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Lack of Effect on Human Lipoprotein–Triacylglycerol on the Function of Perfused Rat Kidney (42450)

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Abstract. We determined whether addition of human lipoprotein-TG to the perfusate for the isolated rat kidney would increase net Na^+ reabsorption or maintain renal tissue K^+ content. Rat kidneys (n = 6) were perfused for 75 min with a perfusate containing 6 g% of substrate-free albumin in Krebs–Ringer bicarbonate and a mixture of human chylomicrons and very low density lipoproteins (human lipoprotein-triacylglycerol (HL-TG)). Control kidneys (n = 6) were perfused in the substrate-limited state, i.e., without any exogenous substrates added to the perfusate. Means (n = 6) for function of control kidneys were GFR = 808 ± 50 μl $g^{-1} \cdot min^{-1}$; %T-Na^+ = 63.3 ± 1.3%. A significant loss of tissue K^+ occurred: tissue K^+ remaining after 75 min of perfusion = 79.1 ± 1.9%. Although kidney tissue contains lipoprotein lipase, HL-TG (n = 6) did not increase %Na^+ reabsorption (64.3 ± 2.6%) or maintain tissue K^+ content (80.6 ± 2.0%). Therefore, the TG might have been hydrolyzed and taken up for biosynthesis, the rat kidney lipoprotein lipase might have been inactive, or the rat kidney might not use lipoprotein-TG for biosynthesis or oxidation. © 1987 Society for Experimental Biology and Medicine.

The kidney utilizes numerous substrates, including nonesterified fatty acids (1). Although plasma triacylglycerols (TG) are utilized by heart, mammary gland, adipose tissue, and skeletal muscle (2–4), the utilization of plasma TG by kidney has not been reported. There is evidence that kidneys may utilize plasma TG to support kidney function. First, lipoprotein lipase activity, which is essential for TG uptake (5), is found in kidney (6–9). Second, Trimble (10) has observed a disappearance of ^{14}C-labeled fatty acids contained in very low density lipoproteins (VLDL) added to the medium of the perfused kidney. Trimble, however, did not measure renal function or determine if the disappearance of the ^{14}C-labeled fatty acids in the VLDL were due to their binding or to their utilization (10). Third, rat kidneys perfused with 1.0 mM palmitic acid had significantly higher fractional Na^+ reabsorption than kidneys perfused with no exogenous substrate (11). Fourth, we have shown (12) that oxygen consumption and fractional sodium reabsorption are both reduced after the addition of 2-tetradecylglycidic acid (2-TGDA) during substrate-free perfusion. 2-TGDA is a specific inhibitor of long chain acylcarnitine transferase I, and hence of the oxidation of long-chain fatty acids (LC-FA). Since the pool of free LC-FA in kidney tissue is small, the LC-FA which are oxidized during substrate-free perfusion must be derived from TG stores in renal tissue. Thus, the kidney can oxidize FFA, the product of TG hydrolysis, to support sodium reabsorption. Taken together this evidence suggests that kidneys can hydrolyze plasma TG, take up the FFA, and oxidize them for support of sodium reabsorption. The direct effect of perfusate TG on kidney function, however, has not been measured.

In a previous study, we showed that isolated rat kidneys perfused with 1 mM lactate had significantly higher sodium reabsorption rates than kidneys perfused with no exogenous substrate (13). Cotransport of Na^+ with lactate would not account for the increase in Na^+ reabsorption. In further studies (14) it was found that only certain substrates, when added to the perfusate, enhanced Na^+ reabsorption and also maintained renal tissue K^+ content, as did lactate. Wittner *et al.* used a similar technique to identify substrates that support net NaCl reabsorption from the lumen of perfused segments of the rabbit cortical thick ascending limb (15). Thus, an increase in tubular function due to the presence of a substrate is an indirect but sensitive (11, 13–15) method for determining if a substrate is taken up and oxidized by the kidney. We have used this method to test if TG in the perfusate can support renal function.

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In this present study, the function of isolated rat kidneys perfused with no exogenous substrate was compared with function of kidneys perfused with human lipoprotein-TG (HL-TG). A mixture of human chylomicrons and very low density lipoproteins (VLDL) was used as the source of TG. These lipoproteins are a water-soluble form of TG, and they contain the peptides that activate lipoprotein lipase.

Materials and Methods. *Perfusion apparatus.* The perfusion apparatus described earlier (16) was used. Two modifications were made. (i) A thin-walled needle (19 gauge, cut to a length of 15 mm) was used as the arterial cannula. The kidney was suspended from the renal arterial cannula over a glass funnel. (ii) The vena cava was not cannulated; thus the venous effluent drained directly into the glass funnel. The stem of the funnel was inserted into the neck of the venous reservoir.

Perfusion medium. For each perfusion we used 200 ml of a Krebs–Ringer bicarbonate (KRB) solution which contained albumin, urea, and inulin. The albumin (fraction V BSA, Miles Laboratory, Cat. No. 81-0003) had been treated with activated charcoal at pH = 3.5, then passed through a millipore filter (0.4-μm pore diam), adjusted to pH 7.0, and dialyzed against glass distilled water (17, 18). It was then lyophilized. The free fatty acid content of the albumin after treatment was 2.66 μEq/g of albumin. The final composition (mM) of the perfusion medium was Na^+, 145; K^+, 5; Ca^{2+}, 2.5; Mg^{2+}, 1; Cl^-, 110; HCO_3^-, 25; SO_4^{-2}, 1; PO_4, 2; urea, 7. Albumin concentration was 60 g/liter; inulin (Pfanstiehl, Kankakee, IL) was 0.50–0.65 g/liter. When gassed with 95% O_2:5% CO_2 the perfusate pH was 7.4.

In half of the perfusions, the mixture of human chylomicrons and VLDL was added to the perfusion medium. These lipoproteins had been separated from 73 ml of serum obtained from a hypertriglyceridemic patient as follows: the lipoproteins were isolated from the serum by ultracentrifugation (19) in a swinging bucket rotor (SW 27 Beckman) at 27,000 rpm for 24 hr. The lipoprotein was removed from the top of the tube, diluted in a saline–EDTA solution (0.85% NaCl and 0.1% Na_2-EDTA · H_2O, pH = 7.2), and centrifuged again under the same conditions as described above. The final volume of the lipoprotein mixture was 21 ml. The long chain free fatty acid concentration (20) in the lipoprotein mixture was 2.43 μEq/ml; no measurable glucose (13) was found after the "milky" lipoproteins had been diluted 1:61 with saline. The lipoprotein fraction was stored at 4°C during the 13 days over which these experiments were done. We added 3.3 ml of the lipoproteins to the perfusion medium (200 ml) before we added the medium to the perfusion circuit. The initial concentration of TG (measured as glyceride–glycerol) in the perfusion medium was 0.75 ± 0.01 mM (n = 6). The concentration of TG after perfusion was 0.93 ± 0.5 mM (n = 6). There was no detectable TG in the urine of kidneys perfused with HL-TG. The TG were measured by the Triglyceride C-37 Rapid Stat Kit (Pierce Chemical Company, Rockford, IL) based on a method described by Biggs *et al.* (21).

Perfusion procedure. For all perfusions, kidneys were taken from fed (Purina rat chow), male, Long–Evans hooded rats (Blue Spruce Farms, Altamont, NY) weighing 500–650 g. Anesthesia was 10 mg/100 g body wt of inactin (5-sec-butyl-5-ethyl-2-thiobarbituric acid, Promonta, Hamburg, Germany). The rats were anesthetized between noon and 2 PM. The cannulation and transfer of the kidney to the thermostated chamber (38°C) have been described (13, 16). Unlike earlier procedures (16), no heparin or other anticoagulent was administered prior to cannulation. The kidney was perfused with a pulsatile pump for 75 min at a mean arterial pressure of 120 mm Hg distal to the arterial cannula (16). We measured kidney functions during three consecutive 20-min observation periods starting at 15 min after cannulation of the renal artery.

Renal function and tissue composition. Glomerular filtration rate (GFR) was assumed to be equal to the clearance of inulin (22). The concentrations of Na^+ and K^+ in the perfusate, urine, and tissue were determined by flame photometry (Instrumentation Laboratories Model 343). The difference between the rates of Na^+ filtered and excreted was used to calculate net rate of Na^+ reabsorption ($\dot{T}$-Na^+). The technique for measurement of K^+ in tissue has been described (16).

Experimental design and statistical analysis. The two groups of kidneys were those perfused without HL-TG (n = 6) and those perfused with HL-TG (n = 6). The 12 perfusions

were done in a random order. Statistical comparisons for functions between the two groups were made with Student's *t* test. A *P* value of less than 0.05 was the criterion for two means to be considered significantly different from each other.

Results and Discussion. Figure 1 shows the time courses for means of GFR, and %$\dot{T}$-Na^+ in the presence (SFA + HL-TG) and absence (SFA) of human lipoprotein-TG in the perfusate. The mean values for kidney function and tissue K^+ content are shown in Table I. There were no significant differences in the means for GFR, $\dot{T}$-Na^+, or %$\dot{T}$-Na^+ between kidneys perfused without HL-TG present and kidneys perfused with HL-TG present. In addition, tissue K^+ was lost (14, 16) to the same extent when kidneys were perfused with or without HL-TG present. The perfusion flow rates, which were high enough to provide adequate oxygenation,[1] were also similar. If those kidneys which were perfused with HL-TG had shown an increase in tubular function and maintenance of tissue K^+ (13, 14, 16), then we could have concluded that the kidney hydrolyzes HL-TG and oxidizes the fatty acids (11) produced. We found no change, however, in either measurement (Table I).

These results suggest that there was no uptake of HL-TG. No decrease in TG content of the perfusion medium would verify this conclusion. Our calculations indicated, however, that under these experimental conditions it was not possible to measure the expected decrease in the mass of TG accurately: oxidation of only 5.6% of the initial pool of TG in the perfusate could have accounted for all of the renal O_2 uptake during perfusion.[2] We cannot detect this small percentage change because the measurement of the substrate content of the perfusate has an error of 3–4% (23). Also, TG binds to the surface of the perfusion apparatus (10, 24–26).

Although we did not measure TG content of the perfusate, we did measure TG concentration: it increased from 0.75 ± 0.01 m*M* (*n* = 6) at 15 min to 0.93 ± 0.05 m*M* (*n* = 6) at the end of the perfusion. There are four processes that could affect TG concentration during the perfusion: utilization of TG by the kidney, binding of TG, release of TG from kidney, and formation of a urine free of TG. The increase in concentration was due in part to this last process, since the increase in the TG concentration was closely correlated (r = 0.99) with the total volume of urine formed (Fig. 2). This rise in the TG concentration is consistent with the lack of uptake and utilization of HL-TG, but does not prove this conclusion. Therefore there are three possible explanations for our observations.

One possibility is that these perfused kidneys hydrolyzed but did not oxidize the glycerol or FFA from lipoprotein-TG. The carbon chains could have been used for biosyntheses (13, 27). We did not measure whether there was entry of portions of the added TG into net synthetic processes in the perfused kidney. This possibility therefore remains to be explored.

A second possibility is that these perfused kidneys did not hydrolyze the lipoprotein-TG because the lipoprotein lipase was inactive. Nutritional and hormonal factors affect the activity of lipoprotein lipase in adipose tissue, mammary gland, and heart (28–31). We do not know how hormonal and nutritional factors affect the activity of the lipoprotein lipase

[1] In previous studies done under similar conditions (13) the PO_2 of the arterial perfusate was at least 500 mm Hg. Thus, using a solubility coefficient for O_2 of 0.9464 μmol O_2/ml perfusate, the O_2 content of the arterial perfusate was ~0.62 μmole/ml (13). At a mean arterial perfusion flow rate of ~37 ml/min · g, the O_2 delivery to the kidney was ~24 μmole O_2/min · g. Since the isolated perfused rat kidney has an O_2 uptake between 6–8 μmole/min · g, from three to four times more O_2 was being delivered to the kidney than could have been utilized.

[2] Calculation of the fraction of the TG pool in the perfusion medium which would be necessary to supply the kidney with all of its oxidative needs. We assumed that the $\dot{Q}O_2$ of the kidney = 6 μmole $O_2 \cdot min^{-1} \cdot g^{-1}$ (13). The average weight of the control kidneys in this present study = 1.7 g. Therefore, the total amount of the O_2 utilized during 1 hr of perfusion is 6 μmole $O_2 \cdot min^{-1} \cdot g^{-1} \times 60$ min $\times$ 1.7 g = 612 μmole O_2. The complete oxidation of 1 μmole of tripalmitin requires 72.5 μmole of O_2. If all the O_2 utilized in 1 hr were due only to tripalmitin oxidation then the total amount of tripalmitin oxidized would be 612 μmole $O_2 \times$ (1 μmole tripalmitin/72.5 μmole O_2) = 8.44 μmole tripalmitin. The above calculated disappearance of 8.44 μmole of tripalmitin represents only 5.6% of the 150 μmole of TG in the initial perfusion medium (μmole/ml).

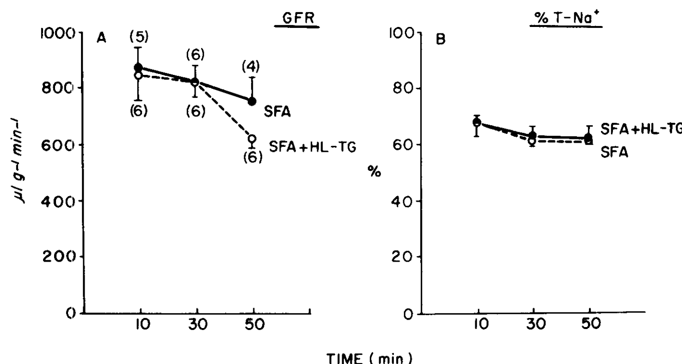

FIG. 1. Renal function with and without HL-TG. Time courses for mean (±SEM) GFR and mean %T-Na^+ in the presence and the absence of a mixture of human chylomicrons and VLDL (HL-TG) in the perfusate for the isolated rat kidney. Numbers in parentheses refer to the number of perfusions on which each mean was based. When $n < 6$, this was due to technical problems either with the assay for GFR or in the quantitative collection of urine.

in the perfused rat kidney. Also to be considered is that the rat kidney lipoprotein lipase may have remained inactive due to species differences in apolipoproteins (32). Perhaps the isolated perfused rat kidney would have utilized lipoprotein-TG obtained from rat plasma. Rat kidney catabolizes only rat high density lipoprotein (HDL) and not human HDL (33). Isolated perfused rat hearts, however, utilize both rat and human VLDL-TG and the utilization of VLDL-TG from both sources occurs at similar rates (2). At present there is no evidence to suggest that rat kidneys could not utilize the lipid part (TG) of human VLDL and chylomicrons. The lipoprotein lipase could have been inactive if the activator polypeptides from plasma lipoprotein were missing from the lipoprotein-TG. For example, Breckenridge *et al.* (34) have reported that the hypertriglyceridemia in a patient was due to the absence of apolipoprotein C-II from the

TABLE I. FUNCTION AND TISSUE K^+ MAINTENANCE IN KIDNEYS PERFUSED FOR 75 min WITHOUT OR WITH A MIXTURE OF HUMAN CHYLOMICRONS AND VERY LOW DENSITY LIPOPROTEINS (HL-TG)

	−HL-TG	+HL-TG[a]
Glomerular filtration rate (μl/min g wet wt)[b]	808 ± 50[c]	797 ± 43
$\dot{T}$-Na^+ (μEq/min g wet wt)	75.5 ± 4.6	74.8 ± 2.9
%$\dot{T}$-Na^+	63.3 ± 1.3	64.3 ± 2.6
perfusion flow rate (ml/min g wet wt)	35.5 ± 2.1	39.5 ± 3.9
%K = remaining in the tissue[d]	79.1 ± 1.9	80.6 ± 2.0

[a] TG concentration in the perfusion medium = 0.75 ± 0.1 m*M* ($n = 6$).

[b] Function of the perfused kidney is expressed per gram wet weight of the unperfused control kidney.

[c] Mean of six perfusions ± SEM. The function of each kidney was calculated as the mean of three consecutive observation periods (each 20 min long). For three of the kidneys in the +HL-TG group there were only two observation periods used to obtain the perfusion mean. In no case were there significant differences between the two groups.

[d] Perfused kidney/control kidney × 100.

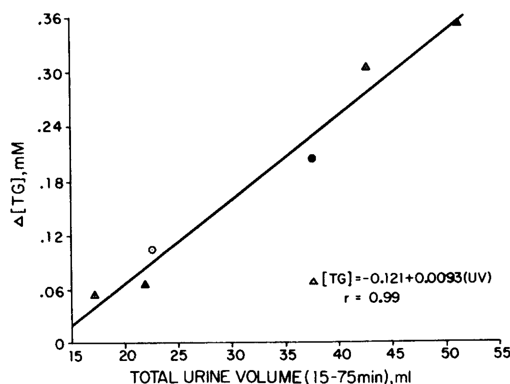

FIG. 2. Δ[HL-TG] vs urine volume. Perfusate concentration of HL-TG increases in proportion to the total volume of urine formed during perfusion. There was no TG in the urine. Each symbol represents a single perfusion done in the presence of HL-TG added to the perfusate.

VLDL. The hypertriglyceridemic plasma used in our experiment was assayed by isoelectric focusing. It contained adequate amounts of apolipoprotein C-II and all the other apoproteins were found to be within normal limits. Thus, factors other than the absence of apolipoproteins must account for the observed lack of an effect of the added lipoprotein-TG on renal function.

A third possibility is that rat kidney does not take up and utilize lipoprotein-TG from the extracellular fluid and that this is a normal characteristic of the intact rat kidney. Until now, however, all tissues that contain lipoprotein lipase have been shown to utilize exogenous lipoprotein-TG *in vivo* and *in vitro*. Thus, in order to distinguish between an inactive lipoprotein lipase and an inability to remove lipoprotein TG from the extracellular fluid, it will be necessary to clearly define the characteristics of the regulation of rat kidney lipoprotein lipase activity.

In summary, our data show that HL-TG, when added to the perfusate, does not support tubular function in the perfused rat kidney or maintain tissue K^+ content. Therefore, the TG might have been hydrolyzed and taken up for biosynthesis, the rat kidney lipoprotein lipase might have been inactive, or the rat kidney might not use lipoprotein-TG for biosynthesis or oxidation.

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