Utilization of fatty acids in perfused hypothermic dog kidney

J. S. HUANG, G. L. DOWNES, and F. O. BELZER

Department of Surgery, University of California, San Francisco, California 94122

ABSTRACT Utilization of oleic acid in whole dog kidneys perfused in vitro for 24 hr at 10°C was studied, and the data were correlated with results on the utilization of oleic acid in kidney slices incubated in the same perfusate at 10°C. Kidneys perfused without added oleate lost 35% of their total lipid content and 27% of their phospholipids. Addition of serum albumin-bound oleate to the perfusate prevented the loss of neutral lipid and reduced the loss of phospholipid to 8%. The kidney slices incorporated 29% of the added oleate into lipid and oxidized 3.2% to CO_2.

Oleate apparently largely replaces endogenous fatty acids which are oxidized to meet the energy requirements of the kidney. The loss of phospholipid from the perfused organ is taken as an indication of cell damage, which may be reduced but is not prevented by the addition of oleate to the perfusate.

SUPPLEMENTARY KEY WORDS oleic acid · oxidation · metabolism

HYPOTHERMIC PERFUSION at 10°C is a successful technique for the preservation of isolated kidneys for subsequent transplantation (1). Although O_2 consumption by canine kidneys at 10°C is only 5% of that at 38°C (2), metabolic needs cannot be ignored if prolonged preservation is to be achieved. In dogs, the kidney cortex accounts for approximately 10% of total body oxygen consumption. Its respiratory quotient is 0.75 (3–5), indicating that oxidation of fatty acids is the main source of energy for this organ (6–9); in vitro studies at body temperature have confirmed this. Fatty acids are rapidly oxidized, whereas the contribution of glucose and amino acids as energy sources is negligible (10–16).

Abbreviations: FFA, free fatty acids.

At present, reimplanted kidneys have a good survival rate after perfusion for 24–72 hr. One of the limiting factors to a longer preservation period may be the depletion of an unknown substrate(s) in the perfusate or in the tissue. Since lipids are crucial in the oxidative metabolism of the kidney, we have investigated (*a*) the change in lipid content and lipid composition in dog kidneys after 24 hr of perfusion at 10°C; (*b*) the concentration of fatty acids in the perfusate before and after perfusion; (*c*) the extent to which oleic acid added to the perfusate is taken up by the kidney; and (*d*) the oxidation of fatty acid by kidney cortex slices incubated in the standard perfusate. The results indicate that the hypothermically perfused dog kidney loses part of its endogenous lipids and that fatty acids from the perfusate can restore or prevent this loss.

MATERIALS AND METHODS

Operative Technique

12 adult mongrel dogs, weighing 20–25 kg, were used as kidney donors after being fasted for approximately 24 hr with free access to water. The animals had previously been fed Purina Dog Chow. Surgery was performed under general anesthesia (Nembutal, 25 mg/kg). Under anesthesia, prior to nephrectomy, diuresis was induced by rapid intravenous infusion of 12.5 g of mannitol in 50 ml of saline. One or both kidneys were removed through a midline incision. The renal artery and vein were mobilized to the aorta and vena cava, and any branches were divided. The ureter was dissected out to the bladder. The renal pedicle was then cut and the kidney was immediately flushed by gravity flow with cold Ringer's lactate solution until the effluent was clear. A cannula was selected which would fit the artery snugly; the Teflon tip was placed 2–3 mm into the vessel

and the distal end of the vessel was securely tied with a 2-0 silk suture. The cannula consisted of a Teflon tip inserted into a 2-inch length of rubber tubing. Cold ischemia time was 8–10 min, and the entire operation was performed in 25–30 min.

Perfusion Apparatus

The perfusion circuit (Fig. 1) consisted of a membrane oxygenator, pulsatile pump, heat exchanger, in-line filter, direct-reading pressure manometer, sampling taps, and kidney chamber. The details were described in a previous publication (17). In this circuit, the perfusate pO_2 was maintained at 130–140 mm Hg and the pH and pO_2 were measured by a Radiometer gas analyzer at 37°C and corrected to 10°C by a factor of +0.34 for pH (18) and 0.6 for pO_2 (19). The pump was set to maintain a systolic perfusion pressure of 60 mm Hg. At this pressure the flow rate was approximately 1 ml/g of kidney tissue/min. Flow rate and perfusion pressure remained constant throughout a 24-hr perfusion.

The kidney was placed in the kidney chamber (Fig. 1) and the renal artery cannula was connected to the arterial inlet. Perfusate drained freely from the renal vein and ureter and returned by gravity to the venous reservoir. At a perfusion pressure of 60 mm Hg the ureteral flow was approximately 0.25 ml/min. At 10°C this effluent was simply a protein-free filtrate of perfusate. In the isolated dog kidney no tubular reabsorption occurred at this temperature.[1]

Perfusion Medium

Cryoprecipitated, homologous plasma was used for both whole organ perfusion and tissue slice incubation. Acid citrate dextrose plasma (A.C.D., solution B, U.S.P.) stored at −20°C was rapidly thawed at 40–41°C and then passed through a sterile filter system (293-mm diameter) composed of a Millipore (Millipore Corp., Bedfore, Mass.) prefilter and three cellulose acetate filters of pore sizes 1.2, 0.45, and 0.22 μm. 8 mmoles of magnesium sulfate, 100 mg of hydrocortisone, 12 mg of phenol red, and 200,000 units of potassium penicillin G were added to each liter of perfusate. Prior to filtration, distilled water, mannitol, and KCl were added to the plasma to adjust the osmolarity to 300 mosm/kg H_2O and the Na^+ and K^+ concentrations to 140 mM and 5 mM, respectively.

The sodium oleate was prepared by adding an equimolar amount of sodium hydroxide (1.42 ml of a 2.5 N solution) to 1 g of oleic acid (Sigma Chemical Co., St. Louis, Mo.) in 10 ml of distilled water. A total of 250 mg of canine plasma albumin (99% pure, Sigma) was

[1] Belzer, F. O., and B. S. Ashby. Unpublished data.

KIDNEY PERFUSION CIRCUIT

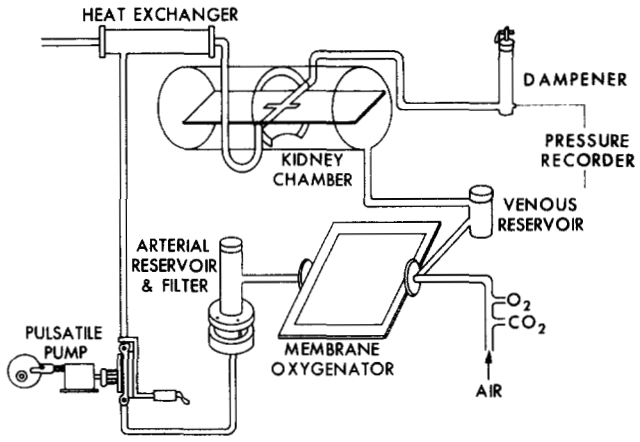

FIG. 1. Kidney perfusion circuit.

dissolved in 50 ml of distilled water and a total of 5 mmoles of sodium oleate was added. The fatty acid infusion had a pH of 7.4 at 25°C. It was introduced into the perfusate throughout the 24-hr period by a constant-infusion pump at a flow rate of 0.038 ml/min. At this flow rate sodium oleate is added to the perfusate medium at a rate of 3.5×10^{-3} mmoles/min. After the 24-hr perfusion, portions of kidney cortex and an aliquot of perfusate were used for lipid analysis.

Lipid Analysis

2–3 g of decapsulated kidney cortex was homogenized in 60 ml of chloroform–methanol 2:1. The extract was filtered and purified according to the procedure of Folch, Lees, and Sloane Stanley (20). Phospholipids and neutral lipids were separated on a silicic acid column (21). Dry weights of both fractions were determined and phosphorus was analyzed according to the method of Bartlett (22). The phospholipids were further separated by thin-layer chromatography on silicic acid plates (Merck, silica gel G). The solvent system was chloroform–methanol–water 60:35:5. The plates were sprayed with a solution of 2,7-dichlorofluorescein in 0.02% ethanol; the separated bands were scraped off carefully and the lipids were eluted with 4 × 3-ml aliquots of chloroform–methanol 2:1. The phosphorus content of each fraction was determined (22).

The neutral lipid fraction was further fractionated by thin-layer chromatography on silica plates using petroleum ether–ether–acetic acid 80:20:1, and the plates were sprayed with 2,7-dichlorofluorescein as above; the bands of triglycerides were scraped off and extracted with 3 × 3-ml aliquots of chloroform–methanol 2:1. The amount of triglycerides in tissue was determined gravimetrically using a Cahn G-2 Electrobalance; the concentration of triglycerides in

perfusates was determined by the method of Van Handel and Zilversmit (23).

The perfusate was circulated through the perfusion system without the kidney for 1–2 hr before an aliquot was removed for analysis as the sample "before perfusion." Lipids in the perfusate were determined as described by Van Handel (24).

Incubation of Kidney Cortex Slices

Oleate-U-^{14}C was obtained as the sodium salt with a specific activity of 1 Ci/mmole (Amersham/Searle Corp., Des Plaines, Ill.); it was complexed with serum albumin as described above. Kidney cortex slices approximately 0.3 mm thick (100–150 mg wet wt) were prepared with a Stadie-Riggs manual microtome, rinsed in cold Ringer's lactate solution, and blotted with hard filter paper. Two of these slices were placed in 50-ml Erlenmeyer flasks containing 5 ml of the standard medium, as used for perfusion, with 8 μmoles of ^{14}C-labeled oleate–albumin. After 2 hr of incubation at 10°C in a Dubnoff metabolic shaker, the slices were removed and blotted, and the tissue lipids were determined as described above.

For determination of the CO_2 derived from oxidation of oleic acid, a glass vial was suspended from the rubber stopper, and at the end of the incubation period (2 hr) 0.5 ml of 1 M Hyamine hydroxide was injected through the rubber stopper into the vial, and 0.5 ml of 2 N sulfuric acid was injected into the incubation medium. The flask was then warmed to 37°C for 1 hr to allow for trapping of CO_2 (25).

Radioactivity of isolated tissue lipids and trapped CO_2 was determined with a Packard Tri-Carb liquid scintillation spectrometer (model 3310). Each counting vial contained 10 ml of toluene, 50 mg of 2,5-diphenyloxazole, and 3 mg of 1,4-bis[2-(5-phenyloxazolyl)]-benzene; quenching was evaluated by use of an internal standard.

RESULTS

In Table 1 the lipid content and composition of kidneys perfused for 24 hr at 10°C with and without added oleate are compared with the values for control kidneys. After perfusion without added oleate there was a 35% loss of total lipid from the tissue. Neutral lipids and triglycerides decreased the most (65 and 58%, respectively); total phospholipids decreased by only 27%. When oleate was added to the perfusate the values for all lipid classes were not significantly different from those of the controls, with the exception of phospholipids, which showed a decrease of 8%. The distribution of individual phospholipids was essentially the same for all three groups of kidneys and was as follows: 52–59% phosphatidylethanolamine; 31–39% phosphatidylcholine; and 9–10% sphingomyelin. Apparently, oleate either prevents the loss of lipids from the tissue or is used for the resynthesis of lipid in the tissue.

Table 2 shows the loss of lipids from the perfusate after 24 hr of perfusion. Without added oleate 25% of the total lipids were lost. The loss occurred mainly in the neutral lipid fraction; the loss from the phospholipid fraction was relatively small. Practically all the loss from the neutral lipid fraction could be attributed to loss of triglycerides. When oleate was added, the loss from the neutral lipid fraction was higher (667 vs. 530 mg), whereas the loss from the triglyceride fraction was only half of that in the perfusate without added oleate (160 vs. 310 mg). Since the added oleate is largely recovered in the neutral lipid fraction, the data may be interpreted

TABLE 1 CHANGE IN LIPID CONTENT OF DOG KIDNEY* CORTEX BEFORE AND AFTER 24-HR PERFUSION AT 10°C

Lipid	Nonperfused Cortex	Perfused Cortex with No Oleate			Perfused Cortex with 5 mmoles of Oleate		
		After 24 hr	Loss	Loss	After 24 hr	Loss	Loss
	mg/g	*mg/g*		%	*mg/g*		%
Total lipids	20.80† ±1.34	13.60 ±0.99	7.20	35‡	21.01 ±1.30	NS§	NS
Phospholipids	15.80 ±0.66	11.57 ±1.42	4.23	27‖	14.51 ±0.86	1.29	8§
Neutral lipids¶	4.80 ±0.34	1.73 ±0.10	3.07	65‡	4.66 ±0.25	0.14	3§
Triglycerides	2.36 ±0.22	1.01 ±0.08	1.35	58‡	2.34 ±0.27	NS	NS

P values are taken from the Student *t* test of paired observations before and after perfusion.
* Average kidney weight was 60.12 ± 17.5 g.
† Values shown are the means ± SEM (four kidneys in each group).
‡ $P = 0.0005$.
§ Not significant.
‖ $P = 0.025$.
¶ Includes FFA, triglycerides, and free and esterified cholesterol.

TABLE 2 LIPID CONTENT OF PLASMA PERFUSATE* BEFORE AND AFTER 24-HR PERFUSION AT 10°C

Lipid	Lipid in Perfusate†				Lipid in Perfusate† with 5 mmoles of Oleate				P Value‡
	Before Perfusion	After Perfusion	Loss	Loss	Before Perfusion	After Perfusion	Loss	Loss	
	mg		*mg*	*%*	*mg*		*mg*	*%*	
Total lipids	2750§ ±25	2050 ±22	700 ±33	25	3408 ±29	2400 ±36	1008 ±47	29	0.005
Phospholipids	1740 ±17	1590 ±33	150 ±37	8	1780 ±26	1710 ±34	70 ±43	4	NS
Neutral lipids‖	1010 ±28	480 ±13	530 ±31	53	1352 ±36	685 ±18	667 ±40	50	0.025
Triglycerides	560 ±23	250 ±13	310 ±26	55	535 ±24	375 ±22	160 ±33	30	0.01

* Method of preparation is described in Ref. 17.

† 800 ml, initial volume of perfusate in system; 650 ml, final volume after 24-hr perfusion. 19% loss in volume was due to evaporation; lipid values after 24-hr perfusion were corrected for evaporation. 5 mmoles of albumin-bound oleic acid is equivalent to 1410 mg.

‡ These P values are taken from the Student t distribution, and represent the probability that the figures for lipid loss with and without added oleate are representative of the same population. NS, no significant difference between groups.

§ Values are the means ± SEM (four kidneys in each group).

‖ Includes FFA, triglycerides, and free and esterified cholesterol.

to show that a large part of the added oleate is taken up by the kidney and that the loss of triglycerides from the perfusate is correspondingly reduced.

Table 3 illustrates the recovery of radioactivity from oleate-U-^{14}C added to kidney cortex slices incubated for 2 hr at 10°C. 29% of the added label was recovered in the total lipid fraction extracted from the tissue, corresponding to an incorporation of 2280 nmoles of oleate/g. 84% of the radioactivity recovered in the lipids was found in the neutral lipids, 49% in triglycerides, and 16% in phospholipids. A total of 3.2% of added radioactivity was recovered as $^{14}CO_2$. This means that of the oleate taken up by the tissue, 11%, or 252 nmoles/g, was fully oxidized, and at least two-thirds of the remaining 90% was incorporated into phospholipids and triglycerides.

TABLE 3 ISOTOPE DISTRIBUTION AFTER 2-HR INCUBATION OF DOG KIDNEY SLICES WITH OLEATE-U-^{14}C AT 10°C

Radioactivity Recovered in:	nmoles/g*	% of Total Lipids	% of Oleate-U-^{14}C
Total lipids	2280 ± 113.04		29
Phospholipids	370 ± 34.78	16	
Neutral lipids†	1904 ± 74.86	84	
Triglycerides	1100 ± 40.24	49	
CO_2 (oleate oxidized)	252 ± 19.02		3.20
Incubation media	5640		
Initial oleate-U-^{14}C	7964		
Total recovered	8172		
Percentage recovered	102		

* g = dry weight of slices; results are means ± SD (four experiments).

† Includes FFA, triglycerides, and free and esterified cholesterol.

DISCUSSION

Under hypothermic conditions, oleate from the incubation medium is incorporated into the lipids of kidney tissue, as demonstrated by the uptake of label in the slice experiment. Only 11% of the total radioactive oleate which was taken up was oxidized to CO_2. We assume that the remaining 89% of the oleate was used mainly to replace endogenous fatty acids that were oxidized to meet the energy requirements of the tissue. This interpretation is supported by the observations on the whole organ, in which oleic acid added to the perfusate largely prevented the loss of lipid from the kidney. Moreover, the loss of lipid from the perfusate (1008 mg/24 hr) was about equal to the combined loss of lipid from the perfusate and the organ (1035 mg/24 hr) when no oleate was added to the system.

The observed loss of lipid in the perfusion experiments was higher than the actual amount metabolized because lipid was trapped in the perfusion system during the experiment. In one experiment, 478 mg of total lipid was extracted from the oxygenator membrane and the silk filter. Also, the measured oxygen consumption of the perfused organ at 10°C was 0.36 ml of O_2/g/hr,[1] which was much too low for complete oxidation of the lipid that was lost from the perfusate and the kidney. We could not correct for this trapping of lipid because it was not possible to extract all parts of the apparatus. Moreover, the trapping of lipid could not be estimated by the lipid lost from the perfusate alone, because under otherwise identical conditions, when no organ was present, loss of lipid from the system was insignificant (50 mg).

The oxygen consumption of the kidney has been reported to be 10 ml of O_2/g/hr at 38°C (26). If we assume that the oxygen consumption of a slice at 10°C is reduced proportionately as in the whole organ, i.e., to 5% (2), we calculate that only 16% of this O_2 consumption is necessary for the production of the observed labeled CO_2, derived from oxidation of added oleic acid. However, since only 11% of the oleic acid taken up was oxidized, the remainder can be assumed to replace endogenous lipid that has been oxidized. Accordingly, the total lipid oxidized and the oxygen consumed seem to match reasonably well. The remaining deficit in oxygen consumption may be explained by incomplete oxidation of the fatty acid, leading to the formation of ketone bodies (27). It seems likely, therefore, that the kidney cortex slices show the same preferential oxidation of fatty acids under hypothermic conditions that is observed at 38°C (27). The same is probably true for the hypothermically perfused organ, except that observed lipid losses were grossly exaggerated due to large losses in the perfusion apparatus that were difficult to estimate.

That such losses occur when a kidney is connected to the perfusion system, but not when the perfusate is circulated without an organ, is an interesting observation. It implies that the kidney during perfusion loses lipid material in an aggregated or easily adsorbable form, or that it changes the lipid in the perfusate to such a form. This may be taken as an indication of cell damage occurring under the perfusion conditions. The considerable loss of phospholipid, which is usually not used as a substrate for oxidation, should probably be interpreted in the same way. That all classes of phospholipids are approximately equally affected further strengthens this interpretation. Apparently, cell membranes or membrane-containing cell organelles break down, and their constituent lipids are either oxidized or lost into the perfusate. The addition of oleate reduces, but does not prevent, this loss of phospholipids. While it is therefore clearly beneficial in restoring the neutral lipids and reducing the phospholipid loss, the addition of oleate is apparently not sufficient to prevent all cell damage, and further means must be sought to improve the preservation of the organ during prolonged perfusion.

The authors are indebted to Drs. Walther Stoeckenius and Richard J. Havel for their helpful advice and discussion. We wish to thank Mr. Donald Loarie, Mr. Robert Hoffman, and Mr. Kenneth Steiper for their excellent technical assistance.

This study was supported in part by the John A. Hartford Foundation, Inc., and by the U.S. Public Health Service, research grant H.D. 03639.

Manuscript received 22 February 1971; accepted 2 June 1971.

References

1. Belzer, F. O., B. S. Ashby, and J. E. Dunphy. 1967. 24-hour and 72-hour preservation of canine kidneys. *Lancet.* **2:** 536–539.
2. Levy, M. N. 1959. Oxygen consumption and blood flow in the hypothermic, perfused kidney. *Amer. J. Physiol.* **197:** 1111–1114.
3. Bainbridge, F. A., and C. L. Evans. 1914. The heart, lung, kidney preparation. *J. Physiol.* (London). **48:** 278–286.
4. Dickens, F., and F. Simer. 1930. The respiratory quotient, and the relationships of respiration to glycolysis. *Biochem. J.* **24:** 1301–1326.
5. Schorr, E., R. O. Loebel, and H. B. Richardson. 1930. The nature of phlorhizin diabetes. *J. Biol. Chem.* **86:** 529–549.
6. Lee, J. B., V. K. Vance, and G. F. Cahill, Jr. 1962. Metabolism of C^{14}-labeled substrates by rabbit kidney cortex and medulla. *Amer. J. Physiol.* **203:** 27–36.
7. Graffen, A. L., and D. E. Green. 1948. The complete oxidation of fatty acids. *J. Biol. Chem.* **176:** 95–115.
8. Geyer, R. P., L. W. Matthews, and F. J. Stare. 1949. Metabolism of emulsified trilaurin (—C^{14}OO—) and octanoic acid (—C^{14}OO—) by rat tissue slices. *J. Biol. Chem.* **180:** 1037–1045.
9. Hohenleitner, F. J., and J. J. Spitzer. 1961. Changes in plasma free fatty acid concentrations on passage through the dog kidney. *Amer. J. Physiol.* **200:** 1095–1098.
10. Baisset, A., A. Boer, D. Diaz, and C. Soula. 1950. La rein et la regulation glycémique. *J. Physiol.* (Paris). **42:** 534–536.
11. Cohn, C., B. Katz, and M. Kolinsky. 1951. Renal gluconeogenesis in the intact dog. *Amer. J. Physiol.* **165:** 423–428.
12. Bollman, J. L., and J. H. Grindlay. 1952. Measurement of renal gluconeogenesis. *Amer. J. Physiol.* **170:** 38–44.
13. Reinecke, R. M. 1956. Renal arteriovenous changes in fructose and blood sugar in the rat with ligated ureters. *Amer. J. Physiol.* **186:** 409–413.
14. Chinard, F. P., W. R. Taylor, M. F. Nolan, and T. Enns. 1959. Renal handling of glucose in dogs. *Amer. J. Physiol.* **196:** 535–544.
15. Söling, H-D., and L. Schmidt. 1960. Vergleichende Untersuchungen des Sauerstoffverbrauchs und der Bearbeitung von Glucose, Milchsäure und Brenztraubensäure im Stoffwechsel der Hundeniere. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* **240:** 140–156.
16. McCann, W. P., and J. R. Jude. 1958. The synthesis of glucose by the kidney. *Bull. Johns Hopkins Hosp.* **103:** 77–93.
17. Belzer, F. O., B. S. Ashby, J. S. Huang, and J. E. Dunphy. 1968. Etiology of rising perfusion pressure in isolated organ perfusion. *Ann. Surg.* **168:** 382–390.
18. Rosenthal, T. B. 1948. The effect of temperature on the pH of blood and plasma in vitro. *J. Biol. Chem.* **173:** 25–30.
19. Hedley-Whyte, J., E. P. Radford, Jr., and M. B. Laver. 1965. Nomogram for temperature correction or electrode calibration during PO_2 measurements. *J. Appl. Physiol.* **20:** 785–786.
20. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.

21. Hirsch, J., and E. H. Ahrens. 1958. The separation of complex lipide mixtures by the use of silicic acid chromatography. *J. Biol. Chem.* **233:** 311–320.
22. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
23. Van Handel, E., and D. B. Zilversmit. 1957. Micromethod for the direct determination of serum triglycerides. *J. Lab. Clin. Med.* **50:** 152–157.
24. Van Handel, E. 1961. Suggested modifications of the micro determination of triglycerides. *Clin. Chem.* **7:** 249–251.
25. Cuppy, D., and L. Crevasse. 1963. An assembly for $C^{14}O_2$ collection in metabolic studies for liquid scintillation counting. *Anal. Biochem.* **5:** 462–463.
26. Taft, J. L., R. E. Marshall, and H. D. A. Schirmer. 1967. Effect of oxygen tension on glucose metabolism in rabbit kidney cortex and medulla. *Invest. Urol.* **5:** 95–97.
27. Weidemann, M. J., and H. A. Krebs. 1969. The fuel of respiration of rat kidney cortex. *Biochem. J.* **112:** 149–166.