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Effect of Palmitate or Lipid Emulsion on Nitrogen Metabolism during Hyperthermic Perfusion of Rat Liver[1] (41526)

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Abstract. Livers of fasted rats were perfused for one hour at 37° or 42°. Endogenous hepatic ureogenesis was increased twofold at 42° and the release of endogenous ninhydrin-reactive amino nitrogen was not changed by hyperthermia. Exogenous amino acids at 10 times the normal plasma concentration stimulated hepatic ureogenesis sevenfold at 37° and over twofold at 42°. Exogenous amino acids stimulated hepatic amino acid uptake at 37°. Uptake of the exogenous amino acids at 42° was about 40% of that observed at 37°. However, exogenous amino acid uptake in the presence of 1 m*M* palmitate was not altered at 42°. Ureogenesis in the presence of supplemental amino acids and palmitate was significantly reduced at 37° and moderately reduced at 42°, suggesting decreased amino acid catabolism. Ten percent Intralipid, a clinically available lipid emulsion primarily consisting of triglycerides in a physical form similar to chylomicrons at a perfusate concentration of 1 ml/100 ml, did not markedly alter hepatic ureogenesis or amino acid dynamics at 37° or 42° in the presence or absence of amino acids. It is concluded that palmitate blocks the adverse effect of hyperthermia on hepatic amino acid uptake.

Hyperthermia is presently undergoing clinical evaluation as a treatment modality in the management of cancer (1, 2). Whole-body hyperthermia may be complicated by circulatory (3, 4) and hepatic (4, 5) dysfunction. However, research directed toward attenuating the effects of hyperthermia on hepatic function is lacking. Previous work from this laboratory (6, 15) showed that palmitate decreased excessive lactate utilization and helped maintain higher maximal rates of gluconeogenesis from lactate, ureogenesis from NH_4Cl, and O_2 consumption under the increased metabolic demands of hyperthermia. Additional experiments demonstrated a temperature-dependent decrease in the incorporation of radioactively labeled leucine into hepatic proteins of normal and regenerating liver (7). Therefore, it was of interest to determine if palmitate provided any protection from thermally induced alterations in hepatic amino acid metabolism. The present study examines the effect of palmitate on hepatic nitrogen metabolism in the presence or absence of supplemental amino acids at 37° and 42°. In addition, Intralipid, a clinically employed lipid emulsion, was also evaluated.

Materials and Methods. *Animals.* Male Sprague–Dawley rats (King Animal Laboratories, Inc., Oregon, Wisc.) weighing 150–200 g were fed laboratory chow *ad libitum* and were fasted 24–30 hr prior to perfusion.

Perfusion methods. The technique for *in situ* perfusion of rat liver with accurate temperature control was previously described by Collins and Skibba (8). In summary, the 150 ml recirculating perfusate contained 2.5% (w/v) bovine plasma albumin, fraction V (Reheis Chemical Co., Phoenix, Ariz.) and 20% (v/v) washed aged human erythrocytes in Krebs–Ringer bicarbonate solution at pH 7.4. The bicarbonate level was adjusted to 25 m*M* with 1 *M* $NaHCO_3$. A membrane oxygenator (Sci-Med Life Systems, Inc., Minneapolis, Minn.) was employed to gas the perfusate with humidified O_2 + CO_2 (95:5). The pH was monitored and maintained at 7.4 ± 0.05 by 1 *M* NaOH. When amino acids were included in the perfusate, 2 ml of a concentrated mixture of 11 amino acids was added to the control perfusate before initiation of the perfu-

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TABLE I. EFFECT OF PALMITATE OR LIPID EMULSION ON PERFUSATE UREA NITROGEN IN THE PRESENCE OR ABSENCE OF SUPPLEMENTAL AMINO ACIDS AT 37° AND 42° [a]

Condition [b]	Urea nitrogen (μmol/g liver/min) 37°	42°
Control	0.10 ± 0.02	0.21 ± 0.04[c]
Palmitate	0.09 ± 0.01	0.17 ± 0.02[c]
Lipid emulsion	0.13 ± 0.03	0.13 ± 0.02[d]
Amino acids	0.71 ± 0.05	0.54 ± 0.04[c]
Amino acids + palmitate	0.50 ± 0.02[d]	0.41 ± 0.04
Amino acids + lipid emulsion	0.56 ± 0.05	0.68 ± 0.07

[a] Mean maximal values ± SEM obtained from four separate liver perfusions. Maximal rates of metabolic change were determined from perfusate samples removed every 30 min during the 90-min experimental period.

[b] Livers of fasted rats were perfused in the presence of 1 m*M* palmitate or 1 ml lipid emulsion/100 ml perfusate (approximately 1 m*M* triglyceride, 3 m*M* glycerol, and 0.2 m*M* phospholipid) in the presence or absence of 10 times the normal rat plasma concentration of 11 amino acids.

[c] Significantly different from ($P < 0.05$) the corresponding 37° value by Student's *t* test.

[d] Significantly different from ($P < 0.05$) the appropriate control value at the same temperature by Student's *t* test.

sion. The final perfusate concentration (m*M*) of the amino acids was 10 times the normal rat plasma concentration (9): arginine, 2.2; asparagine, 0.4; isoleucine, 0.9; leucine, 1.7; lysine, 4.8; methionine, 0.7; phenylalanine, 0.8; proline, 2.4; threonine, 2.9; tryptophan, 0.7; valine, 2.0. Each rat was heparinized (200 U, iv) prior to placement of the portal vein and inferior vena cava cannulas. After placement of the cannulas, the initial 5 ml of perfusate collected from the outflow cannula was discarded to ensure removal of all rat blood. Thermocouple microprobes (Bailey Instrument Co., Saddle Brook, N.J.) were employed to monitor and control the liver, perfusate, and cabinet temperatures. Rat livers were perfused for 120 min. A 30-min equilibration period was followed by a 90-min experimental period. Sodium palmitate or lipid emulsion was added to the perfusate 2 min before the beginning of the experimental period, allowing time for adequate mixing of the substances. Sodium palmitate was heated in an aqueous solution at 65° until the solution was clear. An aqueous solution of bovine plasma albumin (55°) was vigorously mixed with the palmitate solution to a final albumin concentration of 12% (w/v) and the solution, a total of 2 ml, was added immediately to the 150 ml recirculating perfusate. The perfusate concentration of palmitate was 1 m*M* (1 μmole/ml perfusate). The molar ratio of palmitate to albumin in the perfusate was 3 to 1. The albumin vehicle was added to the control experiments since it contains a small amount of free fatty acid. The lipid emulsion contained 10% soybean oil, 2.25% glycerol, and 1.2% phospholipid. The commercial name is Intralipid (Cutter Laboratories, Inc., Berkeley, Calif.). Approximate perfusate concentrations of triglyceride, glycerol, and phospholipid were calculated to be 1, 3, and 0.2 m*M*, respectively, after the addition of 1.5 ml of the lipid emulsion to 150 ml of the perfusate. Amino acids, sodium palmitate, and other reagents were obtained from Sigma Chemical Company, St. Louis, Missouri.

Analytical and statistical methods. Perfusate samples were obtained at 30-min intervals from 0 to 90 min of the experimental period. Protein-free filtrates were prepared from the perfusate samples with perchloric acid. Aliquots of these protein-free filtrates were analyzed for urea by the diacetyl monoxime assay (10), α-amino nitrogen by a modification (11) of the ninhydrin reaction (12) and glucose by the glucose oxidase method (13). Estimates of the maximal rates of metabolite change were determined by regression analysis as described by Hems *et al.* (14). Maximal rates are expressed in micromoles per gram wet liver weight per minute. Leucine was employed as the standard for the ninhydrin reaction. Each value represents the mean ± standard error of four separate experiments. The significance of differences between the means was determined by unpaired Student's *t* test. A *P* value of <0.05 was considered statistically significant.

Results. *Ureogenesis.* Endogenous ureogenesis was not affected by palmitate or the lipid emulsion at 37° (Table I). Hyperthermia significantly increased the rate of endogenous ureogenesis in control and palmitate-supplemented liver perfusions, but no

change in the rate of ureogenesis was found in the presence of lipid emulsion at 42° (Table I). Even though endogenous ureogenesis was stimulated at 42°, these rates are relatively low, i.e., about 10% of the rates of ureogenesis when an exogenous source of ammonia is added to the perfusate (6, 8, 15). Hepatic amino acid metabolism is a primary source of ammonia for ureogenesis (16, 17). Addition of 11 amino acids at 10 times the normal plasma concentration resulted in a sevenfold stimulation of urea formation at 37°. Hyperthermia significantly attenuated the increase in ureogenesis in the presence of amino acids; however, the rate of ureogenesis at 42° remained over twofold higher than the rate obtained without supplemental amino acids (Table I). Although urea formation was stimulated in the presence of amino acids and palmitate at 37°, it was significantly less than the level of urea formation observed in the presence of amino acids alone (Table I). At 42°, urea formation from amino acids was not affected by palmitate. Ureogenesis from supplemental amino acids was not altered by the lipid emulsion at 37° or 42°.

The lipid emulsion contains glycerol and the approximate perfusate concentration was 3 m*M*. Since glycerol is a gluconeogenic substrate in the fasted rat, it was of interest to examine the level of gluconeogenesis in the presence of the lipid emulsion at 37° and 42°. The maximal rate of gluconeogensis in the presence of lipid emulsion was 1.37 ± 0.08 μmole/g/min at 37°, and 1.06 ± 0.15 μmole/g/min at 42°. In the control experiments, endogenous gluconeogenesis was 0.24 ± 0.02 and 0.29 ± 0.03 μmol/g/min at 37° and 42°, respectively. The rates of gluconeogenesis obtained in the presence of the lipid emulsion at 37° and 42° were not significantly different and are comparable to the rates of gluconeogenesis from lactate reported in our earlier studies (6, 15).

Amino acids. Hepatic amino acid release and uptake data are summarized in Table II. Endogenous release of amino acids was not affected by supplemental palmitate or lipid emulsion at 37° or 42°. In experiments in which the perfusate contained 10 times the normal plasma concentration of amino acids, hepatic amino acid uptake was stimulated. However, amino acid uptake at 42° was significantly attenuated. The reduced rate of amino acid uptake at 42° was about 40% of the rate determined at 37°. Supplemental palmitate or lipid emulsion had no effect on amino acid uptake at 37°. However, at 42°, no significant reduction in hepatic amino acid uptake occurred in the presence of amino acids and palmitate. By contrast, amino acid uptake was significantly depressed at 42° in the presence of the lipid emulsion and amino acids, and the rate of amino acid uptake was 36% of the rate obtained at 37° under similar conditions. The level of reduction in the presence of lipid emulsion at 42° was similar to the data obtained from the control perfusions with amino acids at 42°.

TABLE II. EFFECT OF PALMITATE OR LIPID EMULSION ON PERFUSATE AMINO NITROGEN IN THE PRESENCE OR ABSENCE OF SUPPLEMENTAL AMINO ACIDS AT 37° AND 42° [a]

Condition [b]	Amino nitrogen (μmol/g liver/min) 37°	42°
Control	0.38 ± 0.06	0.35 ± 0.04
Palmitate	0.31 ± 0.01	0.32 ± 0.03
Lipid emulsion	0.46 ± 0.09	0.41 ± 0.07
Amino acids	−1.76 ± 0.38	−0.71 ± 0.15 [c]
Amino acids + palmitate	−1.58 ± 0.18	−1.43 ± 0.27
Amino acids + lipid emulsion	−1.48 ± 0.24	−0.53 ± 0.16 [c]

[a] Mean maximal values ± SEM obtained from four separate liver perfusions. Maximal rates of metabolic change were determined from perfusate samples removed every 30 min during the 90-min experimental period.

[b] Livers of fasted rats were perfused in the presence of 1 m*M* palmitate or 1 ml lipid emulsion/100 ml perfusate (approximately 1 m*M* triglyceride, 3 m*M* glycerol, and 0.2 m*M* phospholipid) in the presence or absence of 10 times the normal rat plasma concentration of 11 amino acids.

[c] Significantly different from ($P < 0.05$) the corresponding 37° value by Student's *t* test.

Discussion. A primary aim of this study was to examine the effect of hyperthermia on hepatic amino acid dynamics and urea formation. A previous study by Collins and Skibba (7) showed a temperature-related decrease in the incorporation of radioactively

labeled amino acid into hepatic proteins suggesting an alteration in hepatic nitrogen metabolism by hyperthermia. In the present study, endogenous ureogenesis was stimulated at 42° which suggests an increase in endogenous proteolysis and amino acid catabolism. Amino acid supplementation at 10 times the normal rat plasma level increased ureogenesis sevenfold at 37°. Presumably the amino acids increased the supply of donor nitrogen for ureogenesis. At 42°, ureogenesis increased less than threefold in the presence of exogenous amino acids. These data suggest a decrease in the availability of exogenous nitrogen to support ureogenesis at 42°. Decreased amino acid uptake would be one explanation for a lower level of donor nitrogen for ureogenesis at 42°. Indeed, the data show that amino acid uptake was significantly reduced at 42°. However, ureogenesis in the presence of palmitate and amino acid supplements was reduced at 37° and a similar trend was noted at 42°. The decrease in ureogenesis in the presence of palmitate implies a decrease in the catabolism of the amino acids. Perhaps the beneficial effect of palmitate on the ureogenic pathway is related to an intracellular process such as lysosomal membrane stabilization or provision of energy as was suggested in our previous studies (6, 15) on the relationship between palmitate and gluconeogenesis from lactate. At 37°, other workers reported that exogenous oleate had no effect on the rate of ureogenesis in isolated hepatocytes of fasted rats (18), or that oleate decreased the rate of ureogenesis in perfused rat livers of fasted or diabetic rats (19). Apparently oleate decreased the rate of hepatic protein catabolism as the rate of ureogenesis was reduced (19). The present findings agree with those of Woodside and Heimberg (19). Moreover, amino acid uptake at 42° in the presence of palmitate was the same as that measured at 37°. This finding suggests that palmitate blocks the adverse effect of heat on hepatic amino acid transport. The mechanism by which palmitate alters amino acid transport under hyperthermic conditions is presently not known. It may be due to the intracellular mechanisms previously discussed or to an effect on cellular membrane integrity or plasma membrane transport. Further experiments are planned to examine hepatic membrane and intracellular processes in order to determine the point(s) of biochemical interaction between palmitate and nitrogen metabolism under hyperthermic stress.

A lipid emulsion was evaluated in this study because it is a clinically available lipid supplement. Since the lipid emulsion physically resembles chylomicrons, the expected hepatic uptake by the liver would be minimal (20, 21). Indeed, hepatic ureogenic, and amino acid dynamics were not markedly altered by the presence of the lipid emulsion at 37° or 42°. However, in man, lipid emulsions can substitute for carbohydrate in supplying energy in order to maintain a positive nitrogen balance (22, 23). Presumably, chylomicrons are metabolized to free fatty acids by lipoprotein lipase in the whole animal (24). In addition, isolated perfusion of the liver in the presence of heparin should increase chylomicron metabolism to free fatty acids and chylomicron remnants by releasing hepatic triglyceride lipase (25). Since free fatty acid appears to attenuate the adverse effects of hyperthermia on hepatic functions, it is suggested that exogenous free fatty acids or a lipid emulsion might be beneficial to the liver in cancer patients treated with systemic hyperthermia or regional heparinized thermotherapeutic perfusion of the liver.

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