Original

**Microdialysis detection of lactate in subcutaneous tissue as a reliable indicator of tissue metabolic disorders in an animal sepsis model**

Hitoshi Ohashi¹, Naruo Kawasaki¹, Hirotsugu Komatsu¹, Takafumi Wada¹, Akiko Hosoyama¹, Nobuyoshi Hanyu², Katsutoshi Kobayashi², Maiko Ohashi³ and Yasuhiko Taira¹

¹Department of Emergency and Critical Care Medicine, St Marianna University School of Medicine;
²Department of Surgery, Jikei University School of Medicine;
³Department of Plastic Surgery, St Marianna University School of Medicine, Japan

Received November 11, 2010; Accepted December 24, 2010

**Abstract**

**Purpose:** Tissue dysoxia is thought to be a fundamental cause of the organ failure that occurs as a result of shock. Plasma lactate has been frequently measured as an indicator of the state of systemic tissue metabolism. On the other hand, tissue lactate levels can directly indicate a disorder in the state of cytological tissue metabolism. The continuous monitoring of lactate levels in subcutaneous tissue will reflect the state of tissue dysoxia more precisely than levels of lactate in the plasma lactate. We have investigated the differences in the levels of plasma and tissue lactate using a microdialysis (MD) technique in an animal septic shock model. **Method:** Male 8-week-old Wistar/ST rats were used. We prepared an animal model by injection of lipopolysaccharide (LPS) into the abdominal cavity. LPS was given to 9 animals in the experimental group while physiological saline was given to 6 animals in the control group. A MD probe was used to quantify the lactate levels in the subcutaneous tissue. The mean arterial pressure, blood gas content and lactate levels were measured every 50 min up to 400 min after injection and compared between both groups. **Result:** The MAP of both groups showed similar changes after injection. Plasma lactate levels in the LPS group showed a significant increase after 100 min and reached a plateau from 150 min to 250 min. Subcutaneous lactate in the LPS group showed a significant increase after 150 min. Subcutaneous pyruvate in the LPS group showed a significant increase after 100 min. The lactate/pyruvate (L/P) ratio in the subcutaneous tissue showed a sustained increase from 300 min in the LPS group. **Conclusion:** Monitoring plasma lactate levels is useful for the early assessment of anaerobic metabolism before hypotension. Plasma lactate levels did not increase during some periods. This phenomenon was due to the balance between production and utilization. However, tissue lactate showed a chronological increase. These results suggest that the measurement of tissue lactate levels is reliable for assessing local energy metabolic disturbances. Under conditions of septic shock, an increase in lactate levels was found to be a sensitive marker of tissue metabolism disorder.

Key words: microdialysis, blood capillary, lipopolysaccharide (LPS), lactate, peripheral tissue metabolism

Correspondence to: Dr. Hitoshi Ohashi, Department of Emergency and Critical Care Medicine, St Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki City, Kanagawa 216-8511, Japan Phone: +81-44-977-8111, ext. 80024 Fax: +81-44-979-1522 e-mail: h-ohashi@mygsji.or.jp
Introduction

Shock has been defined as a state of markedly impaired peripheral circulation due to decreased blood pressure. More recently, shock has been defined as a state in which blood flow to important organs cannot be maintained, resulting in cytological tissue metabolism disorder and organ damage (Kruse, 1999). According to Antonelli et al. (2007), hypotension is not required to define shock, and as a result, the presence of inadequate tissue perfusion on physical examination should be the essential factor. Given the current evidence, the only bio-marker recommended for diagnosis or staging of shock is plasma lactate levels. Plasma lactate levels are easily measured, and so have been frequently quantified to ascertain systemic tissue metabolism (Vitek et al., 1971). However, plasma lactate levels are affected by production and metabolism in the whole body, therefore they do not precisely indicate the metabolic process in the local tissue.

We used microdialysis (MD) to measure the local tissue lactate and pyruvate levels in our study. In MD, compounds in blood, cerebrospinal fluid and extracellular fluid are serially recovered via the semi-permeable membrane of a MD probe, and this enables quantification of nerve mediators and metabolites. From measurements of lactate levels in the subcutaneous tissue and the peritoneum, it has been reported that anaerobic metabolism could be detected at an early stage (Ungerstedt, 1991; Jansson et al., 2005). It is known that local tissue lactate levels are raised in the event of tissue metabolism disorder, as blood distribution imbalance takes place to protect important organs. Only a few studies have compared tissue lactate levels with conventional indicators such as plasma lactate, systemic arterial pressure and blood gas data.

We measured lactate and pyruvate levels in subcutaneous tissue, and calculated the lactate/pyruvate (L/P) ratio in the tissue. The first aim of this study was to determine whether tissue lactate levels could provide a more sensitive and earlier detection of septic shock than plasma lactate levels. Secondly, the study examined whether the continuous monitoring of lactate levels in the subcutaneous tissue reflected the state of tissue dysoxia more precisely than plasma lactate levels. And thirdly, the present study was designed to assess whether the monitoring of lactate levels and determination of a L/P ratio in subcutaneous tissue could be used to give a prognosis of septic shock.

Methods

Experimental animal preparation

Male 8-week-old Wister/ST rats (270–330g) (Japan SLC Inc, Shizuoka, Japan) were used for the present study. After 6 h of fasting, 3 mL of diethyl ether was used to induce anesthesia. Anesthesia was maintained by administering 2.0 L/min of 1.2% isoflurane using a systemic anesthesia machine for laboratory animals (TK-5; Neuroscience Inc., Japan). All animals were placed in a supine position and managed by spontaneous respiration. A tube (inner diameter, 0.5 mm; outer diameter, 0.8 mm; SP31: Natsume Seisakusho Co Ltd, Osaka, Japan) for measuring arterial pressure and collecting blood sample was cannulated into the left femoral artery. Mean arterial pressure (MAP) was measured using a blood pressure transducer (Lifekit DX-360; Becton, Dickinson and Company, Tokyo, Japan) via the catheter tube. For MD, the introducer (CMA/20
Continuous monitoring of tissue lactate allows prognosis of shock

14/10 PC probe, CMA Microdialysis AB, Solna, Sweden) was inserted into the subcutaneous tissue in the middle abdominal wall. At the end of the study, the skin was dissected to ensure that the probe had been properly placed in the subcutaneous tissue.

Study design

After confirming systemic body stability, 40 mg/kg of lipopolysaccharide from Escherichia coli (LPS; Sigma Lot No.035k405), adjusted to 5 mg/mL with physiological saline, was injected into the abdominal cavity. LPS was administered to 9 animals in the experimental group while physiological saline was injected into the abdominal cavity of the 6 animals in the control group. Experimental data were obtained at 50 min intervals for a period of 400 min after injection, and the data obtained from the LPS-injected group compared with that obtained from the saline-injected (control) group.

Measurement methods and items

Using the catheter in the femoral artery, 100 µL of blood was collected at 0, 50, 100, 150, 200, 250, 300, 350 and 400 min after injection of either LPS or saline, and the MAP, arterial blood gas and plasma lactate levels measured using an i-STAT portable gas analyzer (i-STAT Corporation, Illinois, U.S.A.). MD was performed to quantify lactate and pyruvate levels in the tissue interstitium of the abdominal subcutaneous tissue. Lactate free Ringer’s solution (Na 147 mEq/L, K 4 mEq/L, Ca 5 mEq/L, Cl 156 mEq/L) was injected as a perfusion solution into the tissue at a rate of 1.0 µL/min using a pump (CMA400, CMA Microdialysis AB, Solna, Sweden). Tissue fluid was collected using the method described by Klaus et al. (Klaus et al., 2004). The dialysate was analyzed spectrophotometrically for concentrations of lactate and pyruvate by the Microdialysis analyzer (CMA600, CMA Microdialysis AB, Solna, Sweden). Lactate and pyruvate in collected fluids were measured at 0, 50, 100, 150, 200, 250, 300, 350 and 400 min after injection. The time course of the L/P ratio was also investigated.

Statistical analysis

Each value was expressed as the mean ± SEM. Data were analyzed by a two-way repeated measures analysis of variance (ANOVA; Excel Statistics ver. 5.0, ESUMI Co., Ltd., Tokyo, Japan). When interaction between time and treatment (saline or LPS) was statistically significant, significance between control and LPS-treated groups at each time point was analyzed by F-analysis followed by Student’s t-test and Aspin-Welch’s t-test for homoscedastic and heteroscedastic data, respectively. P values less than 0.05 were considered to be statistically significant.

Experimental guideline

The above animal study was conducted in accordance with St. Marianna University School of Medicine Animal Study Guidelines and Ethical Committee.
Results

**MAP and arterial blood gases**

Figure 1 shows the time course of the MAP after saline (control) or LPS injection. A two-way repeated measures ANOVA revealed a significant interaction between treatment and time \([F (8, 143) = 4.210, P<0.001]\). A post hoc analysis revealed that the MAP of LPS-treated rats was significantly higher between 200 and 250 min, but lower at 400 min than in control rats at the same time points \((#, P<0.01; *, P<0.05)\).

***Table 1. Arterial blood gas levels***

|          | 0     | 50    | 100   | 150   | 200   | 250   | 300   | 350   | 400 (min) |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-----------|
| **PH**   |       |       |       |       |       |       |       |       |           |
| LPS      | 7.4 ± 0.0 | 7.4 ± 0.0 | 7.4 ± 0.0 | 7.4 ± 0.0 | 7.4 ± 0.0 | 7.4 ± 0.0 | 7.3 ± 0.0 | 7.3 ± 0.0 | 7.3 ± 0.1 | 7.3 ± 0.1 |
| Control  | 7.3 ± 0.0 | 7.3 ± 0.0 | 7.4 ± 0.0 | 7.4 ± 0.0 | 7.4 ± 0.0 | 7.4 ± 0.0 | 7.4 ± 0.0 | 7.4 ± 0.1 | 7.4 ± 0.0 |           |
| **pCO₂ (mmHg)** |       |       |       |       |       |       |       |       |           |
| LPS      | 52.8 ± 4.6 | 50.1 ± 4.1* | 48.4 ± 2.7* | 47.7 ± 1.8 | 46.7 ± 3.5# | 44.0 ± 3.2# | 42.9 ± 5.1# | 40.9 ± 3.6# | 39.8 ± 9.0* |
| Control  | 57.0 ± 5.3 | 57.0 ± 7.1 | 55.8 ± 6.1 | 54.8 ± 6.8 | 54.9 ± 6.8 | 54.5 ± 6.5 | 53.1 ± 7.2 | 51.4 ± 7.5 | 50.8 ± 4.8 |
| **pO₂ (mmHg)** |       |       |       |       |       |       |       |       |           |
| LPS      | 79.6 ± 5.2 | 81.1 ± 5.4 | 86.7 ± 7.3 | 87.3 ± 8.8 | 85.0 ± 7.1 | 82.7 ± 7.7 | 86.1 ± 8.4 | 88.9 ± 10.0 | 94.7 ± 14.1 |
| Control  | 81.5 ± 7.3 | 84.0 ± 8.9 | 82.8 ± 3.7 | 83.2 ± 3.3 | 81.7 ± 9.5 | 83.2 ± 6.7 | 88.2 ± 7.3 | 86.8 ± 6.9 | 87.2 ± 11.9 |
| **HCO₃ (mmol/L)** |       |       |       |       |       |       |       |       |           |
| LPS      | 31.3 ± 1.5 | 29.5 ± 1.3 | 27.6 ± 1.3# | 26.7 ± 1.6# | 26.0 ± 1.9# | 25.1 ± 2.0# | 22.8 ± 2.9# | 20.0 ± 2.0# | 17.8 ± 1.9# |
| Control  | 31.2 ± 1.1 | 31.1 ± 1.9 | 30.7 ± 1.9 | 31.2 ± 1.7 | 30.5 ± 2.2 | 30.2 ± 1.6 | 29.6 ± 0.9 | 29.0 ± 0.9 | 28.7 ± 0.8  |

At the time of injection (0 min), no significant changes were seen in either group. Interactions between time and treatment in both pH and pO₂ were not significant, however, those in pCO₂ and HCO₃ levels were statistically significant. In LPS-treated rats, significant decreases in pCO₂ were observed between 50 and 400 min except at 150 min, suggesting that a respiratory compensation for acidosis had occurred. The HCO₃ levels of LPS-treated rats were significantly lower than those of control rats between 100 and 400 min after LPS injection \((#, P<0.01; *, P<0.05)\).
Continuous monitoring of tissue lactate allows prognosis of shock

The LPS-treated rats was significantly lower than those of control rats at 100 to 400 min after the LPS injection.

Plasma and subcutaneous tissue lactate levels

Figure 2 shows changes in plasma lactate and subcutaneous tissue lactate levels after the injection of either LPS or saline. A two-way ANOVA with repeated measures for plasma lactate revealed a significant interaction between treatment and time [F (8, 143) = 29.935, P<0.001], a significant effect of time [F (8, 143) = 58.022, P<0.001] and a significant effect of treatment [F (1, 143) = 491.739, P<0.001]. A post hoc analysis revealed that the plasma lactate levels of LPS-treated rats were significantly higher than those of control rats at 100 to 400 min after the LPS injection. A two-way ANOVA with repeated measures for subcutaneous tissue lactate yielded a significant interaction between time and treatment, and a significant effect of time [F (8, 143) = 30.461, P<0.001] and a significant effect of treatment [F (1, 143) = 162.189, P<0.001]. A post hoc analysis revealed that LPS-treated rats showed a significant increase in the subcutaneous tissue lactate levels at 150 to 400 min as compared with control rats. The results suggest that plasma lactate levels are a more sensitive indicator of early shock detection than the subcutaneous tissue lactate levels. Interestingly, plasma lactate levels reached a plateau at between 150 and 250 min and rose again after 250 min, while the subcutaneous tissue lactate level increased continuously from 150 to 400 min after the LPS injection.

Lactate/pyruvate (L/P) ratio and pyruvate levels in the subcutaneous tissue

We measured the subcutaneous tissue pyruvate levels in addition to tissue lactate levels and calculated the L/P ratio in the subcutaneous tissue (Figs. 3 and 4). A two-way ANOVA with repeated measures revealed a significant interaction between time and treatment, and a significant...
effect of treatment in the subcutaneous tissue pyruvate and L/P ratio in the subcutaneous tissue. A post hoc analysis yielded that LPS-treated rats showed significant increase in the subcutaneous tissue pyruvate level from 100 to 400 min as compared with control rats (#, $P<0.01$; *, $P<0.05$).

**Discussion**

The present results suggest that continuous monitoring of tissue lactate levels, even with a stable blood pressure, enables the prognosis of shock. Detecting shock at the peripheral tissue in
Continuous monitoring of tissue lactate allows prognosis of shock

In shock, an anaerobic glycolysis occurs, which end up with a metabolic acidosis. Thus arterial blood gas analysis can provide one of the indices of anaerobic metabolism. In this experiment, arterial blood gas analysis showed that interactions between time and treatment in pH and pO$_2$ were not significant, those in pCO$_2$, HCO$_3$ were statistically significant. Especially, HCO$_3$ was statistically significantly lower (Table 1). In clinical settings, HCO$_3$ is frequently measured as a convenient method to detect anaerobic metabolism (Bunker, 1965). HCO$_3$ of LPS-treated rats was significantly lower than those of control rats from 100 to 400 min after the LPS injection. But HCO$_3$ did not change dramatically in the period from 100 to 200 min after injection. In fact, when the changes in HCO$_3$ from 100 to 200 min were examined in individual rats, changes of more than 20% were confined to only 2 out of the 9 animals.

Plasma lactate is thus frequently measured in clinical settings as a convenient method to detect anaerobic metabolism (Bakker et al., 2007; Mikkelsen et al., 2009). In the present study, lactate was significantly higher in the LPS-treated group than in the control group, with the increase in the LPS group occurring soon after LPS injection. Plasma lactate levels in the LPS-treated group showed a significant increase after 100 min ($P<0.01$). On the other hand, subcutaneous lactate levels in the LPS-treated group showed a significant increase after 150 min ($P<0.01$). Our observation confirms that plasma lactate is useful for the early assessment of anaerobic metabolism. In our result, plasma lactate levels reached a plateau for the period from 150 to 250 min after LPS injection (Fig. 2). In addition to the overall observation, when we observed the changes in plasma lactate during this period in individual rats, only 2 of the 9 rats exhibited a change of more than 20%. In consequence, plasma lactate remained at a constant level while the blood pressure was stable. This phenomenon was possibly due to a balance between production and utilization of lactate. The problem is that the assessment of severity could be in error as a result of the masked changes in plasma lactate levels.

Lactate is produced mainly in the skin, skeletal muscle, the gut, leucocytes and red blood cells. It is mainly utilized in the liver and the kidney (Iscra et al., 2002). Levraut reported that the plasma lactate concentration of septic patients was closely linked to the reciprocal of lactate clearance but not to lactate production (Levraut et al., 1988). Hyperlactatemia occurring in a septic patient is mainly due to a defect in the utilization of lactate in the liver, kidney and heart. In the present study, the tissue lactate as assessed by MD showed an increase over time. The subcutaneous tissue lactate was not affected by metabolism. It was shown that dysoxia in subcutaneous tissue occurred even if the blood pressure was maintained. These results suggest that the tissue lactate measurement is useful and reliable for assessing local energy metabolic disturbances.

The L/P ratio has been used to assess tissue circulatory failure and ischemia. Especially, visceral ischemia which is an important factor in multiple organ failure. Both lactate levels and the L/P ratio are well-known markers of anaerobic metabolism and ischemia. We have previously suggested that the L/P ratio in subcutaneous tissue using the MD method is more sensitive and a possible early detection marker for hemorrhagic shock (Ohashi et al., 2009). Lactate levels, and particularly the L/P ratio in subcutaneous tissue, continuously increased. Cells take up as much
glucose as possible in order to produce ATP by anaerobic glycolysis. The decrease in glucose delivery from the capillaries together with the increase in glucose uptake leads to a fall in the glucose concentration in the dialysate. More lactate is produced in order to regenerate nicotinamide adenine dinucleotide (NAD+) which is necessary for keeping up anaerobic glycolysis. The result will be an increase in lactate and a decrease in pyruvate resulting in an increase in the lactate/pyruvate ratio.

In this study, subcutaneous lactate in the LPS-treated group (the septic shock model) showed a significant increase after 150 min ($P<0.01$). On the other hand, pyruvate in the subcutaneous tissue showed an increase from 100 min ($P<0.01$). The L/P ratio in the subcutaneous tissue showed a sustained increase from 300 min ($P<0.01$). Thus, in the septic shock model, lactate and pyruvate were the most sensitive markers, although the L/P ratio was not as sensitive. This was because both pyruvate and lactate levels were elevated concurrently, with the result that the L/P ratio was not a sensitive marker. Thus this result represents tissue circulatory failure prior to collapse of the compensatory mechanisms for the vital signs.

Chew et al. (2008) have studied the local energy and metabolic disturbances that occur during porcine endotoxaemia. Compared with the control group, the endotoxaemic animals displayed significantly decreased left ventricular stroke work and venous oxygen saturation ($SvO_2$), with increased mean pulmonary artery pressure and plasma lactate levels. Despite severe systemic and pulmonary hemodynamic changes, interstitial MD measurements revealed no evidence of anaerobic metabolism in the myocardium of the endotoxaemic pigs. There were, however, changes in the concentrations of both glucose and pyruvate, suggesting local energy and metabolic disturbances. This indicates that there was a either a decrease in ATP production or a depletion of mitochondrial energy stores. Even if oxygen is supplied to mitochondria and the blood pressure is maintained, ATP is not produced in cells, resulting in an increase in tissue pyruvate without a reduced oxygen supply in the tissue.

Levy et al. (2005) reported the use of MD probes which were inserted into the quadriceps muscles of patients with septic shock who had been infused with ouabain. Ouabain, a specific inhibitor of Na$^+$-K$^+$-ATPase, reduces muscle lactate. During shock, plasma epinephrine concentrations are raised. Epinephrine enhances glycogenolysis with a net increase in pyruvate production. Epinephrine induces hyperlactataemia by binding to adrenergic $\beta_2$ receptors in muscle and raising AMP production. Such an increase leads to the coordinated stimulation of both Na$^+$-K$^+$-ATPase activity and glycogenolysis. Activation of Na$^+$K$^+$ ATPase generates ADP, thereby raising phosphofructokinase activity, accelerating aerobic glycolysis, and increasing lactate concentration. This mechanism of lactate generation during septic shock in skeletal muscle occurs during exaggerated aerobic glycolysis rather than during tissue hypoxia.

Szabó et al. (1996) used cultured vascular smooth muscle cells and reported that peroxynitrite (ONOO$^-$), peroxide generated by ischemia and inflammation, hinders ATP production in mitochondria. Peroxynitrite is a reactive oxidant produced from nitric oxide (NO) and superoxide, which reacts with proteins, lipids, and DNA under conditions of inflammation and shock. Peroxynitrite can initiate toxic oxidative reactions in vitro and in vivo. Initiation of lipid peroxidation, direct inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane Na$^+$-K$^+$-ATPase activity,
inactivation of membrane sodium channels, and other oxidative protein modifications contribute to the cytotoxic effect of peroxynitrite. In addition, peroxynitrite is a potent trigger of DNA strand breakage, with subsequent activation of the nuclear enzyme poly-ADP ribosyl synthetase, with eventual severe energy depletion of the cells (Szabó, 1996).

Cytokines have been suggested as one of the factors involved in the increase of lactate levels. Vary et al. (1999) have reported that administration of IL-1 to rats increased lactate levels in skeletal muscle and lowered the levels of activated pyruvate dehydrogenase, resulting in a lactate metabolism disorder. Tredget et al. (1988) administered TNF-α and IL-1β to rabbits and reported increased lactate levels, an elevated glycolytic system and decreased oxygen utilization.

So far, it is impossible to measure inflammatory cytokines, ATP and NAD⁺ directly to understand the state of oxygen metabolism disorders in tissues. In addition, there is no gold standard to investigate disorders of tissue oxygen metabolism directly at this stage either.

In the present study, the increase in tissue lactate levels in the LPS-treated group might represent a dysfunction in the utilization of oxygen, rather than a reduced oxygen supply. The tissue lactate levels would indicate local energy metabolic disturbances in the surrounding cells. Quantifying lactate levels in the subcutaneous tissue by MD is thus, less invasive, and an effective and convenient method of assessing the state of oxygen metabolism disorder in tissues.

References
Antonelli, M., Levy, M., Andrews, P.J., Chastre, J., Hudson, L.D., Manthous, C., Meduri, G.U., Moreno, R.P., Putensen, C., Stewart, T. and Torres, A. (2007). Hemodynamic monitoring in shock and implications for management. *Intensive Care Med.* 33: 575–590.
Bakker, J. and Jansen, T.C. (2007). Don’t take vitals, take a lactate. *Intensive Care Med.* 33: 1863–1865.
Bunker, J. (1965). The Great Trans-Atlantic Acid-Base Debate. *Anesthesiology* 25: 591–594.
Chew, M.S., Johansson, A., Anderson, C., Ersson, A. and Tønnesen, E. (2008). Decreases in myocardial glucose and increases in pyruvate but not ischemia are observed during porcine endotoxaemia. *Acta Anaesthesiol. Scand.* 52: 959–968.
Isca, F., Gullo, A. and Biolo, G. (2002). Bench-to-bedside review: Lactate and the lung. *Crit. Care* 6: 327–329.
Jansson, K., Jansson, M., Andersson, M., Magnuson, A., Ungerstedt, U. and Norgren, L. (2005). Normal values and differences between intraperitoneal and subcutaneous microdialysis in patients after non-complicated gastrointestinal surgery. *Scand. J. Clin. Lab. Invest.* 65: 273–281.
Klaus, S., Heringlake, M. and Bahlmann, L. (2004). Bench-to-bedside review: microdialysis in intensive care medicine. *Crit. Care* 8: 363–368.
Kruse, J.A. (1999). Searching for the perfect indicator of dysoxia. *Crit. Care Med.* 27: 469–471.
Levraut, J., Ciebiera, J.P., Chave, S., Rabary, O., Jambou, P., Carles, M. and Grimaud, D. (1988). Mild hyperlactatemia in stable septic patients is due to impaired lactate clearance rather than overproduction. *Am. J. Respir. Crit. Care Med.* 157: 1021–1026.
Levy, B., Gibot, S., Franck, P., Cravoisy, A. and Bollaert, P.E. (2005). Relation between muscle Na⁺ K⁺ ATPase activity and raised lactate concentrations in septic shock: a prospective study. *Lancet* 365: 871–875.
Mikkelsen, M.E., Militades, A.N., Gaieski, D.F., Goyal, M., Fuchs, B.D., Shah, C.V., Bellamy, S.L. and Christie, J.D. (2009). Serum lactate is associated with mortality in severe sepsis independent of organ failure and shock. *Crit. Care Med.* 37: 1670–1677.
Ohashi, H., Kawasaki, N., Fujitani, S., Kobayashi, K., Ohashi, M., Hosoyama, A., Wada, T. and Taira, Y.
(2009). Utility of microdialysis to detect the lactate/pyruvate ratio in subcutaneous tissue for the reliable monitoring of hemorrhagic shock. *J. Smooth Muscle Res.* **45**: 269–278.

Szabó, C. (1996). The pathophysiological role of peroxynitrite in shock, inflammation, and ischemia-reperfusion injury. *Shock* **6**: 79–88.

Szabó, C., Zingarelli, B. and Salzman, A.L. (1996). Role of poly-ADP ribosyltransferase activation in the vascular contractile and energetic failure elicited by exogenous and endogenous nitric oxide and peroxynitrite. *Circ. Res.* **78**: 1051–1063.

Tredget, E.E., Yu, Y.M., Zhong, S., Burini, R., Okusawa, S., Gelfand, J.A., Dinarello, C.A., Young, V.R. and Burke, J.F. (1988). Role of interleukin 1 and tumor necrosis factor on energy metabolism in rabbits. *Am. J. Physiol.* **255**: 760–768.

Ungerstedt, U. (1991). Microdialysis—principles and applications for studies in animals and man. *J. Intern. Med.* **230**: 365–373.

Vary, T.C., O’Neill, P., Cooney, R.N., Maish, G. 3rd and Shumate, M. (1999). Chronic infusion of interleukin 1 induces hyperlactatemia and altered regulation of lactate metabolism in skeletal muscle. *JPEN J. Parenter. Enteral. Nutr.* **23**: 213–217.

Vitek, V. and Cowley, R.A. (1971). Blood lactate in the prognosis of various forms of shock. *Ann. Surg.* **173**: 308–313.