hepatocytes or heart mitochondria from neonatal rats with these reactive species, or with cytokines, leads to impairment of mitochondrial oxidative function, and in an animal model of neonatal sepsis similar results to the *in vitro* findings have been demonstrated. Recent *in vivo* studies, using indirect calorimetry of suckling rat pups, show that during endotoxaemia there is a profound hypometabolism, associated with hypothermia. Having determined that cellular oxidative function may be impaired during sepsis, it is of great importance to try to identify therapeutic measures. Much interest has been shown in glutamine, which may become essential during sepsis. It has been shown that hepatic glutamine is rapidly depleted during endotoxaemia. When hepatocytes from endotoxaemic rats were incubated with glutamine, there was a restoration of mitochondrial structure and metabolism. *In vivo*, intraperitoneal injection of glutamine into endotoxic suckling rats partially reversed hypometabolism, markedly reduced the incidence of hypothermia and improved clinical status. These results suggest that glutamine has a beneficial effect during sepsis in neonates.

Neonatal sepsis: Energy metabolism: Glutamine: Therapeutic strategies

Sepsis remains a major cause of morbidity and mortality in adults (Friedman *et al*. 1998), children (Anderson & Blumer, 1997) and neonates (Ford & Rowe, 1998). Increased risk factors for sepsis in the neonatal period include prematurity (Stoll *et al*. 1998), low birth weight (Fanaroff *et al*. 1998), surgery (Ford & Rowe, 1998), requirement for mechanical ventilation (Mehr *et al*. 2002), the use of parenteral nutrition (Okada *et al*. 2000) and the presence of abnormal gastrointestinal flora (Pierro *et al*. 1996, 1998). Much of the mortality associated with neonatal sepsis is a result of multiple organ system failure (hepatic–renal–cardiac–pulmonary–microvascular; Smith *et al*. 1991; Morecroft *et al*. 1994; Avanoglu *et al*. 1997), as it is in adults (Deitch, 1992). Suitable therapies and support for these patients are needed both to prevent sepsis and to prevent multiple organ failure where sepsis does occur.

Whole-body metabolism in sepsis

There are many metabolic and pathophysiological alterations associated with sepsis (Vlessis *et al*. 1995); however, the existing knowledge on the metabolic response to sepsis in infants is limited. Adults respond to sepsis, surgery and trauma with a hypometabolic ‘ebb’ phase followed by a

Corresponding author: Dr S. Eaton, fax: +44 20 7404 6181, email s.eaton@ich.ucl.ac.uk

prolonged hypermetabolic response (Cuthbertson, 1945; Monk *et al*. 1996; Plank *et al*. 1998). Although infants undergoing surgery have an increased energy expenditure, which is associated with increased heart rate and respiratory rate, this increase is short lived and energy expenditure returns to pre-operative levels after 12–24 h (Jones *et al*. 1993, 1994, 1995). Whether critically-ill infants and children are hypermetabolic is less certain. Children with head trauma have been shown to be hypermetabolic, with energy expenditure higher than predicted (Phillips *et al*. 1987; Tilden *et al*. 1989), but other workers have shown that energy expenditure of critically-ill infants and children may be close to, or even lower than, the predicted BMR (Chwals *et al*. 1988; Briassoulis *et al*. 2000; White *et al*. 2000), or similar to a control group (Jaksic *et al*. 2001). However, another recent study has suggested that > 50 % of critically-ill children are hypermetabolic (Coss-Bu *et al*. 2001). Most of these studies were conducted in heterogenous patient groups, so the aim was to establish whether patients with systemic inflammatory response syndrome, sepsis or septic shock had any alteration in energy expenditure compared with weight-matched controls (Turi *et al*. 2001). As shown in Fig. 1, there was no significant alteration in energy expenditure in these patient groups, nor was there any significant day-to-day variation in energy expenditure, any difference in RQ or any correlation between paediatric risk of mortality score and energy expenditure. In a neonatal animal model of sepsis, however, it was shown that hypometabolism does occur soon after sepsis (Garrett-Cox *et al*. 2003; Fig. 2). Hence, it is possible that there is hypometabolism early in human neonatal sepsis, which had consequently not been observed in the clinical studies mentioned earlier.

In adults fat becomes a preferred fuel for oxidation (Samra *et al*. 1996) that together with increased gluconeogenesis (Wolfe, 1997) can contribute to the hyperglycaemia observed in sepsis (Mizock, 2001). However, fat mobilisation greatly exceeds utilisation under these conditions, resulting in considerable cycling (Nordenstrom *et al*. 1983; Wolfe & Martini, 2000). This outcome could be a result of inhibition of lipoprotein lipase (Robin *et al*. 1981; Lanza-Jacoby *et al*. 1997; Picard *et al*. 2001), an increase in VLDL production (Wolfe *et al*. 1985), decreased LDL clearance (Liao *et al*. 1996) or decreased oxidation of non-esterified fatty acids (Memon *et al*. 1998). Very little information is available on the ability of infants and children to oxidise fat during sepsis or critical illness, although some authors have suggested that hypermetabolic critically-ill infants preferentially oxidise fat (Coss-Bu *et al*. 2001). Recently, an investigation of whether infants and children with sepsis are able to oxidise exogenous fat, using an Intralipid® (Fresenius Kabi Ltd, Runcorn, Cheshire, UK) utilisation test, was conducted (Pierro *et al*. 1989). It was found that most infants and children with sepsis were able to oxidise exogenous fat efficiently, but a few subjects did not appear able to oxidise exogenous Intralipid® (E Caresta, A Pierro, A Petros, M Peters and S Eaton, unpublished results). Further work is necessary to define substrate utilisation more closely in sepsis in the paediatric population.

Free radical production during parenteral nutrition and sepsis

An increased production of free radicals is thought to occur during parenteral nutrition of infants, specifically linked to the infused lipids (Wispe *et al*. 1985; Pitkanen *et al*. 1991; Andersson *et al*. 1992; Pitkanen, 1992) and it has been shown that this effect is exacerbated during critical illness (Basu *et al*. 1999*b*). Infused lipids are composed of a high proportion of polyunsaturated fatty acids, which renders them particularly susceptible to peroxidation and free radical production. It has been hypothesised that, although lipid peroxidation can occur before infusion, much of the peroxidation occurs in the circulation and that it would be decreased if the lipid was efficiently oxidised. It has been shown in infants on parenteral nutrition that at the same rates of lipid infusion free radical production could be decreased

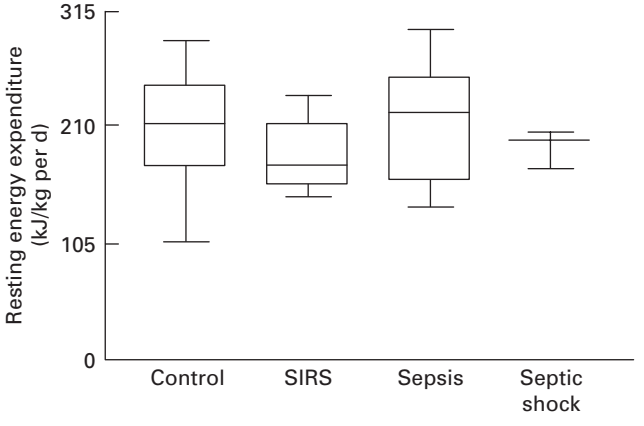

Fig. 1. Resting energy expenditure in critically-ill infants and controls. Indirect calorimetry was performed on infants and children with systemic inflammatory response syndrome (SIRS; *n* 8), sepsis (*n* 10), septic shock (*n* 5) and controls (*n* 23). Results are expressed as median, range (represented by vertical bars) and interquartile range (□). There were no significant differences between the groups. (See Turi *et al*. 2001.)

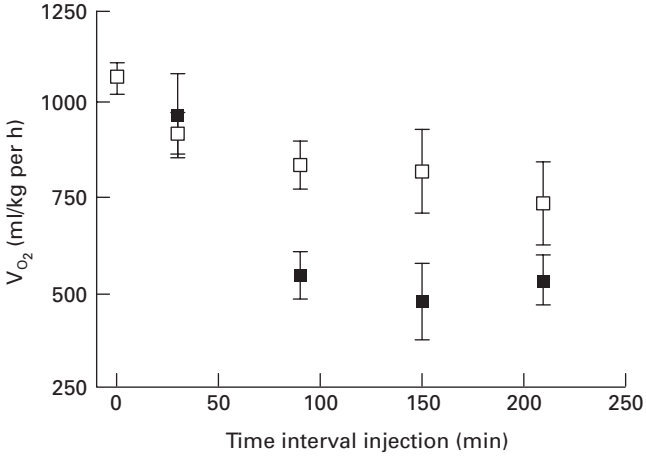

Fig. 2. Hypometabolism in endotoxic neonatal rats. Oxygen consumption (V_{O_2}) of rat pups injected with saline (9 g NaCl/l; □) or saline plus endotoxin (■). Values are means with their standard errors represented by vertical bars. (Data from Garrett-Cox *et al*. 2003.)

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by decreasing the amount of carbohydrate given, thereby promoting fat oxidation and preventing peroxidation (Basu *et al*. 1999*a*). It has also been hypothesised that infusion of a mixture of medium- and long-chain triacylglycerols, which as they contain fewer polyunsaturated acyl groups are less susceptible to peroxidation, would decrease lipid peroxidation *in vivo*. However, the opposite was found to be true; i.e. although the medium- and long-chain triacylglycerol mixture was efficiently oxidised (Donnell *et al*. 2002), free radical production was greater with the medium- and long-chain triacylglycerol mix than with long-chain triacylglycerols alone (Basu *et al*. 2000). Whether this difference is the result of rapid oxidation of medium-chain triacylglycerols leading to long-chain triacylglycerols remaining in the circulation for longer (and thus being more available for peroxidation), or other specific effects, warrants further investigation.

The early events that follow the host response to bacterial infection are represented by production of a cascade of pro-inflammatory cytokines such as interleukins 1 and 6 and tumour necrosis factor α. These cytokines trigger the anti-microbial activity of macrophages and neutrophils, resulting in production of considerable quantities of a battery of cytotoxic reactive oxygen and nitrogen species such as H_2O_2, NO, superoxide and peroxynitrite (Vlessis *et al*. 1995). The main function of these compounds is, of course, killing bacteria. However, where there is considerable local infiltration and activation of macrophages and neutrophils, excess production of these highly-reactive compounds can lead to host tissue damage.

These reactive oxygen and nitrogen species are known to inhibit mitochondrial function and/or cause mitochondrial damage in adult liver (Karbowski *et al*. 1997; Riobo *et al*. 2001), kidney (Davis *et al*. 2001) and heart (Borutaite & Brown, 1996; Janero & Hreniuk, 1996; Nulton-Persson & Szweda, 2001). It has been shown that incubation of either hepatocytes or heart mitochondria from neonatal rats with these reactive species, or with cytokines, leads to impairment of mitochondrial oxidative function (Romeo *et al*. 1999, 2000; Fukumoto *et al*. 2002*a*, 2003*a*; New *et al*. 2001). Similarly, mitochondrial dysfunction resulting from endotoxaemia and/or sepsis has been implicated in the pathogenesis of organ failure in the liver (Kantrow *et al*. 1997), heart (Gellerich *et al*. 1999; Trumbeckaite *et al*. 2001) and kidney (Kang *et al*. 1995; Messner *et al*. 1999). In a recent study it was shown that the extent of mitochondrial impairment was correlated with outcome in human septic shock (Brealey *et al*. 2002). However, it is not known whether this mitochondrial dysfunction occurs in neonatal sepsis, as mitochondrial metabolism alters greatly during the transition from newborn to adult (Valcarce *et al*. 1988; Girard *et al*. 1992; Lionetti *et al*. 1998). In an animal model of neonatal sepsis results consistent with the *in vitro* findings have been demonstrated, i.e. endotoxaemia caused inhibition of the oxidative function of hepatocytes and cardiac mitochondria, although kidney mitochondria appeared to be more resistant (Fukumoto *et al*. 2002*a*, 2003*b*; Markley *et al*. 2002). These results are in keeping with the finding of whole-body hypometabolism in endotoxic rat pups (Garrett-Cox *et al*. 2003). Impairment of cardiac carnitine palmitoyl transferase I (a rate-controlling enzyme of fatty acid oxidation; Eaton *et al*. 2001; Eaton, 2002) activity was paralleled by nitration of tyrosine residues (Fukumoto *et al*. 2002*b*), characteristic of protein damage by peroxynitrite (Beckman & Koppenol, 1996). Tyrosine nitration of other enzymes has been demonstrated during sepsis (Kooy *et al*. 1997; Marcondes *et al*. 2001; Barreiro *et al*. 2002). The anti-oxidant glutathione is thought to be particularly important in the protection of mitochondrial respiratory chain complexes (Clementi *et al*. 1998; Bolanos *et al*. 1996), and recently it was shown that in adult patients with sepsis complex I activity is positively correlated with mitochondrial glutathione content and is lower in non-survivors than survivors (Brealey *et al*. 2002).

Possible therapies for mitochondrial damage in neonatal sepsis

Having determined that cellular oxidative function may be impaired during sepsis, it is of great importance to try to identify therapeutic measures. As the inflammatory response itself serves a function in protection against infection, therapeutic measures directed against pro-inflammatory cytokines, such as anti-tumour necrosis factor antibodies, do not appear to be effective (Arndt & Abraham, 2001), although more sophisticated approaches to modulation of the inflammatory–intravascular coagulation–endothelium axis appear promising in severe sepsis (Bernard *et al*. 2001; Grinnell & Joyce, 2001). Similarly, although high doses of antioxidants may prevent secondary organ damage by free radicals, they could also diminish the killing of bacteria by free radical-dependent mechanisms. Hence, strategies aiding immune function and aiding the body's own antioxidant defences whilst preserving the balance between pro- and anti-inflammatory cytokines may be beneficial. This approach is the basis of immunonutrition, which includes *n*-3 poly-unsaturated fatty acids, glutamine, arginine, S-amino acids and nucleotides (Beale *et al*. 1999; Grimble, 2001; Heyland *et al*. 2001).

The non-essential amino acid glutamine is the most abundant amino acid in plasma, having several important physiological roles (Haussinger, 1998). During sepsis endogenous glutamine stores are mobilized, gut glutamine uptake is diminished and the liver and immune system become its major consumers such that net glutamine utilization exceeds production and glutamine becomes 'conditionally essential' (Lacey & Wilmore, 1990; Souba & Austgen, 2001). The enhanced hepatic glutamine extraction in sepsis has been attributed to increased hepatic gluconeogenesis, ureagenesis, glutathione production and synthetic and proliferative activities (Ardawi, 1992; Ewart *et al*. 1995). The provision of exogenous glutamine improves N balance, attenuates skeletal muscle proteolysis (Hammarqvist *et al*. 1989; Stehle *et al*. 1989), reduces bacterial translocation from the gut (Souba *et al*. 1990; McAndrew *et al*. 1999) and is essential for the immune system to mount an adequate immune response (Ardawi & Newsholme, 1990). Glutamine is not routinely included in paediatric parenteral nutrition but has been shown to have beneficial effects in premature neonates (Lacey *et al*. 1996). It has been shown that hepatic and plasma glutamine are rapidly depleted during neonatal endotoxaemia (Vejchapipat *et al*. 2002; RG Garrett-Cox, A Pierro and

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S Eaton, unpublished results). Addition of 10 mM-glutamine to hepatocytes isolated from endotoxic neonatal rats *in vitro* restored mitochondrial oxidative metabolism and the drastic ultrastructural changes to mitochondria were completely reversed (Markley *et al*. 2002). In further experiments it was shown that glutamine acts by providing a substrate for glutathione synthesis (Babu *et al*. 2001) and that the addition of glutamine enhanced fatty acid oxidation in both control and endotoxaemic hepatocytes (Kim *et al*. 2002). However, these experiments were all undertaken with isolated hepatocytes incubated with exogenous glutamine. It is important to determine whether glutamine has any beneficial effects *in vivo*. In recent experiments using indirect calorimetry of suckling rats it was shown that intraperitoneal injection of either glutamine or leucine into control animals caused an increase in resting energy expenditure (nutrient-induced thermogenesis; Garrett-Cox *et al*. 2003). Injection of glutamine or leucine into endotoxaemic animals partially reversed the hypometabolism induced by endotoxin (Fig. 3), but only glutamine reduced the incidence of hypothermia and improved the clinical status of these animals (Garrett-Cox *et al*. 2003). These results suggest that glutamine has a beneficial effect on endotoxaemia that may be related to thermoregulation rather than thermogenesis and the metabolism of glutamine *per se*. Further work is necessary in this area to clarify the mechanism of this novel action of glutamine. In addition, the relationship between glutamine and glutathione metabolism during neonatal sepsis requires further investigation, as the supply of cysteine, rather than the glutamate moiety that can be provided by glutamine, is thought to be rate limiting for glutathione synthesis under most conditions (Sen, 1997). Other compounds that ensure adequate glutathione status, such as *N*-acetyl-cysteine, L-2-oxothiazolidine-1-carboxylate and glutathione esters are also of potential clinical interest (Anderson *et al*. 1985; Moberly *et al*. 1998; Poon *et al*. 1998; Grattagliano *et al*. 1999).

Although generalised antioxidant therapies during sepsis are unlikely to be clinically effective, because of the involvement of free-radical mechanisms in the process of killing bacteria and the many physiological functions of free radicals, therapeutic measures directed against peroxynitrite may find useful application as, unlike NO and other reactive oxygen and nitrogen species, peroxynitrite does not have a well-defined physiological role (Beckman & Koppenol, 1996). Recently, a series of porphyrin compounds that scavenge superoxide and/or peroxynitrite have been synthesised and shown to be effective in relevant animal models (Salvemini *et al*. 1998, 1999; Cuzzocrea *et al*. 2000, 2001). However, whether these compounds have utility clinically is as yet unknown. A summary of potential nutritional approaches to therapy for sepsis is shown in Fig. 4.

Conclusions

New possible therapeutic strategies to prevent multiple organ failure during sepsis, involving both pharmaceuticals and 'nutraceuticals', appear promising. However, as there appear to be marked differences in the metabolic response of neonates to infection, these new approaches require careful evaluation in the paediatric population.

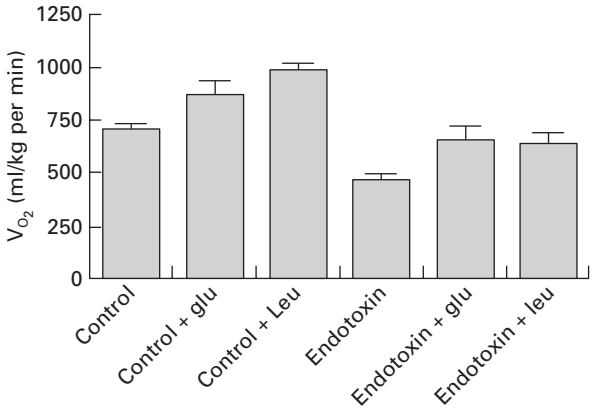

Fig. 3. Effects of endotoxin, glutamine (glu) and leucine (leu) on oxygen consumption (V_{O_2}) of neonatal rats.Rats were injected with saline (control) and endotoxin, glu or leu as indicated. Values are means with their standard errors represented by vertical bars. (Data from Garrett-Cox *et al*. 2003.)

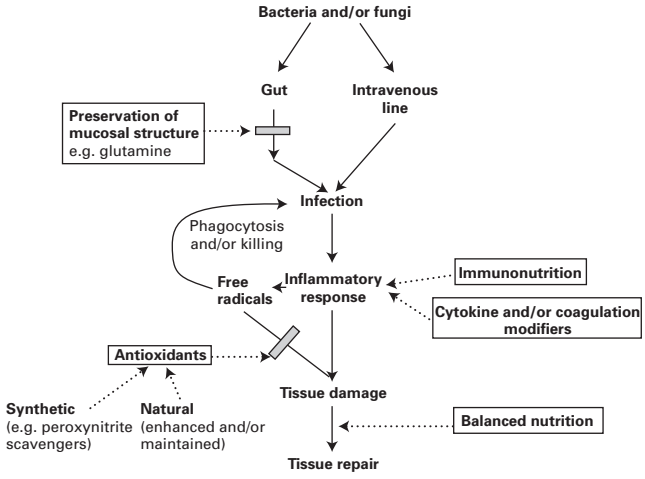

Fig. 4. Potential nutrient therapies for neonatal sepsis.

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