GLUCONEOGENESIS IN ISOLATED HEPATOCYTES FROM FED AND FORTY-EIGHT-HOUR STARVED RATS

D.L. Story, J.A. O'Donnel, F.M. Dong[1] and R.A. Freedland

Department of Physiological Sciences, School of Veterinary Medicine
University of California, Davis, California

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Summary: Isolated rat hepatocytes from fed and starved rats synthesized net glucose from various precursors at similar rates. [3-^{14}C]-lactate incorporation into glucose was also similar in hepatocytes from fed and starved rats, as was ketone body formation from oleate and octanoate. Rates of gluconeogenesis in hepatocytes from fed rats compare to rates seen in perfused livers from starved rats rather than perfused livers from fed rats. Thus metabolic rates and possibly controls may be different between perfused livers and isolated hepatocytes when using fed rats.

Studies of gluconeogenesis using isolated rat hepatocytes have become prevalent since the development of the technique by Berry and Friend (1). Since modifications of this technique have appeared widely in the literature, it is important that the metabolic characteristics be well defined. Using the data in the literature relating to the perfused liver technique, a comparison can be made between the perfused liver and isolated hepatocytes.

Rates of gluconeogenesis and ketogenesis are two parameters that have been studied in perfused livers. Ross, et al. (5) have shown that livers from fasted rats synthesize glucose from lactate at twice the rate of livers from fed rats pretreated with phloridzin and glucagon. Similarly, Exton, et al. (6), have found that perfused livers from starved rats incorporate more radioactivity into glucose from [^{14}C]lactate than do livers from fed rats. Krebs, et al. (11), have found that perfused livers from starved rats synthesize ketone bodies at rates that are higher than those seen in perfused livers from fed rats. Since starved animals appear to have greater glucose and ketone body synthetic capacities, it would be expected that hepatocytes from starved rats should have greater capacities than hepatocytes from fed rats.

Garrison and Haynes (4) have compared isolated hepatocytes to perfused liver in respect to such properties as glycogenolysis, gluconeogenesis from lactate and pyruvate, and glucagon stimulation of gluconeogenesis. They have found both preparations to yield similar rates. In their study determinations

[1]Present address: Department of Nutrition, University of Washington, Seattle.

Table 1. Rates of Gluconeogenesis from Various Precursors in Hepatocytes From Fed and Forty-Eight Hour Fasted Rats

Substrate	Glucose Formation (nmoles/min/mg DNA)		
	Fed + Glucagon + Phloridzin	Starved + Glucagon + Phloridzin	Starved With no Treatment
Endogenous	(7) 18 ± 2[a]	(7) 5 ± 1[b]	(6) 10 ± 2[c]
10 mM Lactate: Pyruvate (9:1)	(7) 309 ± 18[a]	(7) 186 ± 19[b]	(6) 242 ± 19[b]
10 mM Lactate	(7) 191 ± 11[a]	(6) 122 ± 22[b]	(6) 177 ± 20[ab]
10 mM Fructose	(7) 625 ± 47[a]	(7) 251 ± 36[b]	(6) 469 ± 50[c]
10 mM Serine	(6) 75 ± 7[a]	(7) 81 ± 10[a]	(6) 100 ± 14[a]
10 mM Pyruvate	(5) 248 ± 17[a]	(7) 175 ± 27[b]	(6) 186 ± 5[b]
10 mM Sorbitol	(7) 220 ± 20[a]	(7) 96 ± 16[b]	(6) 167 ± 16[a]
10 mM Dihydroxy-acetone	(4) 368 ± 73[a]	(4) 109 ± 42[b]	(6) 264 ± 38[a]
10 mM Glutamate	(5) 7 ± 2[a]	(7) 7 ± 2[a]	(6) 5 ± 1[a]
10 mM Glycerol	(7) 87 ± 9[a]	(6) 43 ± 8[b]	(6) 55 ± 8[b]

Within substrates, values with similar superscripts are similar at $p \leq .05$. Hepatocytes were incubated with the above listed substrates for one hour or in the absence of substrate to determine endogenous rates. Endogenous glucose production has been subtracted.

of rates of gluconeogenesis from lactate and pyruvate in isolated hepatocytes were limited to starved rats only.

This present study was designed to compare rates of gluconeogenesis and ketogenesis between both fed and starved rats in the isolated hepatocytes with data reported in the literature using the perfused liver technique. From these studies it can be determined if hepatocytes from fed rats are suitable models for the study of metabolic regulation in fed animals.

Materials and Methods: All experiments were conducted using 150-275 g male Sprague Dawley rats fed laboratory chow ad libitum. Food was withheld from the starved rats for 48 hours prior to cell isolation. Phloridzin and glucagon treated rats were injected with 100 mg of phloridzin dissolved in 2,3-butanediol (100 mg/ml)/100 g body weight and 50 µmoles of glucagon/kg body weight three hours prior to cell isolation. Cells were isolated by the method of Berry and Friend (1), as modified by Cornell, et al. (7). Cell preparations and incubations were performed as per Cornell, et al. (7). DNA content was determined by the method of Burton (8). Glucose was measured by

Table 2. Gluconeogenesis from [^{14}C]lactate as Determined Enzymatically and Isotopically in Hepatocytes from Fed and Forty-Eight Hour Starved Rats

Treatment	Glucose Formed		
	Glucose Oxidase (μmoles/mg DNA)	[^{14}C]lactate Incorporation (μmoles/mg DNA)	(DPM/μg DNA)
Fed	(2) 9.2 ± 1.1	(3) 4.7 ± 0.74	(3) 260 ± 21
Fed + Glucagon + Phloridzin	(3) 11.0 ± 1.4	(3) 5.6 ± 0.24	(3) 313 ± 9
Starved	(2) 7.7 ± 0.02	(2) 4.4 ± 0.18	(2) 241 ± 7
Starved + Glucagon + Phloridzin	(2) 6.7 ± 0.13	(2) 3.7 ± 0.45	(2) 196 ± 11

Hepatocytes were incubated in triplicate with 10 mM lactate:pyruvate (9:1) containing .025 μCi/μmole [3-^{14}C]-lactate for forty-five minutes or with no substrate present to determine endogenous rates. Endogenous glucose production has been subtracted for rates determined with glucose oxidase.

the method of Krebs, et al. (9) and ketone bodies were measured by the method of Williamson, et al. (10). In studies using [3-^{14}C]-lactate, lactate : pyruvate (9:1) was present at 10 mM and [3-^{14}C]-lactate was present at a specific activity of .025 μci/μmole. In the isotopic experiments the cell contents were not neutralized after acidification but rather centrifuged at 500 g and an aliquot (3 ml) was put on a 1.2 cm X 5 cm column of AG50 W-X8 (H^+, 200-400 mesh) on top of a 1.2 cm X 5 cm column of Dowex 1X8 (acetate, 100-200 mesh). The columns were washed with distilled water until 30 ml were collected and 2 ml was added to 15 ml of scintillation fluid and counted in a scintillation counter. The scintillation fluid was prepared as described by Bray (14), with 0.01% Na-diethyldithiocarbamate added.

Results: Rates of gluconeogenesis in hepatocytes from fed and starved rats are shown in Table 1. The substrates used in this experiment were those studied by Ross, et al. (5). The hepatocytes from fed rats pretreated with glucagon and phloridzin synthesized glucose from all substrates at rates equal to or greater than hepatocytes from untreated starved rats. Hepatocytes from pretreated, starved rats exhibited lower rates of gluconeogenesis than hepatocytes from pretreated, fed rats using lactate:pyruvate, pyruvate, lactate, fructose, sorbitol, dihydroxyacetone, glycerol, and endogenous sources. Furthermore, glucagon and phloridzin treatment in starved rats had an inhibitory effect on the rate of gluconeogenesis from fructose, sorbitol, dihydroxyacetone, and endogenous sources, when compared to untreated starved rats.

Table 3. Ketone Body Formation from Oleate and Octanoate in Hepatocytes from Fed and Forty-Eight Hour Starved Rats

Treatment	Ketone Body Formation (nmoles/min/mg DNA)		
	Endogenous	Octanoate	Oleate
Fed	75 ± 8[a]	658 ± 19[a]	380 ± 22[a]
Fed + Glucagon + Phloridzin	—	489 ± 76[ab]	297 ± 56[ab]
Starved	191 ± 6[b]	620 ± 33[a]	423 ± 18[a]
Starved + Glucagon + Phloridzin	—	343 ± 38[b]	232 ± 22[b]

Within substrates values with similar superscripts are similar at $p \leq .05$. Hepatocytes were incubated with 2 mM Octanoate, 1 mM Oleate, or with no substrate for forty-five minutes. n equals three for all values.

In order to determine the percent contribution of lactate to the net glucose synthesized by hepatocytes from fed and starved rats and to further evaluate the effects of phloridzin and glucagon pretreatment on gluconeogenesis from lactate, [^{14}C]lactate incorporation was measured. Hepatocytes from fed rats incorporated similar amounts of [^{14}C]lactate into glucose as hepatocytes from starved rats (Table 2). Phloridzin and glucagon pretreatment increased [^{14}C]lactate incorporation into glucose by hepatocytes from fed rats but decreased [^{14}C]lactate incorporation by hepatocytes from starved animals. Hepatocytes from pretreated, fed rats synthesized glucose at the greatest rate, as measured isotopically and enzymatically (Table 2). Rates of net glucose synthesis have been corrected for endogenous glucose formation. This endogenous glucose release was considerable in the untreated, fed rats due to glycogenolysis, but minimal in the other three treatments. The rates determined enzymatically correspond well to rates determined isotopically and the data show that about fifty percent of the net glucose synthesized by hepatocytes from both starved and fed rats comes from lactate.

Since rates of ketogenesis have been well defined in perfused livers from starved and fed rats (11), these data may be compared to rates of ketogenesis from oleate and octanoate in isolated hepatocytes. Table 3 shows that hepatocytes from fed rats synthesize ketone bodies from oleate or octanoate at rates similar to hepatocytes from starved rats. Endogenous ketogenesis was 2.5 times greater in hepatocytes from starved rats than in hepatocytes

from fed animals. Pretreatment with glucagon and phloridzin significantly decreased ketogenesis in the hepatocytes from starved rats only. In hepatocytes from fed rats pretreatment also appeared to decrease the rates of ketogenesis but the decrease was not statistically significant.

Discussion: The finding that hepatocytes from fed rats synthesize glucose from various precursors as rapidly as hepatocytes from starved rats is in contrast with observations made in the perfused liver. Using perfused liver Ross, et al. (5), have found that net glucose synthesis from several precursors in starved rats is approximately twice as fast as that seen in fed rats. Glycerol was the only substrate studied which gave comparable rates between fed and starved rats. Our data are also in conflict with those of Wagle, et al. (15) who found that hepatocytes from starved rats synthesize glucose faster than hepatocytes from fed rats when alanine, lactate, fructose, and pyruvate are used as precursors.

Further differences between hepatocytes and perfused livers can be seen in the rates of ketogenesis. Krebs, et al. (11) have shown in perfused livers that endogenous ketogenesis is six times greater in starved rats compared to fed rats, while ketogenesis from octanoate is 3 times greater and from oleate 2 times greater in the starved rats. Our data show that endogenous ketogenesis in hepatocytes from starved rats is only 2.5 times as great as in hepatocytes from fed rats while ketogenesis from octanoate or oleate is similar. As some metabolic intermediates have been shown to be antiketogenic (Rose and Freedland, unpublished) it is possible that the high rates of ketogenesis seen in hepatocytes from fed rats could be due to a loss of some of these metabolites during cell preparation.

Net glucose synthesis from lactate:pyruvate was found to be greater in hepatocytes from fed rats compared to that seen in hepatocytes from starved animals (Table 1). The possibility exists that the cells from fed rats are using endogenous sources for glucose synthesis instead of lactate. As a result, gluconeogenesis from lactate may not be as great as the data indicates. To investigate this, [^{14}C]lactate incorporation into glucose was measured. The data show that hepatocytes from fed rats incorporate [^{14}C]lactate (as lactate:pyruvate) at a slightly greater rate than hepatocytes from starved rats (Table 2). This observation is in contrast with that of Exton, et al. (6) using perfused livers. They found that perfused livers from twenty hour starved rats incorporate up to twice as much [^{14}C]lactate into glucose as do perfused livers from fed rats. The data in Tables 1 and 2 show that the differences in gluconeogenesis from lactate and other precursors seen in perfused livers from fed and starved rats are not seen in isolated hepatocytes.

Table 4. Ratios of Gluconeogenesis in Perfused Livers and Isolated Hepatocytes from Fed and Forty-Eight Hour Starved Rats

Substrate	Fed hepatocytes / Fed perf. liver	Fed hepatocytes / Starved perf. liver	Starved hepatocytes / Starved perf. liver
10 mM Lactate	2.2	.95	1.2
10 mM Fructose	1.6	1.04	1.04
10 mM Glycerol	.81	1.08	.88
10 mM Pyruvate	—	1.2	1.2
10 mM Dihydroxy-acetone	2.1	.80	.77

Rates from hepatocytes from fed rats expressed per wet weight by using a factor of 4.2 mg DNA/g wet weight of liver.

Rates from hepatocytes from starved rats expressed per wet weight by using a factor of 5.6 mg DNA/g wet weight of liver.

Rates from perfused livers from Ross, et al. (5).

Glucose formation from added lactate accounts for approximately one half of the net glucose formed in hepatocytes from both fed and starved rats (Table 2). This would suggest that lactate oxidation is sparing equal amounts of endogenous precursors for glucose formation in both preparations. It is of great interest that this much potential glucose is available in the livers of both fed and starved rats but is apparently used for other purposes when lactate is not present. Other data from our laboratory show that addition of different physiological effectors alters the percentage of glucose that comes from lactate in perfused livers (Kramer and Freedland, unpublished). Thus, when studying the effects of different effectors on gluconeogenesis, interpretation of the data may vary depending upon whether net glucose synthesis or [^{14}C]lactate incorporation is measured.

In contrast to the observations of Ross, et al. (5) that phloridzin and glucagon treatment did not affect gluconeogenesis in perfused liver, we found that hepatocytes from pretreated, starved rats synthesized glucose at slower rates than hepatocytes from untreated, starved rats (Table 1). Gluconeogenesis from fructose, sorbitol, dihydroxyacetone was decreased by the glucagon and phloridzin pretreatment. Pretreatment also decreased [^{14}C]lactate incorporation into glucose by hepatocytes from starved rats, but increased isotope incorporation into glucose by hepatocytes from fed rats. It is possible that increased gluconeogenesis in the hepatocytes from pretreated, fed rats could

be due to glucagon stimulation of gluconeogenesis. The absence of the phloridzin and glucagon stimulation in the starved animals could be the consequence of an increased basal stimulation by the high levels of glucagon seen during starvation (13). When interpreting rates of gluconeogenesis from other precursors in hepatocytes from treated fed rats, consideration should be made of the fact that glucagon and phloridzin treatment could result in a slight stimulation of gluconeogenesis, independent of the precursors.

Expressing the data on a wet weight basis (5.6 mg DNA/gm wet weight) we obtain rates of gluconeogenesis in hepatocytes from starved rats that are similar to rates seen in perfused livers from a number of substrates (Table 4). On a wet weight basis the hepatocytes from fed and starved rats synthesize ketone bodies at rates greater than that seen in perfused livers (11). Krebs, et al., however, report similar rates of ketogenesis in both perfused livers and hepatocytes from starved rats (12). It is possible that entrance of substrate into the cell of the perfused liver is limiting and by using isolated hepatocytes this restriction is alleviated. An alternative explanation for the high rates of ketogenesis seen in isolated hepatocytes compared to perfused liver could be due to a loss of antiketogenic metabolites from cells of both fed and starved rats, during preparation. The cells from fed rats might have lost more of these intermediates and this could explain the marked increase in ketogenesis in hepatocytes from fed rats compared to perfused livers from fed rats. The increases we observed in ketogenesis are not unique since urea formation from lactate, NH_4Cl, and ornithine is also greater in hepatocytes than perfused liver (12).

Our data suggest that hepatocytes from fed rats are acting more like hepatocytes from starved rats with respect to gluconeogenesis and ketogenesis. In the perfused liver, which should be considered more physiological than isolated hepatocytes, gluconeogenic rates from fed rats are approximately one half of the rates seen in starved rats. However, in hepatocytes the rates from fed animals are equal to or greater than the rates seen in starved rats. The argument that hepatocytes from starved rats could be acting like hepatocytes from fed rats, that is, slowing down to the rate of the hepatocytes from fed rats, can be disregarded. If the data in Table 1 is expressed on a wet weight basis and compared to the rates seen in the perfused liver, it can be seen (Table 4) that hepatocytes from fed and starved rats are synthesizing glucose at rates comparable to those observed in perfused livers of starved rats (5). Comparing rates of gluconeogenesis in hepatocytes from fed rats and perfused livers, it can be seen that these hepatocytes are synthesizing glucose at a rate similar to the perfused livers from starved rats. Ketogenesis in hepatocytes follows a similar trend. Ketone body formation in

the hepatocytes from fed rats is far greater than that seen in the perfused livers from fed rats, while ketogenesis in hepatocytes from both fed and starved rats are more similar to ketogenesis in perfused livers from starved rats. Since our data show that hepatocytes from fed rats are not responding similarly to perfused liver in regard to several metabolic phenomenon, it is suggested that caution be used in studies of metabolic regulation using isolated hepatocytes from fed rats.

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References:

1. Berry, M. N., and Friend, D. S. (1969) J. Cell Biol, 43, 506-520.
2. Veneziale, C. M., and Lohmar, P. H. (1973) J. Biol. Chem, 248(22), 7786-7791.
3. Johnson, M. E. M., Das, N. M., Butcher, F. R., and Fain, J. N. (1972) J. Biol. Chem, 247(10), 3229-3235.
4. Garrison, J. C., and Haynes, R. C. Jr. (1973) J. Biol. Chem, 248(15), 5333-5343.
5. Ross, B. D., Hems, R., Freedland, R. A., and Krebs, H. A. (1967) Biochem. J, 105, 869-875.
6. Exton, J. H., Mallette, L. E., Jefferson, L. S., Wong, E. H. A., Friedmann, N., Miller, T. B., and Park, C. R. (1970) Rec. Prog. Horm. Res, 26, 411-461.
7. Cornell, N. W., Lund, P., Hems, R., and Krebs, H. A. (1973) Biochem. J, 134, 671-672.
8. Burton, K. (1956) Biochem. J, 62, 315-323.
9. Krebs, H. A., Bennett, A. M., deGarquet, P., Gascoyne, T., and Yoshida, T. (1963) Biochem. J, 86, 22-27.
10. Williamson, D. H., Mellonby, J., and Krebs, H. A. (1962) Biochem. J, 82, 90-96.
11. Krebs, H. A., Wallace, P. G., Hems, R., and Freedland, R. A. (1969) Biochem. J, 112, 595-600.
12. Krebs, H. A., Cornell, N. W., Lund, P., and Hems, R. (1974) Regulation of Hepatic Metabolism, pp. 727-750, Academic Press, New York.
13. Unger, R. H. (1974) The Pancreas as a Regulator of Metabolism Endocrine Physiology, Vol. 5, pp. 179-204, Butterworths University, Park Press.
14. Bray, G. A. (1960) Analyt. Chem, 1, 279-285.
15. Wagle, S. R., Ingebretsen, W. R. Jr., and Sampson, L. (1975) Acta Diabetol. Lat, 12(3-4), 177-262.