The Effect of Pyruvate or Dihydroxyacetone on Parenterally Induced Liver Lipid Accumulation in the Rat (43169)

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Abstract. **Orally fed pyruvate (pyr) and dihydroxyacetone (DHA) have been shown to decrease liver lipid accumulation in animal models. These compounds lessen the degree of fatty liver in ethanol-fed rats and in a genetic strain of hens predisposed to fatty liver. Total parenteral nutrition can result in liver dysfunction, including fatty infiltration of the liver. In this study, rats were assigned to either control, pyr, or DHA groups. All rats were fitted with jugular vein catheters, and following a 3-day recovery, were infused continuously for 7 days. The infusate provided adequate nutrition (including 7% kcal as fat) with 5% pyr or 5% DHA (g/liter) substituted for dextrose in the experimental groups. Plasma triglycerides were lower in the pyr groups relative to controls: 62.2 ± 34.7 (SE) vs 96.8 ± 44.3 mg/dl, though this was significant only at $P < 0.10$. Neither pyr nor DHA decreased liver lipids. Pyr and DHA were administered intravenously in this study, and therefore passed through the heart and to peripheral tissues first. These compounds may need to be fed orally, passing via the portal system, to produce the liver lipid-lowering effects seen in other studies.** [P.S.E.B.M. 1991, Vol 196]

The consumption of alcohol is associated with the accumulation of lipid in the livers of both humans and animals (1, 2). The metabolism of alcohol occurs primarily in the liver and results in the production of reducing equivalents. Excessive alcohol consumption may therefore lead to an overall reduced state of the liver. In the late 1970s, Stanko *et al.* (3) undertook studies to determine if naturally occurring oxidants could prevent ethanol-induced liver lipid accumulation in rats. Favorable results were obtained by feeding pyruvate (pyr), dihydroxyacetone (DHA), and riboflavin. These results were confirmed by Goheen *et al.* (4) and Rao *et al.* (5) in the early 1980s.

More recent studies suggest that this prevention of lipid accumulation may be a more generalized phenomenon. Stanko and Adibi (6) fed pyr and DHA to non-alcoholic rats. Compared with control animals, experimental animals had the same energy intake but gained less fat, resulting in a lower body weight (6). Johnstone *et al.* (7) fed pyr and DHA to hens gavaged with ethanol. Ethanol feeding resulted in increased liver size and total liver lipids. Total serum and high-density lipoprotein cholesterol, and serum triglycerides were also increased by ethanol feeding. These increases were ameliorated by pyr and DHA (7).

Humans and animals receiving total parenteral nutrition (TPN) may also suffer hepatic dysfunction, including fatty infiltration of the liver, which may be accompanied by elevated plasma liver enzyme levels (8, 9). Using an intravenously fed rat as a model for TPN, we undertook a study to determine whether pyr or DHA given intravenously could prevent TPN-induced liver lipid accumulation. The results, taken together with those in which these compounds are fed orally, might suggest whether route of administration affects the lipid lowering effects of pyr and DHA.

Materials and Methods

Male Wistar rats (Hilltop Laboratories, Scottsdale, PA) were housed in individual stainless steel cages with a constant temperature (24°C) and 12-hr light/dark cycle. Animals were adapted to a specially designed jacket for 1 week, during which time they had free access to stock laboratory diet and water. Following adaptation, rats were fasted overnight and anesthetized

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by intramuscular injection of ketamine (50 mg/kg), xylazine (5 mg/kg), and acepromazine (0.75 mg/kg). The process of catheter insertion has been described previously (10). A Silastic catheter (i.d. 0.20 in) with PE 50 tip was inserted into the jugular, secured with sutures, and tunneled to exit subscapularly. During surgery, the catheter was irrigated intermittently with sterile saline to prevent occlusion, and aseptic techniques were used as described by Popp and Brennan (11).

Catheters were protected by a spring, which was attached to the jacket via a metal button. The spring and catheter were connected to a freely rotating infusion swivel, allowing relatively unrestricted movement.

Recovery was 2–3 days, during which time an increasing volume of 5% dextrose was infused and free access to stock diet and water allowed. Diet was subsequently removed and animals were then continuously infused at 3 ml/hr for 7 days. The infusate was nutritionally adequate (12, 13); the composition is listed in Table I. The caloric level provided 74.3 kcal/day and has been shown to support a growth of 3 g/day (13); pyr or DHA was substituted for dextrose in the experimental groups to provide 5% of energy.

Following the 7-day infusion period, animals were again anesthetized (pentobarbital, 30 mg/kg). Blood was collected via the inferior vena cava into syringes containing EDTA and plasma obtained by centrifugation. Livers were removed and freeze-clamped.

Plasma Assays. Plasma triglyceride levels were determined by glycerol release (Sigma kit no. 336-10) and cholesterol by the cholesterol oxidase method (14). Nonesterified fatty acids were measured by an enzymatic, colorimetric method (Wako kit no. 990-75401) as was glucose (Gilford Clinical Analyzer 400E). Liver function was assessed with the measurement of plasma alanine aminotransferase, aspartate aminotransferase, protein, and albumin (Gilford Analyzer).

Liver Assays. Liver glycogen was measured on fresh tissue by a modification of the method of Hansen *et al.* (15). Liver lipids were extracted with chloroform:methanol. Phospholipid was calculated from measurements of phosphorus (16), triglyceride by glycerol release (17), and cholesterol according to the method of Carlson and Golfarb (18). Lactate was quantitated by conversion to pyr (19).

Statistical Analysis. The data presented are expressed as mean ± SE. Comparisons were made using analysis of variance and Fisher's least significant differences. Results were considered significant at $P < 0.05$.

Table I. Composition of Control Infusion

Ingredient	Amount/liter
Calories	1032
Dextrose[a]	240 g
Amino acids[b]	38 g
Lipid	7 g
Multivitamins[d]	10 ml
Choline (choline chloride)	350 mg
Trace elements[e]	5 ml
Iodine	40 μg
Calcium[f]	9 mEq
Potassium[g]	69.7 mEq
Phosphorus	13.5 m*M*

[a] Four-hundred milliliters of 60% dextrose (Abbott Laboratories, North Chicago, IL).
[b] Four-hundred fifty milliliters of 8.5% Aminosyn + Electrolytes (Abbott).
[c] Thirty-five milliliters of 20% Liposyn (Abbott).
[d] M.V.C. 9 + 3 (½ vial 1 + vial 2; Lyphomed, Inc., Melrose Park, IL). Additional vitamin B_{12} (10 μg) as cyanocobalamin (Ivenex, Chagrin Falls, OH). Additional vitamin E (2.5 IU) as DL-α-tocopherol acetate (Sigma).
[e] Multiple trace element additive (Abbott).
[f] Calcium gluceptate (Abbott).
[g] Aminosyn + Electrolytes, potassium chloride (Abbott).

Results

Rats in all three groups gained weight at the same rate during the infusion period (Table II).

Plasma glucose did not differ among the three groups. Plasma protein, albumin, and aspartate aminotransferase also did not differ among the groups, and all were within normal range. Although the DHA animals had significantly lower plasma alanine aminotransferase, all three groups were within a normal range (Table III) (20). The pyr group had lower plasma triglycerides, but this was significant only at $P < 0.10$. There were no differences in either plasma cholesterol or triglycerides (Fig. 1) and no differences in nonesterified fatty acids (data not shown).

Liver size was the same for all rats, expressed both as grams and as percentage of body weight. Liver glycogen was the same for all three groups. Compared with controls, the DHA animals had a higher liver content of lactate (Table IV). Liver triglyceride, cholesterol, and phospholipid were not decreased by DHA or pyr, and in fact there was a tendency toward increased liver triglycerides in the experimental groups (Fig. 2).

Table II. Weight Gain of Rats during the Experimental Period[a]

	Weight gain (g)		
	Control ($n = 8$)	pyr ($n = 8$)	DHA ($n = 8$)
Presurgery	249 ± 4	249 ± 7	247 ± 4
Final	273 ± 3	279 ± 5	276 ± 3
Gain/day	2.4 ± 0.2	2.9 ± 0.3	2.8 ± 0.4

[a] Values are mean ± SE.

Table III. Plasma Values for Rats at Conclusion of Experimental Period[a]

	Control ($n = 8$)	pyr ($n = 8$)	DHA ($n = 8$)
Protein (g/dl)	6.0 ± 0.2	6.1 ± 0.1	6.2 ± 0.1
Albumin (g/dl)	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1
AST[b] (IU/liter)	55.4 ± 3.9	61.5 ± 6.4	56.3 ± 4.1
ALT[b] (IU/liter)	20.1 ± 1.9	17.4 ± 1.0	15.0 ± 1.4[c]
Glucose (mg/dl)	152.0 ± 10.6	147.5 ± 4.6	130.8 ± 4.0

[a] Values are mean ± SE.
[b] AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[c] Significantly different from control, $P < 0.05$.

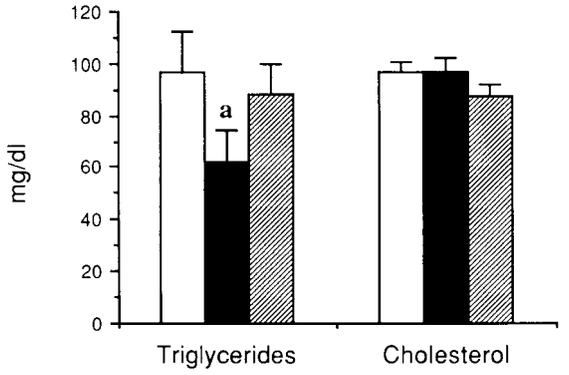

Figure 1. Effect of pyr or DHA on plasma lipids of parenterally fed rats. Values are mean ± SE. a, Significantly different from control, $P < 0.10$. □, control; ■, pyr; ▨, DHA.

Table IV. Liver values for Rats at Conclusion of Experimental Period[a]

	Control ($n = 8$)	pyr ($n = 8$)	DHA ($n = 8$)
Liver (% body wt)	3.1 ± 0.07	3.2 ± 0.05	3.3 ± 0.1
Glycogen (% liver)	3.0 ± 0.3	2.6 ± 0.4	3.1 ± 0.5
Lactate (μmol/g)	2.2 ± 0.2	2.2 ± 0.2	2.7 ± 0.5[b]

[a] Values are mean ± SE.
[b] Significantly different from control, $P < 0.05$.

Discussion

In our study, we were not able to demonstrate that pyr or DHA given intravenously could decrease TPN-induced liver lipid accumulation. Rats of comparable body weight fed stock diet have previously been shown to have liver triglyceride levels of 6.9 ± 0.5 and cholesterol of 2.54 ± 0.13 (21). Hence, the parenteral feeding protocol used in the present study does result in increases in liver lipids relative to comparable stock-fed animals. It is possible that a larger increase is necessary to demonstrate any effect of pyr or DNA. The level of pyr and DHA given is comparable to that of studies in which decreases in liver lipids were obtained (3, 5, 7).

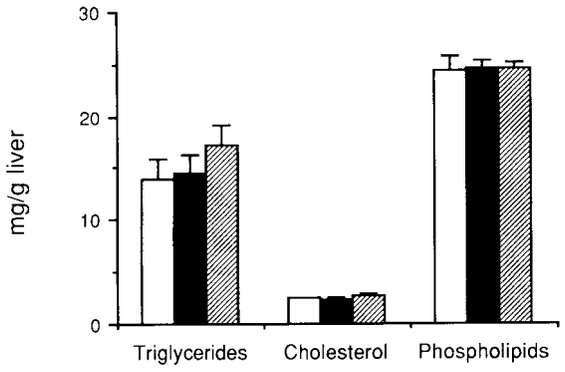

Figure 2. Effect of pyr or DHA on liver lipids of parenterally fed rats. Values are mean ± SE. □, control; ■, pyr; ▨, DHA.

When decreases have been obtained, pyr and DHA (6, 7), or pyr fed alone (5), has been fed orally. With oral feeding, pyr and DHA move through intestinal cells and pass via the portal system to the liver. This more direct path may be required to achieve alterations in liver lipids. With intravenous administration of the pyr and DHA, the compounds passed through the heart and circulated to peripheral tissues before uptake by the liver.

Pyr is a glycolytic intermediate and was likely taken up and metabolized by the heart and other tissues. DHA is a precursor to glycerol phosphate, a substrate for triglyceride synthesis, and so would more likely be taken up by the liver. This may explain the tendency toward higher, versus lower liver lipids with the addition of DHA to the intravenous feeding. In addition, DHA can be converted to lactate, resulting in the significantly higher lactate levels observed in the livers of the DHA animals.

The results of this study, taken together with those in which pyr and DHA were fed orally, suggest that route of administration is important for alterations in liver lipids to occur. Pyr or DHA given orally versus intravenously will pass through intestinal cells, perhaps undergoing some processing necessary to cause reduced liver lipids. Alternatively, the compounds may reach the liver in higher concentrations, which are needed to alter lipid accumulation. The liver lipid and lactate levels of the DHA animals suggest that DHA did reach the liver but resulted in a tendency for higher liver lipids. Rao *et al.* (5) have shown decreases in liver lipids with pyr fed alone but not with DHA fed alone. Other studies demonstrating liver lipid-lowering effects have fed pyr and DHA in combination (6, 7). Therefore, pyr may be the compound that is responsible for the prevention of fatty liver that has been observed in other studies.

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