

## Dose dependency of the effect of ornithine $\alpha$ -ketoglutarate on tissue glutamine concentrations and hypercatabolic response in endotoxaemic rats

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The optimal dosage of ornithine  $\alpha$ -ketoglutarate (OKG) for repleting tissue glutamine (Gln) concentrations and maintaining N homeostasis after injury is unknown. We set out to perform ‘dose-ranging’ of OKG supplementation after an endotoxaemic challenge. Sixty-one male Wistar rats were injected with 3 mg lipopolysaccharide (LPS) from *Escherichia coli*/kg (*n* 50) or saline vehicle (9 g NaCl/l; controls *n* 11). After a 24 h fast, survivors were fed by gavage for 48 h with a polymeric standard diet (879 kJ/kg per d and 1.18 g N/kg per d) supplemented with non-essential amino acids (control, *n* 11; LPS-OKG-0.0, *n* 9), or with 0.5 g OKG/kg per d (LPS-OKG-0.5, *n* 12), 1.5 g OKG/kg per d (LPS-OKG-1.5, *n* 11) or 4.5 g OKG/kg per d (LPS-OKG-4.5, *n* 10). The diets for all groups were made isonitrogenous with the LPS-OKG-4.5 diet by adding an appropriate amount of non-essential amino acids. Rats were killed on day 3 for blood and tissue sampling (muscle, jejunum mucosa, liver). Urine was collected daily for 3-methylhistidine and total N assays. The OKG dose was correlated with Gln concentrations in every tissue and with cumulative N balance (Spearman test,  $P < 0.01$ ). 3-Methylhistidine excretion was increased in endotoxaemic groups compared with controls (ANOVA,  $P < 0.05$ ) except in the LPS-OKG-4.5 group. Only the LPS-OKG-4.5 group achieved a positive post-injury N balance (*t* test,  $P < 0.05$ ). In conclusion, OKG exerted a dose-dependent effect on tissue Gln concentration and N balance, but only the highest dosage counteracted myofibrillar hypercatabolism and caused a positive N balance.

### Ornithine $\alpha$ -ketoglutarate: Glutamine: Endotoxaemia: Injury

Pharmaco-nutrition denotes the use of metabolic regulating substances to correct functional and metabolic disorders induced by injury (Cynober, 1995; De Bandt & Cynober, 1998). Ornithine  $\alpha$ -ketoglutarate (OKG) has been used for this purpose in various pathological situations (Cynober, 2004). OKG is a salt, stable in solution, which can be given by oral, enteral or parenteral routes; it is formed of one molecule of  $\alpha$ -ketoglutarate and two of ornithine (Orn). This pharmac-nutrient is characterized by both anti-catabolic and anabolic activities (De Bandt & Cynober, 1998; Cynober, 2004); it acts as an efficient immunomodulator (Roch-Arveiller *et al.* 1996; Robinson *et al.* 1999; Moirand *et al.* 2002) and as a key promoter of wound healing and tissue repair (Coudray-Lucas *et al.* 2000). OKG supplementation results in an improvement of nutritional and biochemical status and of the clinical outcome of injured patients (De Bandt & Cynober, 1998; Cynober, 2004). OKG action is complex, involving its components, Orn and  $\alpha$ -keto-glutarate, and its metabolites generated *in vivo*, namely glutamine (Gln), arginine (Arg), proline (Pro) and polyamines

(Le Boucher & Cynober, 1998). Moreover, a specific interaction between the two components is involved in the effects of OKG (Cynober *et al.* 1990; Le Boucher & Cynober, 1998), leading to an increase in Gln pools *in vivo* that is not observed when Orn is replaced by Arg (Le Boucher *et al.* 1997). Besides clinical trials (for reviews, see Cynober, 1999; Cardenas *et al.* 2002; Blondé-Cynober *et al.* 2003), numerous experimental studies have found beneficial metabolic effects of OKG in experimental models of injury, such as burns (Vaubourdolle *et al.* 1991), trauma (Jeevanandam *et al.* 1992), cancer (Le Bricon *et al.* 1994), endotoxaemia (Lasnier *et al.* 1996) and intestinal ischaemia (Duranton *et al.* 1998). These studies used different doses from 0.5 to 5.0 g/kg per d. However, there was no strong rationale for the doses of OKG used in most of these experiments. ‘Dose-ranging’ has already been carried out in human subjects (Le Bricon *et al.* 1997; De Bandt *et al.* 1998). However, limited information was obtained, because the effects of OKG were evaluated non-invasively only, in particular by measuring plasma OKG metabolites.

**Abbreviations:** Arg, arginine; Gln, glutamine; LPS, lipopolysaccharide; OKG, ornithine  $\alpha$ -ketoglutarate; Orn, ornithine; Pro, proline.

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In the present study, the adequate dosage of OKG to be used was determined on the basis that the dosage should reproduce the main beneficial effects of OKG on hypercatabolic states. We focused on the tissue level to investigate OKG action on tissues according to dose more closely. For this purpose, we administered different doses of OKG covering the range of OKG doses previously used in experimental models; we used a severe endotoxaemia model as a reproducible model of hypercatabolism (Fink & Heard, 1990).

## Materials and methods

### Animals

Sixty-one male Wistar rats (Centre d'Élevage René Janvier, Saint-Denis-Les-Laval, France) 8 weeks old and weighing 180–200 g were used. They were housed individually in stainless-steel wire-bottomed metabolism cages with a reversed 12 h light–dark cycle (lights off 08.00 to 20.00 hours) in a thermostatically controlled room (22°C). They were acclimatized to standard laboratory conditions for 5 d with free access to water and rat chow (UAR A03; Usine d'Alimentation Rationnelle, Epinay-sur-Orge, France) before the beginning of the experiments. Animal care complied with the rules of the Saint-Antoine Hospital animal facility. Three of the investigators (L. C., A. J. and C. C. -L.) are authorized by the French Ministry of Agriculture to use this experimental model.

### Study design

After acclimatization (day 0), rats were allocated in two experimental groups: a lipopolysaccharide (LPS)-injected group and a control group (Table 1). Endotoxin from *Escherichia coli* 0127:B8 dissolved in saline (9 g NaCl/l; 10 ml/kg body weight) was injected intraperitoneally at a dose of 3 mg/kg in the LPS-treated group, while the control rats received only saline vehicle. After injection the rats were fasted for 24 h (from day 0 to day 1) with free access to water. On day 1, survivors of the LPS group were randomized into four subgroups defined by the daily dose of OKG (Chiesi SA, Courbevoie, France) mixed with their enteral nutrition (see later). Rats were

enterally fed for 48 h (from day 1 to day 3) in three daily gavages (08.00, 13.00 and 18.00 hours) using a standard polymeric diet (Osmolite®; Abbott, Rungis, France) as previously described (Vaubourdolle *et al.* 1991; Lasnier *et al.* 1996). Enteral nutrition supplied 879 kJ/kg per d (1.18 g N/kg per d) with no additional daily OKG dose (control group and LPS-OKG-0.0 group) or an additional dose of 0.5 (LPS-OKG-0.5 group), 1.5 (LPS-OKG-1.5 group) or 4.5 (LPS-OKG-4.5 group) g OKG/kg per d. The diets for all groups were made isonitrogenous with the LPS-OKG-4.5 group by adding an appropriate amount (see Table 1) of a mixture of non-essential amino acids (glycine, alanine and serine, each amino acid supplying one-third of the extra N needed; Sigma, Saint-Louis, MO, USA). OKG and non-essential amino acids were dissolved in the enteral nutrition solution. The rats were killed by decapitation on day 3 (6 h after the last gavage).

Urine was collected daily from day 0 to day 3. Sodium ethylmercurithiosalicylate solution (1 mg/l, 50 µl; Prolabo, Paris, France) was added to each collection container to prevent bacterial growth. Samples were then stored at –20°C until determination of N and 3-methylhistidine excretion. At killing, blood was collected on heparinate from the neck. Blood was centrifuged (10 min, 3500 g). Plasma was then deproteinized with sulfosalicylic acid (50 mg/ml) and centrifuged. The supernatant fractions were stored at –80°C. Liver and *anterior tibialis* muscles were promptly removed, weighed and frozen in liquid N<sub>2</sub>, and then stored at –80°C. A 100 mm segment of jejunum located 100 mm from the pylorus was removed, washed with 4°C saline (9 g NaCl/l), weighed and then opened longitudinally on a glass board placed on ice. The jejunum mucosa was scraped off and weighed, frozen in liquid N<sub>2</sub> and stored at –80°C until analysis.

### Measurements

**Urinary nitrogen.** Total urinary N levels were measured by pyrochemiluminescence on an Antek 5000 N (Antek Instrument, Düsseldorf, Germany). N balance was calculated for each day (N intake – urinary N excretion) and cumulated for the 2 d of the re-nutrition period.

**Urinary 3-methylhistidine.** In the rat, 3-methylhistidine is chiefly excreted in urine in acetylated form. Samples

**Table 1.** Definition of the five experimental groups according to the presence or absence of endotoxaemia and the dose of ornithine  $\alpha$ -ketoglutarate supplementation

Group...	Control (n 11)	LPS (n 50)	LPS-OKG-0.0 (n 9)	LPS-OKG-0.5 (n 12)	LPS-OKG-1.5 (n 11)	LPS-OKG-4.5 (n 10)
Day 0 to day 1: injections and fasting						
Endotoxin*	–	+				
Saline†	+	–				
Day 1 to day 3: nutrition						
Osmolite®‡	+		+	+	+	+
OKG (g/kg per d)	0.0		0.0	0.5	1.5	4.5
NEAA (g/kg per d)	3.8		3.8	3.4	2.5	0.0

LPS, lipopolysaccharide; OKG, ornithine ( $\alpha$ -ketoglutarate); NEAA, non-essential amino acids. + and –, presence and absence of administration of the stated substance.

\* The endotoxin was LPS from *Escherichia coli* 027:B8 (3 mg/kg).

† 9 g NaCl/l, 90 mg/kg.

‡ Abbott, Rungis, France; 879 kJ/kg per d, 1.18 g N/kg per d.

were therefore hydrolysed with 6M-HCl at 80°C for 24 h before quantification by ion-exchange chromatography and ninhydrin detection using a model 6300 Beckman analyser (Beckman, Palo Alto, CA, USA).

**Plasma and tissue amino acids.** Plasma was separated by centrifugation, deproteinized with sulfosalicylic acid (50 mg/l) and stored at -80°C until amino acid analysis. *Anterior tibialis*, liver and jejunum mucosa were homogenized in TCA (100 ml/l) containing 0.5 mmol EDTA/l. After centrifugation, the supernatant fractions were stored at -80°C until amino acid analysis. Amino acid (Arg, Gln, Orn, Pro) concentrations in plasma and supernatant fractions were measured by ion-exchange chromatography on a Beckman 6300 analyser (Beckman).

### Statistical analysis

Results are expressed as mean values with their standard errors. For organ weights, N balance, 3-methylhistidine excretion and plasma amino acid concentrations, comparisons between five groups were made using one-way ANOVA. When an overall significant difference among the five groups was found, the Newman-Keuls test was performed to determine which groups differed. In endotoxaemic groups, correlations between OKG dose and N balance, 3-methylhistidine excretion and plasma amino acid concentrations were sought using linear regression (Spearman test). A one-sample *t* test compared the mean of N balance against a hypothetical mean of 0 (i.e. the hypothesis that average balance does not differ from zero). Statistical analyses were done using Graphpad Prism (version 3.02; GraphPad Software Inc., San Diego, CA, USA). Differences were considered significant at  $P < 0.05$ . Significance levels quoted were two-sided.

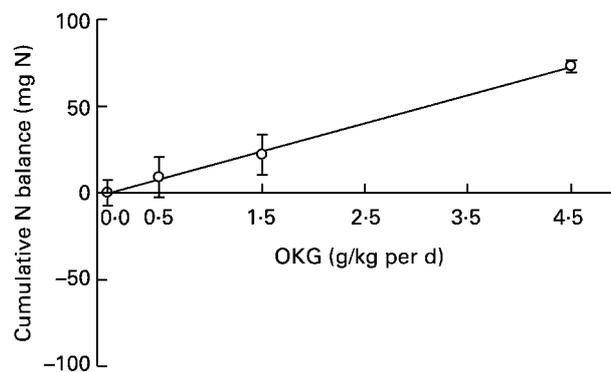
## Results

### Mortality rate

In the LPS group, eight rats (16%) died after endotoxin injection; forty-two rats were therefore randomized for nutritional supplementation on day 1.

### Nitrogen balance

From day 0 to day 1, N balance in the LPS group (-276 (SE 53) mg) was statistically more negative than that in the control group (-214 (SE 87) mg);  $P < 0.05$ . From day 1 to day 3, cumulative N balance was not different between groups (mg): control 53 (SE 24); LPS-OKG-0.0 0 (SE 20); LPS-OKG-0.5 9 (SE 23); LPS-OKG-1.5 22 (SE 35); LPS-OKG-4.5 73 (SE 12). When the mean values of cumulative N balances were tested against a hypothetical mean of 0 (one-sample *t* test), only the LPS-OKG-4.5 group showed a positive balance ( $P < 0.05$ ). Interestingly, a statistical correlation between OKG dose and cumulative N balance in endotoxaemic groups ( $y = 16.2x - 0.3$ ,  $r = 0.99$ ) was observed (see Fig. 1).



**Fig. 1.** Linear regression between ornithine  $\alpha$ -ketoglutarate (OKG) dose and day 1 to day 3 cumulative nitrogen balance. For details of diets and procedures, see Table 1 and pp. 628–629. Values are mean with their standard errors shown by vertical bars.  $y = 16.2x - 0.3$ ;  $r = 0.99$ ,  $P < 0.001$  (Spearman rank test).

### 3-Methylhistidine excretion

Results are reported in Fig. 2. Cumulative 3-methylhistidine excretion was statistically higher in the LPS-OKG-0.0, LPS-OKG-0.5 and LPS-OKG-1.5 groups than in the control group, whereas the LPS-OKG-4.5 group was not different from controls. No statistical correlation was found between OKG dose and 3-methylhistidine excretion in the endotoxaemic groups.

### Organ weights

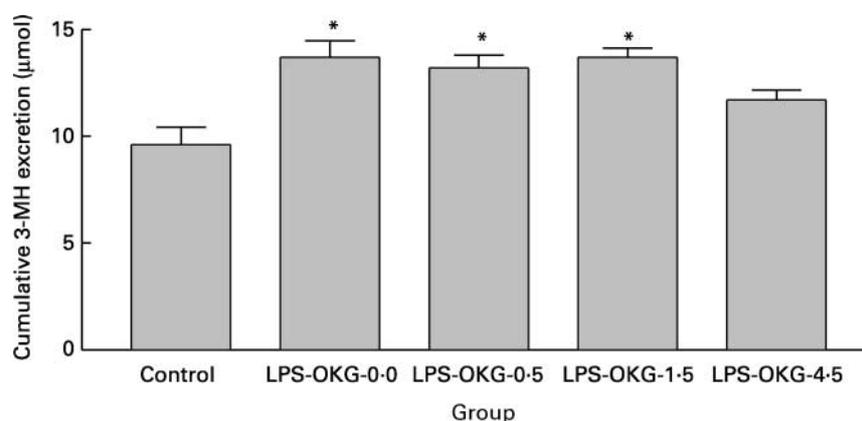
The weights of jejunum and *anterior tibialis* at killing (results not shown) were not statistically different between groups. Liver weight (expressed as % total body weight) increased in all endotoxaemic groups (LPS-OKG-0.0 4.0 (SE 0.4), LPS-OKG-1.5 4.0 (SE 0.3), LPS-OKG-1.5 4.1 (SE 0.4), LPS-OKG-4.5 4.0 (SE 0.3)) compared with the control group (3.6 (SE 0.2)).

### Plasma and tissue amino acid concentrations

**Glutamine.** Results for Gln concentrations are reported in Table 2. In plasma, Gln concentrations were greater in the LPS-OKG-4.5 group than in the four other groups. In jejunum mucosa and in liver, Gln concentrations were greater in the LPS-OKG-4.5 group than in the control, LPS-OKG-0.0 and LPS-OKG-0.5 groups, and also greater in the LPS-OKG-1.5 group than in the control and LPS-OKG-0.0 groups. Muscle Gln concentrations in LPS-OKG-0.0 and LPS-OKG-0.5 groups were lower than those in the control group, whereas OKG at 1.5 and 4.5 g/kg per d counteracted Gln depletion.

In endotoxaemic rats, a significant positive linear relation was found between administered OKG dose and Gln concentration in plasma, *anterior tibialis*, liver and jejunum mucosa (Fig. 3).

**Ornithine.** Results are reported in Table 3. Orn concentrations in the LPS-OKG-4.5 group were significantly greater than those in the four other groups in plasma, *anterior tibialis*, jejunum mucosa and liver. Moreover, in endotoxaemic rats, a significant ( $P < 0.01$ ) positive linear relation was found between administered OKG dose and



**Fig. 2.** Day 1 to day 3 cumulative 3-methylhistidine (3MH) urinary excretion following injury. LPS, lipopolysaccharide; OKG,  $\alpha$ -ketoglutarate. For definition of groups, and details of diets and procedures, see Table 1 and pp. 628–629. Values are means with their standard errors shown by vertical bars. Mean values were significantly different from that of the control group: \* $P < 0.05$  (ANOVA followed by a Newman–

**Table 2.** Plasma and tissue glutamine concentrations at killing\*†  
(Mean values with their standard errors)

Group...	Control		LPS-OKG-0-0		LPS-OKG-0-5		LPS-OKG-1-5		LPS-OKG-4-5	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma ( $\mu\text{mol/l}$ )	880 <sup>a</sup>	33	793 <sup>a</sup>	17	794 <sup>a</sup>	33	864 <sup>a</sup>	17	990 <sup>b</sup>	36
Jejunum mucosa (nmol/g)	662 <sup>a</sup>	52	632 <sup>a</sup>	64	672 <sup>ab</sup>	76	796 <sup>bc</sup>	54	940 <sup>c</sup>	69
Liver (nmol/g)	6362 <sup>a</sup>	234	5889 <sup>a</sup>	190	6336 <sup>ab</sup>	347	6782 <sup>bc</sup>	154	7382 <sup>c</sup>	353
Anterior tibialis (nmol/g)	4094 <sup>a</sup>	184	3127 <sup>b</sup>	268	3071 <sup>b</sup>	122	3397 <sup>ab</sup>	100	4144 <sup>a</sup>	389

LPS, lipopolysaccharide; OKG, ornithine  $\alpha$ -ketoglutarate.

<sup>a,b,c</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* For details of groups and procedures, see Table 1 and pp. 628–629.

† For each variable, when an overall significant difference among the five groups was found (one-way ANOVA), the Newman–Keuls *post hoc* test was performed.

Ons concentration in plasma ( $y = 39x + 25$ ;  $r = 0.74$ ), anterior tibialis ( $y = 108x + 3$ ;  $r = 0.74$ ) and jejunum mucosa ( $y = 19x + 72$ ;  $r = 0.71$ ).

**Proline.** Results are reported in Table 4. Concentrations of Pro in the LPS-OKG-4-5 group were significantly greater in plasma and anterior tibialis than in the four other groups, and in the control and LPS-OKG-1-5 groups in the liver ( $P < 0.05$ ). Liver Pro concentrations in endotoxaemic groups were significantly greater than in the control group. In endotoxaemic rats, a significant ( $P < 0.01$ ) positive linear relation was found between OKG administered dose and Pro concentrations in plasma ( $y = 32x + 170$ ;  $r = 0.72$ ) and anterior tibialis ( $y = 77x + 266$ ;  $r = 0.81$ ). No differences between groups were found for jejunum mucosa.

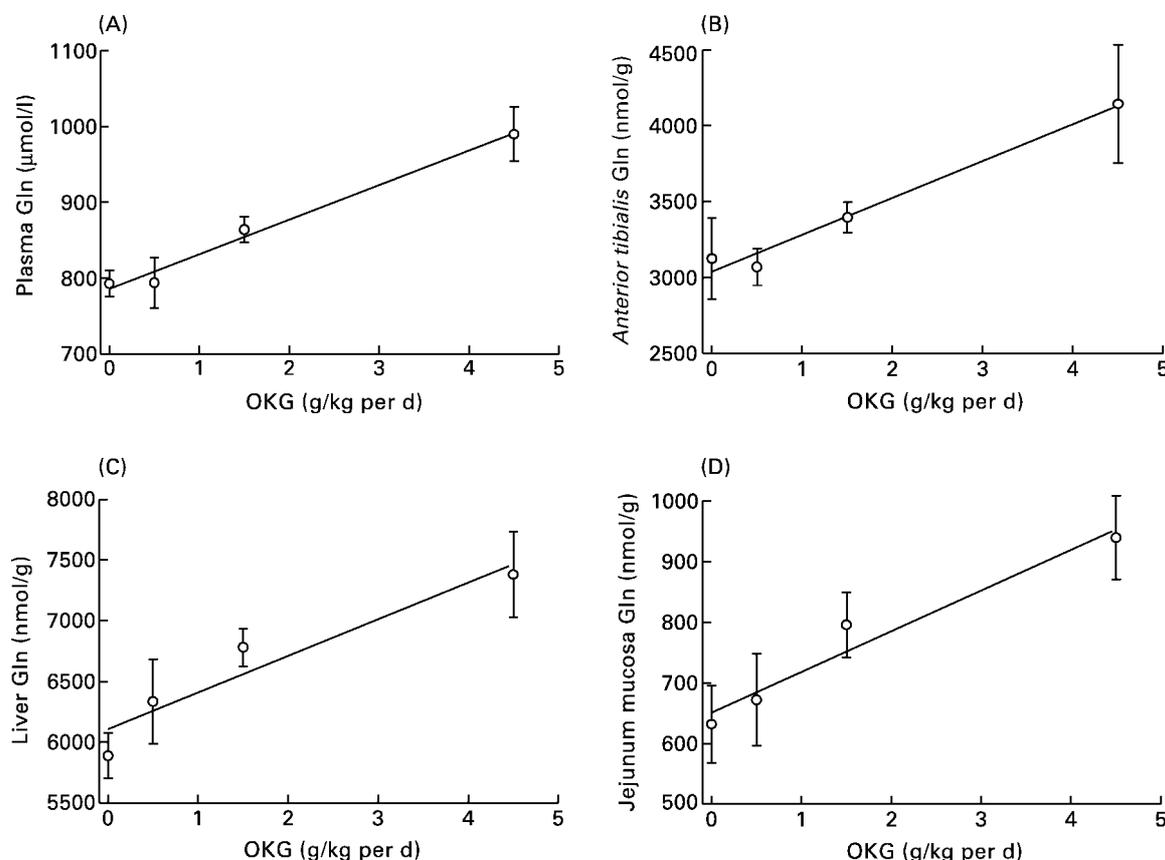
**Arginine.** Results are reported in Table 5. Arg concentrations in the control and LPS-OKG-4-5 groups were significantly greater in anterior tibialis than in the LPS-OKG-0-5 and LPS-OKG-1-5 groups ( $P < 0.05$ ). No differences between groups were found in plasma or jejunum mucosa. Arg concentrations in liver were undetectable.

## Discussion

'Dose-ranging' studies are mandatory to assess the effects of pharmaconutrients, yet very few such studies are available in the literature. To evaluate the effects of OKG

using controlled nutrition, we had to choose an experimental model that would induce a reproducible state of injury. For this purpose, we used an endotoxaemic rat model, since endotoxaemia may be involved in the pathogenesis of sepsis and is known to induce a marked reproducible hypercatabolic state (Lasnier *et al.* 1996). Endotoxaemia, and caecal ligation and puncture, induce the same pattern of pro-inflammatory cytokine production (P Wischmeyer, personal communication). The dose of endotoxin from *E. coli* 0127:B8 used in the present study (3 mg/kg) has been shown to induce a mortality rate of 20% in young rats (Pernet *et al.* 1999). These results are close to the mortality rate of 16% observed in the present experiments and confirm that this dose of LPS inflicts major injury. However, because rats are able to recover quickly from injuries, they were subsequently fasted to potentiate and prolong the effects of the endotoxaemia (Vaubourdolle *et al.* 1991) in order to evaluate the beneficial effects of the 2 d nutrition. This controlled nutrition was made isonitrogenous to avoid bias of N intake, and a mixture of non-essential amino acids, considered to be the most inert N source, was used as an isonitrogenous control (Chambon-Savanovitch *et al.* 1999). High doses of a single amino acid used as isonitrogenous control, such as glycine (Hall, 1998), may exert metabolic effects and induce bias of interpretation.

In previous experimental studies in rats, OKG doses ranged from 0.5 (Lasnier *et al.* 1996) to 5.0 g/kg per d



**Fig. 3.** Linear regression of  $\alpha$ -ketoglutarate (OKG) dose *v.* glutamine (Gln) concentrations in plasma (A), muscle (B), liver (C) and jejunum mucosa (D) in endotoxaemic rats at killing. For details of diets and procedures, see Table 1 and pp. 628–629. Values are means with their standard errors shown by vertical bars. (A),  $y = 46x + 786$ ,  $r = 0.99$ ,  $P < 0.01$ ; (B),  $y = 242x + 3041$ ,  $r = 0.98$ ,  $P < 0.05$ ; (C),  $y = 302x + 6107$ ,  $r = 0.95$ ,  $P < 0.05$ ; (D)  $y = 67x + 651$ ,  $r = 0.97$ ,  $P < 0.05$ ; Spearman test.

**Table 3.** Plasma and tissue ornithine concentrations at killing\*†  
(Mean values with their standard errors)

Group...	Control		LPS-OKG-0.0		LPS-OKG-0.5		LPS-OKG-1.5		LPS-OKG-4.5	
	Mean	SEM								
Plasma ( $\mu\text{mol/l}$ )	40 <sup>a</sup>	2	41 <sup>a</sup>	3	45 <sup>a</sup>	1	60 <sup>a</sup>	3	208 <sup>b</sup>	38
Jejunum mucosa (nmol/g)	79 <sup>a</sup>	5	84 <sup>a</sup>	7	77 <sup>a</sup>	5	87 <sup>a</sup>	8	161 <sup>b</sup>	27
Liver (nmol/g)	236 <sup>a</sup>	8	241 <sup>a</sup>	9	234 <sup>a</sup>	8	236 <sup>a</sup>	4	311 <sup>b</sup>	22
Anterior tibialis (nmol/g)	47 <sup>a</sup>	4	59 <sup>a</sup>	6	58 <sup>a</sup>	4	78 <sup>a</sup>	9	516 <sup>b</sup>	106

LPS, lipopolysaccharide; OKG, Ornithine  $\alpha$ -ketoglutarate.

<sup>a,b</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* For details of groups and procedures, see Table 1 and pp. 628–629.

† For each variable, when an overall significant difference among the five groups was found (one-way ANOVA), the Newman–Keuls *post hoc* test was performed.

(Vaubourdolle *et al.* 1991; Kalfarentzos *et al.* 1996; Le Boucher *et al.* 1997a), with various intermediate dosages. However, there was no real rationale for the doses used. Accordingly, to determine the optimal dose, we performed ‘dose-ranging’ with a progression common ratio of 3, from 0.5 to 4.5 g/kg per d, to cover the full range of doses of OKG reported in the literature. We administered OKG as boluses because it has been shown that for a given dose, boluses are more efficient than continuous intragastric administration, especially for biological markers of protein turnover and wound healing (Le Bricon *et al.* 1997;

De Bandt *et al.* 1998). In addition, as OKG is a Gln precursor (Cynober, 2004), the use of boluses was considered relevant since a high dose of Gln as a bolus gives protection from endotoxaemic shock in rats (Wischmeyer *et al.* 2001). In addition, fractionation of the OKG dose in boluses permits high dosages without increasing side-effects such as diarrhoea (De Bandt *et al.* 1998).

The metabolic effects of OKG are linked to the metabolites released *in vivo* from Orn and  $\alpha$ -ketoglutarate (Le Boucher & Cynober, 1998). OKG acts in two major metabolic cycles: the Krebs cycle with its  $\alpha$ -ketoglutarate

**Table 4.** Plasma and tissue proline concentrations at killing\*†  
(Mean values with their standard errors)

Group...	Control		LPS-OKG-0.0		LPS-OKG-0.5		LPS-OKG-1.5		LPS-OKG-4.5	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma ( $\mu\text{mol/l}$ )	162 <sup>a</sup>	7	182 <sup>a</sup>	13	186 <sup>a</sup>	7	199 <sup>a</sup>	10	320 <sup>b</sup>	25
Jejunum mucosa (nmol/g)	599	50	646	55	666	49	769	60	662	67
Liver (nmol/g)	144 <sup>a</sup>	6	223 <sup>bc</sup>	15	215 <sup>bc</sup>	12	187 <sup>b</sup>	10	232 <sup>c</sup>	15
Anterior tibialis (nmol/g)	241 <sup>a</sup>	12	293 <sup>b</sup>	17	305 <sup>b</sup>	11	342 <sup>b</sup>	24	628 <sup>c</sup>	51

LPS, lipopolysaccharide; OKG, ornithine  $\alpha$ -ketoglutarate.<sup>a,b,c</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* For details of groups and procedures, see Table 1 and pp. 628–629.

† For each variable, when an overall significant difference among the five groups was found (one-way ANOVA), the Newman–Keuls *post hoc* test was performed.**Table 5.** Plasma and tissue arginine concentrations at killing\*†  
(Mean values with their standard errors)

Group...	Control		LPS-OKG-0.0		LPS-OKG-0.5		LPS-OKG-1.5		LPS-OKG-4.5	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma ( $\mu\text{mol/l}$ )	115	8	96	6	96	6	99	6	105	6
Jejunum mucosa (nmol/g)	220	30	183	15	198	24	229	25	193	28
Anterior tibialis (nmol/g)	153 <sup>a</sup>	12	113 <sup>b</sup>	15	94 <sup>b</sup>	6	89 <sup>b</sup>	8	143 <sup>a</sup>	15

LPS, lipopolysaccharide; OKG, ornithine  $\alpha$ -ketoglutarate.<sup>a,b</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* For details of groups and procedures, see Table 1 and pp. 628–629.

† For each variable, when an overall significant difference among the five groups was found (one-way ANOVA), the Newman–Keuls *post hoc* test was performed.

moiety and the urea cycle with its Orn moiety. OKG metabolites include Gln, Arg and Pro derived from Orn metabolism and OKG stimulates hormone (insulin and human growth hormone) secretion (Cynober *et al.* 1984; Cynober, 1991).

Our present ‘dose-ranging’ study clearly shows a linear relationship between dose of OKG administered and Gln, Orn and Pro tissue repletion. OKG is able to enhance Gln pools linearly in all tissues, confirming the major role of OKG as a Gln precursor and/or as sparing endogenous Gln stores (Le Boucher & Cynober, 1998). Our present results add to those observed in malnourished rats, showing a dose-related effect on Gln concentrations in plasma and muscle (Ziegler *et al.* 1991).

It is noteworthy that although OKG provides Orn directly, only the highest dose induced a significantly increased concentration in all tissues compared with controls. This may be related to the fact that Orn is heavily used in gluconeogenesis, a pathway that is activated in the present stress model. Thus, only 4.5 g OKG/kg per d was able to increase both Gln and Orn concentrations in jejunum mucosa; these substances act as an enterocyte fuel and purine precursor, and as a polyamine precursor respectively. We did not study intestinal function, but this observation is in line with results obtained in different experimental models showing that OKG can improve intestinal trophicity and brush border enzyme content (Duranton *et al.* 1998) or stimulate villi hyperplasia after intestinal resection (Dumas *et al.* 1998).

Pro, which is involved in tissue repair (Young & El-Khoury, 1995), showed variations that were also linearly

linked to OKG dose in plasma, muscle and liver at the highest dose. The action of Pro in collagen synthesis, a key step in wound healing, has been involved in OKG beneficial effects after burns (Le Bricon *et al.* 1997). Arg, which is an OKG metabolite mainly via Orn metabolism in ureagenesis, has been shown to increase in several tissues in endotoxaemic (Lasnier *et al.* 1996) and burned (Roch-Arveiller *et al.* 1996) rats treated with OKG. This increase was also found in our present study, restricted to muscle. This confirms that although OKG leads to Arg production *in vivo*, its main property is to act as a Gln precursor.

Important properties of OKG are improvement of N balance (Le Bricon *et al.* 1995; De Bandt *et al.* 1998; Donati *et al.* 1999) and reduction of myofibrillar catabolism (Le Boucher *et al.* 1997b; De Bandt *et al.* 1998; Coudray-Lucas *et al.* 2000). N balances were negative from day 0 to day 1 in the control and LPS groups because of the fasting state, but, as expected, were more negative in the endotoxaemic group. After injury, we found a positive linear relationship between OKG dose and cumulative N balance. However, only the highest dose allowed a positive mean value, whereas other OKG-treated groups and controls had a mean cumulative N balance not different from equilibrium, i.e. null. Of course, it is hopeless to obtain a positive N balance in stressed patients during the ‘flow phase’. However, it has been shown that pharmaconutrients can limit N loss in stressed patients; since the rat is more sensitive than humans to nutritional manipulation, the results we obtained highlight the ability of OKG to modulate protein metabolism. Likewise, a beneficial effect of OKG on myofibrillar catabolism was only observed with

the highest dose in the LPS-OKG-4.5 group, which was not different from the control group for urinary 3-methylhistidine excretion. We can hypothesize that the lack of correlation between OKG dose and 3-methylhistidine excretion may show that muscle Gln replenishment is required to obtain an OKG effect on myofibrillar catabolism, since the LPS-OKG-4.5 group was the only one that maintained muscle Gln concentration.

In conclusion, in this experimental model we found beneficial effects exerted by OKG in the context of injury and a dose-related effect on tissue Gln concentrations and N balance. This 'dose-ranging' study is new and provides data on OKG metabolite generation at the tissular level that cannot be obtained in human subjects for obvious ethical reasons, and which confirm the major role of OKG in generating Gln in tissues. Given that only the highest dose of 4.5 g/kg per d was able to counteract the myofibrillar catabolism, achieve positive N balance, restore Gln pools and increase tissue Orn, Pro and Arg concentrations, we recommend a daily dose of OKG in this range for further animal studies designed to elucidate the mechanisms of action of OKG.

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