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The Metabolism of an Exogenous Lipid Source during Septic Shock in the Puppy

ARNOLD G. CORAN, MD.,* ROBERT A. DRONGOWSKI, M.S.,† GRACE S. LEE, B.S.,‡
MICHAEL D. KLEIN, M.D.,§ AND JOHN R. WESLEY, M.D. ||

From the Section of Pediatric Surgery, Mott Children's Hospital and University of Michigan Medical School, Ann Arbor, Michigan

ABSTRACT. Septic shock induces physiologic and hemodynamic responses that may alter the host's ability to metabolize an exogenous source of lipids. The present study examined the metabolic changes occuring during septic shock in the young animal receiving an intravenous fat emulsion. Five 8-wk-old male Beagle puppies were studied. Each animal served as his own control twice, during which time ^{14}C-palmitic acid alone or with a 10% fat emulsion (Liposyn) was administered. Septic shock (cardiac output less than 50% of control) was induced with an intravenous bolus of live *Escherichia coli*. During shock the puppies received intravenous ^{14}C-palmitic acid and Liposyn. The mean respiratory quotient for the shock dogs (0.96 ± 0.01) was significantly ($p < 0.05$) higher than that of the controls (0.83 ± 0.06). The amount of expired $^{14}CO_2$ was 25.0 ± 15.9% for the shock animals, 53.9 ± 28.1% for the Liposyn controls, and 75.7 ± 30.5% for the controls receiving only ^{14}C-palmitic acid (these differences are all significant, $p < 0.05$). After the onset of shock, serum triglyceride levels peaked within 2 min at 851 ± 540 mg/100 ml and remained elevated at 333 ± 213 mg/100 ml. Triglyceride levels in the Liposyn control animals returned to baseline values (54 ± 13 mg/100 ml) at the end of the 4-hr experimental period. Free fatty acids in the shock dogs reached a maximal level of 1.44 ± 0.09 mEq/liter at 1 hr and remained at this elevated value for a significantly longer period of time than in the Liposyn control puppies. Glycerol value followed a similar pattern and cholesterol remain unchanged. Plasma insulin in the shock animals steadily rose to a peak of 826 ± 358 μU/ml at the end of the experimental period; control animals showed no plasma insulin changes. The results of this study suggest that exogenous lipids administered during septic shock may not be metabolized as well as during the nonshock state. (*Journal of Parenteral and Enteral Nutrition* **8**:652–656, 1984)

The effect of sepsis and septic shock on lipid metabolism has not been fully delineated. Many authors have documented hypertriglyceridemia, elevated levels of free fatty acids, and other abnormalities of lipid metabolism in a variety of animal species exposed to gram-negative sepsis, endotoxin, or infection with various other bacteria.[1-9] There are conflicting reports concerning the ability of animals and humans to utilize intravenous fats as an energy substrate during sepsis. Wannemacher et al[10] studied patients and monkeys with pneumoccocal sepsis and reported a protein-sparing effect associated with the infusion of a lipid emulsion. Similarly, Nordenstrom et al[11] have shown that intravenously administered fat emulsions are cleared from plasma and oxidized at accelerated rates in patients with trauma or infection compared with normal subjects. In contrast, Long et al[12] reported minimal protein-sparing from fat emulsions in patients with severe burns.

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Reprint requests to: Arnold G. Coran, M.D., Head, Pediatric Surgery, Mott Children's Hospital F-7516, Box 66, Ann Arbor, MI 48109.
* Professor of Surgery and Head of the Section of Pediatric Surgery, University of Michigan Medical School, Surgeon-in-Chief, Mott Children's Hospital.
† Research Associate, Pediatric Surgery Research Laboratories, Mott Children's Hospital.
‡ Medical Student, University of Michigan Medical School.
§ Assistant Professor of Surgery, Section of Pediatric Surgery, University of Michigan Medical School.
|| Associate Professor of Surgery, Section of Pediatric Surgery, University of Michigan Medical School.

This study was undertaken to determine the effects of severe *Escherichia coli* bacteremic shock on lipid metabolism in puppies administered an intravenous fat emulsion.

MATERIALS AND METHODS

Five 8-wk-old, colony-bred puppies were used in this study. Two control studies and one experimental study were established for each puppy, with a 1-wk recovery period between experiments. All dogs were anesthetized with rompum (Bayvet, Shawnee, KS), 2.5 mg/kg sc, and pentobarbitol, 25 mg/kg iv. Food was withheld for 8 hr prior to each experiment but water was allowed *ad libitum*.

One femoral vein was cannulated for lipid administration and blood sampling. The other femoral vein was catheterized for measuring cardiac output with a pediatric, balloon-tipped thermodilution catheter passed into the pulmonary artery. The femoral artery was catheterized for monitoring systemic blood pressure.

A 1-hr equilibration period was established for all three animal groups. After this period, each of the five puppies received 50 μCi of ^{14}C-palmitic acid iv (group A). Two days later, the same animals received the same dose of ^{14}C-palmitic acid plus a fat emulsion (Liposyn 10%, Abbott Laboratories, North Chicago, IL) at 0.5 g of fat/kg iv (group B). After a 2-day rest, the five puppies were given an iv bolus of liver *E. coli* (10 ml/kg of 109 colonies *E. coli*/ml, an LD_{100} dose) to induce septic shock (cardiac output 50% or less than baseline values). Ten minutes

after septic shock was established, the animals were given the same doses of both ^{14}C-palmitic acid and the lipid emulsion used in the first two experiments (group C).

Blood samples were taken at intervals during the 4-hr experimental period for measurement of glycerol, triglyceride, cholesterol, glucose, free fatty acids (FFA), and insulin.

All animals were intubated with a cuffed endotracheal tube fitted with a Wright respirometer (British Oxygen Company, Harlow, England) for tidal volume determinations and were allowed to respire room air spontaneously. The expired air was collected in intermittent mandatory ventilation bags (OEM Medical, Inc., Richmond, VA) and was bubbled through 50 ml of ethanolamine/ethylene glycol monomethyl ether/toluene (1:8:10) solution for absorption of all carbon dioxide.[13] The solution was replaced hourly. A 1-ml aliquot of this solution was then added to 10 ml of aqueous counting scintillant (Amersham, Arlington Heights, IL) and was counted for 10 min in a Beckman LS-230 liquid scintillation counter for determination of radioactive $^{14}CO_2$ in the expired air. In addition, one intermittent mandatory ventilation bag was analyzed every 15 min for expired carbon dioxide with a Beckman LB-2 Medical Gas Analyzer. Expired oxygen was measured with a S-3A Oxygen Analyzer (Applied Electrochemistry, Inc., Sunnyvale, CA). The respiratory quotient was then calculated from the CO_2 produced and the O_2 consumed.[14, 15] The rate of oxidation of ^{14}C-palmitic acid was calculated from the cumulative $^{14}CO_2$ production, which was obtained by integrating the area under the curve of expired radioactive CO_2. This rate was then expressed as a percentage of the initially administered dose of ^{14}C-palmitic acid oxidized within 4 hr.[11]

Triglycerides, glycerol, cholesterol, glucose, and FFA were analyzed using standard laboratory methods. Insulin was measured with a standard radioimmunoassay technique.

Statistical significance was determined using Student's *t*-test and analysis of variance with *p* values of less than 0.05 were considered significant.

RESULTS

The mean respiratory quotient for the shock animals (group C), 0.96 ± 0.01, was significantly higher than that of the controls (groups A and B), 0.83 ± 0.06 (Fig. 1). The mean values for the actual O_2 consumption and CO_2 production over a 3-hr period were 19.7 ± 7.8 and 16.8 ± 6.3 liters for group A, 27.7 ± 9.0 and 22.4 ± 8.5 for group B, and 46.6 ± 19.2 and 44.7 ± 11.2 for group C. These data indicate that the shock puppies were clearly not hypometabolic in comparison with the other two groups. The amount of expired $^{14}CO_2$, expressed as percentage of the initially administered ^{14}C-palmitic acid, was 75.7 ± 30.5% in the puppies receiving only ^{14}C-palmitic acid, 53.9 ± 28.1% in the Liposyn controls, and 25.0 ± 15.9% in the shock animals. The differences between all three groups were statistically significant (Fig. 2).

The triglyceride levels in the control dogs receiving only radioactive palmitic acid remained unchanged during the experiment. In the puppies receiving the fat

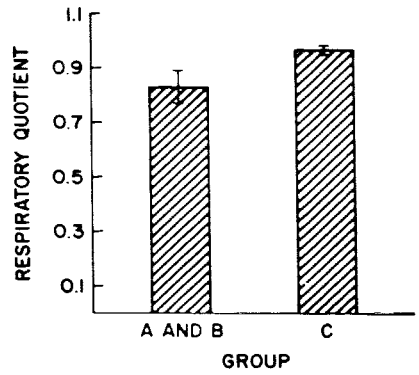

FIG. 1. Respiratory quotient.

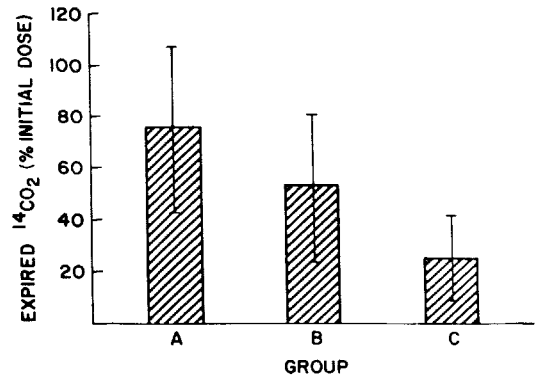

FIG. 2. Expired $^{14}CO_2$.

emulsion, the triglycerides rose to 1040 ± 162 mg/100 ml 2 min after the fat infusion and returned to baseline levels 2.5 hr later (83 ± 30 mg/100 ml). In contrast, in the shock animals, triglyceride levels 2 min after the lipid infusion were 851 ± 539 mg/100 ml and remained at or above 333 ± 213 mg/100 ml throughout the study period. When compared with both control groups, the triglyceride levels in the shock animals at the conclusion of the experiment were significantly higher (Fig. 3).

FFA values in group A remained unchanged during the first 3 hr of the experiment. A statistically insignificant rise occurred during the last hour of the experiment. FFA levels in the Liposyn control animals rose to peak values of 1.49 ± 1.18 mEq/liter 30 min after the lipid infusion and returned to baseline 2 hr later. The FFA levels in the shock animals remained elevated for 2.5 hr after the lipid infusion and returned toward baseline levels only at the end of the experiment. There were no significant differences in the FFA levels in any of the three experimental groups at the conclusion of the study (Fig. 4).

Glycerol concentrations in the nonliposyn controls showed an insignificant increase to 13 ± 20 mg/100 ml after ^{14}C-palmitic acid was administered. The remaining glycerol levels in this control group were not elevated above baseline. Glycerol levels rose to a peak value of 35 ± 5 mg/100 ml at the 2-min sampling period and remained significantly elevated for 1.5 hr after the fat infusion in the Liposyn control animals. Glycerol values in the shock puppies increased to a peak value of 37 ±

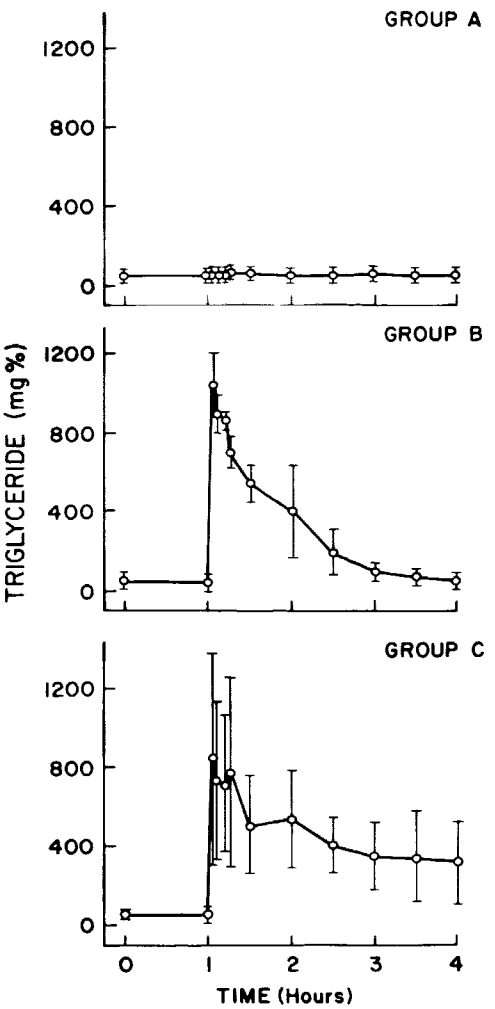

FIG. 3. Serum triglycerides.

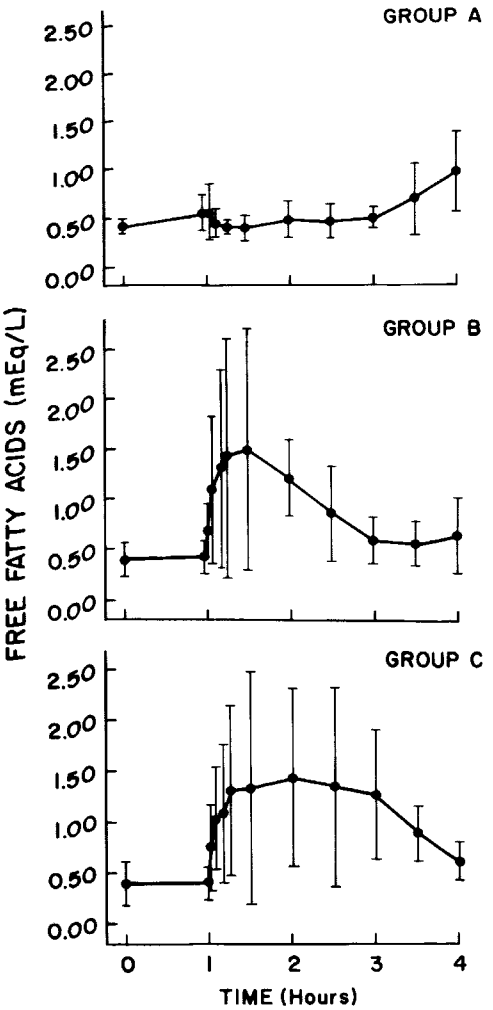

FIG. 4. Serum FFA.

18 mg/100 ml at 2 min and remained significantly elevated throughout the experiment. In the shock animals, glycerol levels remained significantly higher than in the Liposyn control puppies at 1.5 hr after the lipid infusion and for the remainder of the experiment. All the glycerol levels in the shock animals were significantly higher than those seen in the non-Liposyn control group following the equilibration period (Fig. 5).

Although the shock puppies tended to have higher levels of glucose after the lipid infusion than the two control groups, these differences were not statistically significant (Table I). There were no significant changes in cholesterol (Table II).

Although there were no significant changes in plasma insulin noted in the two control groups, the value increased steadily in the shock animals after the lipid infusion to a maximum of 826 ± 358 μU/ml at the end of the experimental period, which was significantly higher than the baseline value and the levels seen in the two control groups (Fig. 6).

DISCUSSION

Optimal treatment of septic shock remains controversial, but usually includes steroid and fluid administration to combat the severe metabolic and hemodynamic disturbances.[16-19] In addition, complete nutritional support is usually required in the clinical situation. Intravenous fats are usually part of the nutritional program. The conflicting data regarding the ability of animals and humans to utilize lipid in various septic states prompted this investigation.[1-12]

The mean respiratory quotient of 0.96 in the shock animals compared with 0.83 for the control group indicates that glucose was the principal energy substrate being utilized by the shock puppies. The low value of $^{14}CO_2$ (25%) expired (*vs* 53.9% in the control groups) by the shock animals is further evidence that less lipid is being oxidized in the septic animals. The higher levels of triglyceride, glycerol, and FFA in the shock group indicate a reduced clearance and/or utilization of administered fat in these animals. This is in sharp contrast to the studies of Wannemacher et al[10] and Nordenstrom et al[11] on septic subjects not in shock and is consistent with the experience of Long et al[12] in burned patients. One must use extreme caution, however, in extrapolating these data to the clinical situation because of the extreme severity of our shock model.

Both glucose and insulin levels were increased in the

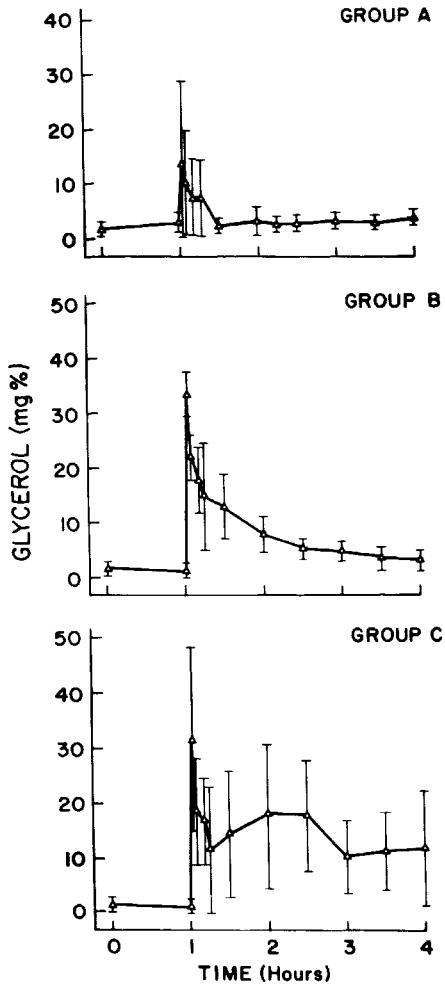

FIG. 5. Serum glycerol.

TABLE I
Blood glucose (mg/100 ml)

	Time (min)											
Group	0	60	62	65	70	75	90	120	150	180	210	240
A	135	172	198	223	217	227	211	220	204	195	189	170
B	116	167	197	168	192	212	229	206	183	177	159	145
C	186	240	277	297	303	268	241	282	289	384	449	337

TABLE II
Serum cholesterol (mg/100 ml)

	Time (min)											
Group	0	60	62	65	70	75	90	120	150	180	210	240
A	186	227	194	218	199	231	268	242	235	253	240	232
B	282	201	228	248	234	218	226	281	281	266	241	255
C	232	202	283	267	262	260	263	269	270	234	209	220

shock puppies; however, the increase in insulin to 826 μU/ml at the conclusion of the experiment was dramatic. Munro[20] and Beisel[21] have observed similar increases in both glucose and insulin in patients with sepsis and fever and have suggested that the high insulin levels inhibit flow of FFA from adipose tissue, and thereby reverse the ketosis.

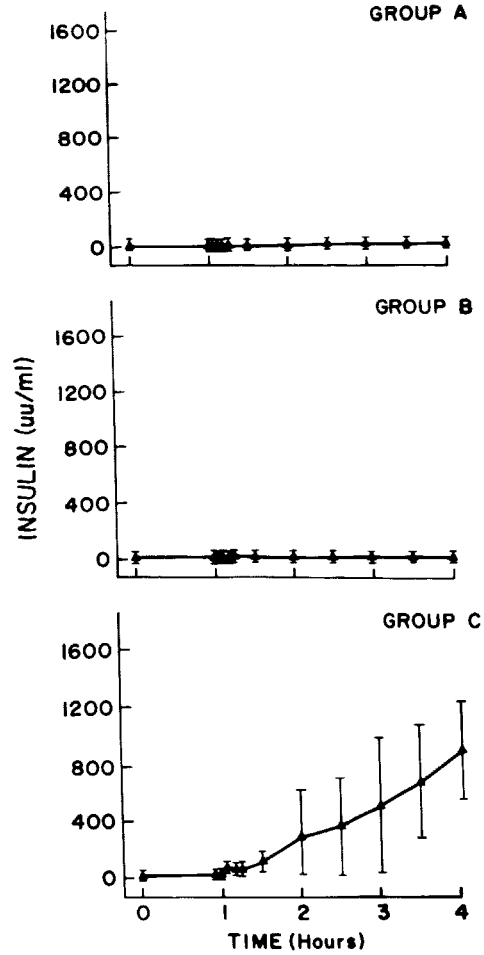

FIG. 6. Serum insulin.

It is possible that the *E. coli* bacteria themselves inhibited the activity of lipoprotein lipase, the principal enzyme involved in the hydrolysis of triglycerides, as reported by Lanza-Jacoby et al[2] in rats. This would obviously result in decreased lipid clearance and elevated levels of serum triglycerides.

The high levels of triglyceride, FFA, and glycerol could also be attributed to the decreased peripheral perfusion seen in this severe septic shock model. The decreased liver blood flow could account for a lowered clearance of lipids with the resultant elevated levels of triglyceride, FFA, and glycerol. In addition, the increased catecholamine response to the *E. coli* shock probably contributed to the elevated lipid levels.[6, 22] However, these two factors would not account for the lowered rate of $^{14}CO_2$ production or the high respiratory quotients observed in the septic puppies. Therefore, our data strongly suggest that the septic animals in this study are unable to metabolize and oxidize an exogenous source of neutral fats as well as the control puppies and that they preferentially burn carbohydrate during septic shock.

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