On the carbohydrate metabolic response to an experimental infection with *Brachyspira hyodysenteriae* (swine dysentery) in pigs

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Summary
The carbohydrate metabolic response to experimentally induced swine dysentery was studied in crossbreed pigs. Twelve pigs, with a mean weight of ~20 kg, were orally inoculated with *Brachyspira hyodysenteriae* strain B204. After an incubation period of 6-20 days, five animals developed swine dysentery with haemorrhagic diarrhoea and two animals developed non-haemorrhagic diarrhoea. Five animals remained healthy throughout the study. Blood samples from the animals with clinical signs of disease were collected before inoculation, several times during the course of the dysentery and finally after recovery. Blood samples from animals that remained healthy were obtained before inoculation and at slaughter four weeks later. Glucose, lactate and cortisol concentrations did not differ between sampling occasions in the healthy animals. In the sick animals, higher concentrations were observed when haemorrhagic diarrhoea occurred (mean peak value ± SD: glucose 7.6 ± 0.7 mmol/L; lactate 4.5 ± 1.7 mmol/L; cortisol 278 ± 86 nmol/L) compared to before inoculation (mean value ± SD: glucose 5.1 ± 1.2 mmol/L; lactate 1.3 ± 0.5 mmol/L; cortisol 24 ± 11 nmol/L). At slaughter, tissue samples from *m. biceps femoris, m. longissimus dorsi,* myocardium and liver were collected from 10 pigs and glycogen analysis was performed. Glycogen concentrations did not differ between the healthy pigs and those that developed swine dysentery: concentrations were highest in the liver and lowest in the heart. In conclusion, experimental infection with *B. hyodysenteriae* results in alteration of the carbohydrate metabolism, which is characterised by a transient increase in blood glucose and lactate concentrations during the initial phase of the haemorrhagic period of the disease.

Introduction
In biomedical research, the pig is becoming more important as a large-animal model, and currently five to six times more pigs than dogs are used in Sweden *(The Swedish National Board for Laboratory Animals, www.CFN.se)*. The pig is extensively used in studies concerning cardiovascular research, the digestive system, immunology, metabolism, nutrition, surgical training and experimental infections *(Mount & Ingram, 1971; Trott et al., 1996)*.

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In the present study, the pig was chosen as an animal model for swine dysentery, a well-known disease in pig production. Swine dysentery, which is caused by the microbe *Brachyspira hyodysenteriae*, can induce severe mucohaemorrhagic diarrhoea *(Smith et al., 1990)*. The lesions in the large intestines are characterised by haemorrhages and necrosis. Affected herds often suffer devastating production losses *(Windsor & Simmons, 1981)*. A mortality of approximately 50% among untreated animals has been reported *(Fellström, 1996)*. Although several aspects of the disease have been thoroughly elucidated it is unknown if metabolic changes occur.

Mobilization of energy from substrate stores is needed when the body is exposed to stress situations e.g. trauma, injury, burns and infection. In human patients with progressive organ failure, hypermetabolism occurs, which is characterised by suppression of glycogen synthesis, increased glycogenolysis, gluconeogenesis and increased insulin resistance *(Cerra, 1987; Mizock, 1995)*. These metabolic alterations lead to hyperglycaemia and hyperlactatemia *(Mizock, 2001)*. The regulation of carbohydrate metabolism is mediated by mechanisms associated with the activation of the hypothalamic-pituitary-adrenal axis, adrenergic and cytokines systems *(McCann et al., 2000; Mizock, 2000; 2001)*. Cortisol a catabolic hormone, serves to stimulate gluconeogenesis and enhance glycogenolysis *(Spitzer et al., 1988)*. This hormone is a frequently used marker in pigs for various stress situations such as physical stress *(Jensen-Waern & Nyberg, 1993; Jensen-Waern & Fossum, 1993)* and surgical stress *(Dalin et al., 1993)*.

The aim of the present experiment was to determine if alteration in carbohydrate metabolism occurred during the course of experimentally induced swine dysentery. During dysentery, analyses of glucose, lactate and cortisol concentrations in plasma were repeatedly performed. At slaughter, glycogen concentration in skeletal muscles, heart and liver was analysed.

Materials and Methods

Animals and experimental design

The experimental design was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden. The animals used in the study consisted of twelve commercially purchased and clinically healthy crossbreed pigs (Yorkshire x Swedish Landrace) of both sexes. On arrival at the Department of Large Animal Clinical Sciences, SLU, the pigs were ~11 weeks old and weighed ~20 kg. The animals were kept in groups of three pigs per pen, given free access to water and were fed twice daily with a commercial diet ±Singel Veg SPK, Fori HB, Lidköping, Sweden). All pigs were acclimatized for at least one week prior to onset of the experiment.

All pigs were orally inoculated once a day for three consecutive days with 30 ml of BHI broth containing 10^7-10^9 B. *hyodysenteriae* strain B204/ml. Prior to infection, the culture was analysed with phase contrast microscope for purity and growth. Clinical examinations were performed daily and the following variables were recorded: general appearance, rectal temperature, faecal consistency and signs of diarrhoea. From the first day of inoculation until slaughter, daily faecal samples were collected for bacteriology.

Before inoculation and using the anaesthetic and surgical protocol described by Jacobson *et* al. (2001), five pigs (pigs A, C, D, E and G) were fitted with an intestinal cannula in the caecum. The surgical cannulation was performed to enable repeated endoscopic monitoring and biopsy collection from the large intestine. These morphological results will be reported in a separate article. After cannulation, these pigs were kept in single pens throughout the study and a two-week post-surgical recovery period was allowed before inoculation. Seven out of the twelve pigs developed clinical signs of swine dysentery after an incubation period of 6-20 days. These pigs were therefore named the experimental group and five of them developed haemorrhagic diarrhoea (pigs A, B, C, D and E) while two of them non-haemorrhagic diarrhoea (pigs F and G). Blood samples ±5 ml), obtained either in the pen or in connection with the biopsy sampling, were collected from the jugular vein into heparinized vacutainer tubes, before inoculation and between four to seven times after onset of clinical signs of diarrhoea (Fig.1). These samples were centrifuged at 4°C and 3000 rpm for 10 min and the plasma was collected and stored at -80°C until analyses.

Blood samples (5 ml) from the five pigs that did not develop any clinical signs of swine dysentery were taken from the jugular vein into heparinized vacutainer tubes on two occasions, before inoculation and 4 weeks later, at slaughter.

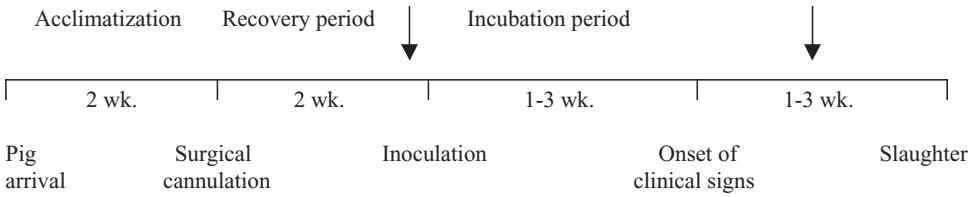

Figure 1. Design of the experimental infection

Three pigs with diarrhoea and a poor body condition (pigs A, B and C) were slaughtered during infection and four pigs (pigs D, E, F and G) were slaughtered after a recovery period of one to four days: all pigs were euthanized with a captive bolt and exsanguination. At slaughter, tissue samples for glycogen analyses were collected from *m. biceps femoris, m. longissimus dorsi,* myocardium and liver from 10 pigs. The biceps femoris muscle was incised over the middle of the muscle belly and a sample was taken from the centre of the muscle. The longissimus dorsi muscle was incised from a point between the last rib and the iliac crest, and a sample was taken from the centre of the muscle belly. The myocardium sample was obtained from the left papillary muscle. The liver sample was taken from the medial lobe. All tissue samples were ~ 1 cm^3 in size and were immediately frozen in liquid nitrogen and stored at -80°C until analyses.

Glucose, lactate and cortisol analyses

Plasma glucose concentration was analysed fluorometrically (fluorometer FL 600®, Bio-Tek Instruments, Inc., Vermont, USA), with a modified method by Lowry and Passonneau (1972). The concentrations were expressed as mmol/L.

Plasma lactate concentration was determined using an enzymatic lactate analyser (Analox, GM7, Analox Instruments Ltd., London, UK), according to the manufacturer's instruction. The values were given as mmol/L.

Plasma cortisol concentration was determined with a solid-phase radioimmunoassay kit (Coat-A-Count® Cortisol, Diagnostic Products Co., Los Angeles, CA). The concentrations were expressed as nmol/L.

Glycogen analyses

The tissues were freeze-dried for 24 hours and the muscles were dissected free from blood, fat and connective tissue under a microscope. Approximately 1-2 mg of the sample was weighed and put into a glass tube. One ml 1 mol/L HCl was added to each tube that was then sealed and boiled in a water bath for 2 hours. With a modified method by Lowry and Passonneau (1972), glycogen was then analysed fluorometrically (fluorometer FL 600®, Bio-Tek Instruments, Inc., Vermont, USA) for glucose residues. The concentrations were expressed as mmol/kg dry weight.

Faecal analysis

Faecal samples were transported in Amies medium and cultured as described by Fellström *et al.* (1996) for confirmation of *Brachyspira hyodysenteriae*. Culturing was performed at the Department of Bacteriology, National Veterinary Institute, Uppsala, Sweden.

Statistical analysis

All values were expressed as mean ± SD. The Wilcoxon Signed Rank test was used to compare differences within groups. The Mann-Whitney U test was used to compare differences between groups. Differences were regarded as significant at $P < 0.05$. The statistical analysis was performed with SigmaStat® statistical software version 2.0 (SPSS® Science, Chicago, USA).

Results

The mean daily weight gain for the pigs that remained healthy throughout the study was 0.6 kg. Faecal samples were cultured positive for *Brachyspira*

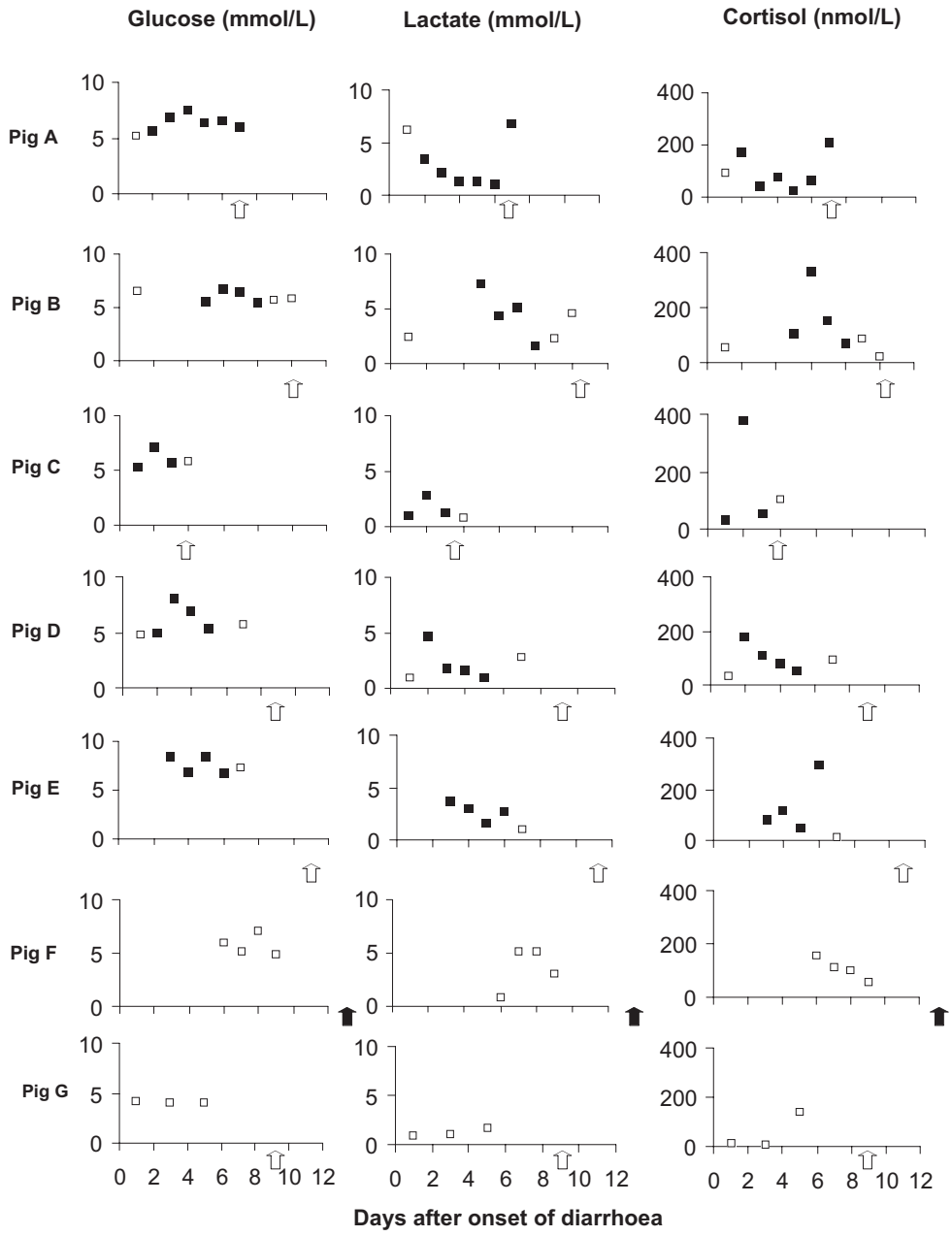

Figure 2. Individual plasma glucose, lactate and cortisol values from pigs with swine dysentery. Blood samples were taken repeatedly after onset of diarrhoea.

▫ Non-haemorrhagic diarrhoea; ■ Haemorrhagic diarrhoea;

⇧ Slaughter date for pig A-E, G; ⬆ Slaughter date for pig F ±21 days after onset of diarrhoea)

spp. for all but one pig.

The pigs in the experimental group displayed clinical signs of diarrhoea for at least one week and during this period the peak rectal temperatures were 39.4-40.6 °C. Some of the pigs had a slightly decreased appetite and their body weight decreased or remained unchanged during the period of diarrhoea.

Glucose

In the healthy pigs, the mean plasma glucose concentrations did not differ between the samples taken before inoculation (6.0 ± 0.9 mmol/L) and four weeks later at slaughter (5.3 ± 0.9 mmol/L). The initial value did not differ from the mean glucose value (5.1 ± 1.2 mmol/L) before inoculation in the experimental group. As seen in Fig. 2, the highest individual glucose value was observed in the beginning of the haemorrhagic diarrhoea period and the mean peak value was 7.6 ± 0.7 mmol/L. Pigs D and E recovered from haemorrhagic diarrhoea and glucose values were 5.9 mmol/L after 4 days in pig D and 5.8 mmol/L after 5 days in pig E. For the two pigs that developed non-haemorrhagic diarrhoea, pig F had a peak glucose value of 7.0 mmol/L after 8 days. Pig G had no alterations in glucose values during the period of diarrhoea and after 4 days of recovery the value was 4.9 mmol/L.

Lactate

In the healthy pigs, the mean plasma lactate concentrations did not differ between the samples taken before inoculation (4.7 ± 2.3 mmol/L) and four weeks later at slaughter (3.0 ± 2.3 mmol/L). In the experimental group, the mean plasma lactate concentration before inoculation was 1.3 ± 0.5 mmol/L. This value was lower than the initial value for the healthy pigs. As seen in Fig. 2, the highest individual lactate value was observed in the beginning of the haemorrhagic diarrhoea period and the mean peak value was 4.5 ± 1.7 mmol/L. Pigs D and E recovered from haemorrhagic diarrhoea and lactate values were 2.3 mmol/L after 4 days in pig D and 2.4 mmol/L after 5 days in pig E. Pig F had a peak lactate value of 5.2 mmol/L after 8 days of diarrhoea. Pig G had low lactate values during the period of diarrhoea and after 4 days of recovery the value was 1.7 mmol/L.

Cortisol

In the healthy pigs, the mean plasma cortisol concentrations did not differ between the samples taken before inoculation (127 ± 63 nmol/L) and four weeks later at slaughter (93 ± 34 nmol/L). In the experimental group, the mean cortisol concentration before inoculation was 24 ± 11 nmol/L. This value was lower than the initial value for the healthy pigs. As seen in Fig. 2, the highest individual cortisol value was observed during the haemorrhagic diarrhoea period and the mean peak value was 278 ± 86 nmol/L. Pigs D and E recovered from haemorrhagic diarrhoea and cortisol values were 119 nmol/L after 4 days in pig D and 138 nmol/L after 5 days in pig E. The peak individual cortisol values for pigs F and G with non-haemorrhagic diarrhoea were 157 and 138 nmol/L, respectively. After 4 days of recovery, pig G had a value of 62 nmol/L.

Tissue analysis

Glycogen

The glycogen concentrations of skeletal muscles, myocardium and liver for the healthy pigs did not differ from those obtained from the experimental group: the highest glycogen concentration was found in the liver and the lowest in the myocardium (Table 1).

Discussion

The results show a transient increase in plasma glucose and lactate concentrations during the initial phase of haemorrhagic diarrhoea induced by *B. hyodysenteriae*. This indicated an alteration in carbohydrate metabolism when the colon was severely affected by the infection.

We conclude that the experimental infectious model was appropriate and swine dysentery was induced after an incubation period of 6-20 days. Endoscopic monitoring of the large intestines of the cannulated

	Healthy pigs (n=4)	Experimental group (n=6)
Biceps femoris	153 ± 27	251 ± 120
Longissimus dorsi	223 ± 51	226 ± 88
Myocardium	9 ± 3	11 ± 13
Liver	1165 ± 496	1013 ± 164

Values are given as means ± SD.

Table 1. Glycogen concentrations (mmol/kg d.w.) in skeletal muscles, myocardium and liver. Samples were taken at slaughter from healthy pigs and from pigs in the experimental group.

pigs revealed mucus, blood and necrotic lesions during the period of haemorrhagic diarrhoea *(Jacobson et al., unpublished observations)*. In some animals, the lesions healed within a few days and the pigs recovered. Alterations of the gut wall with haemorrhages and mucofibrinous pseudomembranes are considered as a stressful state for the pigs, and this was supported by the high cortisol levels measured during the haemorrhagic diarrhoea period.

Stressful situations such as severe injury, sepsis or infection in human patients are associated with a hypermetabolic state, which gives rise to an enhanced peripheral glucose uptake and utilization; hyperlactatemia; increased gluconeogenesis; depressed glycogenesis; and increased insulin resistance *(Mizock, 1995)*. If this hypermetabolic state with hyperglycaemia cannot be controlled it may be detrimental to the patient. The mortality among non-diabetic patients with hyperglycaemia in intensive care decreased significantly after intensive insulin therapy *(Van den Berghe et al., 2001)*. This emphasises the importance of glycaemic control. In the present study, the infected pigs were not treated with antimicrobials but apparently were able to maintain glycaemic control since only a transient increase was observed in plasma glucose levels.

Even though lesions of the above type are generally superficial, the local immune response is activated, which increases the demand for energy *(Fellström, 1996)*. It is possible that the rate of glucose utilization by phagocytic cells increases when the immune response is activated due to infection *(Newsholme & Leech, 1983)*. The reason for the initial glucose increase observed in the present study may thus be related to a greater energy requirement in the body due to the inflammatory response and repairing processes in the gut.

On the eighth day of diarrhoea, a notable increase was observed in glucose and lactate concentrations for pig F (Fig. 2) that might have been due to haemorrhages even if no obvious signs of blood were registered; however, dark faeces were observed in this pig. Pig G displayed no signs of haemorrhagic diarrhoea and blood glucose levels were unchanged over time: this pig was not as affected as the other pigs, as emphasized by the low lactate and cortisol levels.

In human patients, it has been shown that blood lactate increases concurrently with the degree of stress *(Cerra, 1989)*. Similarly, in those pigs that experienced haemorrhagic diarrhoea, increased lactate levels were observed. The lactate produced can be used as a substrate for gluconeogenesis in the liver and thus glucose production. The increased lactate production may reflect tissue hypoxia and/or be due to an increase in glycolysis and glycogen breakdown. The phagocytes involved in the immune response of the gut may also contribute to lactate production *(Mizock, 2001)*.

At slaughter, pig A had elevated lactate and cortisol values (Fig. 2), possibly related to a stressful slaughter situation rather than the metabolic stress induced by the infection as both lactate and cortisol levels in blood increase in connection with physical stress *(Pösö and Jensen-Waern, 1992; Jensen-Waern and Nyberg, 1993)*. The initial resting levels of lactate and cortisol differed between the healthy pigs and pigs in the experimental group. Lactate values in the healthy pigs were in good agreement with previous observations *(Håglin & Essén-Gustavsson, 1992; Jensen-Waern & Nyberg, 1993).* The experimental pigs with cannulas were housed individually and thus experienced more frequent handling, which could explain the lower stress response reflected in lower initial concentrations of lactate and cortisol.

The present results show that liver and skeletal muscles had high glycogen values compared with myocardium, which was in agreement with previous findings *(Håglin et al., 1994).* During clinical signs of disease, the pigs showed a slight reduction in appetite and their body weight was either unchanged or decreased slightly. During this catabolic stage, glucose may be supplied by the breakdown of glycogen in muscle and liver *(Kaneko, 1997)*. At slaughter, glycogen concentrations in these tissues did not differ between the healthy and experimental groups; thus, energy supply was not limited by the glycogen stores.

In conclusion, this study demonstrates that alterations in the carbohydrate metabolism occur during an experimental infection with *B. hyodysenteriae* in pigs. These alterations are characterised by a transient increase in blood glucose and lactate concentrations during the initial phase of the disease.

The results contribute to the understanding of the pathogenesis of swine dysentery and the metabolic response to infectious diseases in general.

Acknowledgements

This study was financially supported by The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning. Somchit, A. received support from The Swedish Foundation for International Co-operation in Research and Higher Education (STINT).

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