Movement of lipids into and out of the blood during hyperlipidemia induced in rabbits by pituitary extract and Fraction H

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ABSTRACT Rabbits were rendered hyperlipidemic by the subcutaneous injection of an alkaline aqueous extract of mammalian pituitary gland or a partially purified, concentrated fraction derived therefrom, designated Fraction H. Eight hours after the injection of Fraction H, arteriovenous (A–V) differences in plasma triglyceride (TG) were measured across five body areas.

A large negative A–V difference in plasma TG was consistently found across the liver (increase in plasma concentration), and a moderate positive A–V difference across the perirenal fat depot (decrease in plasma concentration). Most values obtained across the intestines and mesentery and the leg were positive. Differences across the kidney were mostly small and almost equally divided between positive and negative values. Similar results were obtained 7–12 and 16–18 hr after injection of the pituitary extract. It is concluded that the liver is the principal source of the TG for this kind of hyperlipidemia, and that the plasma TG levels are reduced principally in passage through adipose tissue.

A consistent negative A–V difference in plasma free fatty acid concentration (net increase) was found across the kidney 7–12 hr after injection, suggesting that the kidney hydrolyzes its accumulated TG and mobilizes it in the form of free fatty acids.

KEY WORDS hyperlipidemia · pituitary extract · Fraction H · rabbit · A–V differences · triglycerides · liver · adipose tissue · kidney · free fatty acids · fatty liver

HYPERLIPIDEMIA CAN be produced in rabbits by the subcutaneous injection of an alkaline aqueous extract of hog pituitary glands, or a partially purified, concentrated fraction derived therefrom, designated Fraction H (1, 2) or Peptide II (3). Addition of either the crude extract or the concentrated fraction to rabbit adipose tissue in vitro accelerates the hydrolysis of triglycerides (TG) to free fatty acids (FFA). In the living animal, the initial response to a single subcutaneous injection of either material is a 5- to 10-fold rise in the plasma FFA concentration within 30 min, followed in the first 6 hr by a 4- to 8-fold increase in the TG content of the liver and kidney. Six to eight hr after injection a lactescent hyperlipidemia begins to develop which lasts until the 18th–24th hr (2).

Previously we have shown (4) that the initial increase in plasma FFA is due to mobilization of FFA from adipose tissue into the blood, as indicated by a marked increase in the plasma FFA concentration that occurs across the perirenal fat depot 1 and 2 hr after injection. At the same time, a substantial proportion of the newly mobilized FFA is taken up by the liver and kidney and synthesized into hepatic and renal TG. In the experiments to be described, the net movement of lipids into and out of the blood was determined in rabbits during development of the hyperlipidemia and during the return of plasma lipid levels to preinjection control values. This was done by measuring the A–V differences in plasma TG and FFA concentration across five tissues and organs 7–12 and 16–18 hr after an injection of pituitary extract. In this manner (*a*) the fate of the TG in the liver and kidney, (*b*) the source of the hyperlipidemia, and (*c*) a mechanism capable of returning the plasma lipid levels to preinjection values were elucidated.

MATERIALS AND METHODS

Animals

Male and female rabbits of mixed breeds weighing

Abbreviations: FFA, free fatty acids; TG, Triglyceride; A–V, arteriovenous.

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2800–3800 g were used. The animals had free access to Purina Rabbit Chow Checkers and water until they were anesthetized for the surgical procedures.

Adipokinetic Materials

Intact, lyophilized hog pituitary glands were extracted in 0.1 M sodium phosphate buffer, pH 9, for 5 hr at 4°C. The insoluble residue was removed by centrifugation; the turbid supernatant fraction was designated pituitary extract. Each rabbit received a single subcutaneous dose of 10 ml of this solution, representing the extract from 100 mg of lyophilized glands. The amounts of the various recognized pituitary hormones extracted by this procedure have been shown (5) to be too small to produce any significant increase in rabbit plasma triglyceride concentration at this dose level. Some of the experiments utilized Fraction H, a partially purified, concentrated fraction of pituitary extract prepared by the method of Rudman, Seidman, and Reid (5). This material has been shown (5) to contain less than 0.8% of any of the recognized pituitary hormones. The dose, given subcutaneously in saline, was 3–5 mg per rabbit.

Procedures

Each rabbit received 40 ml of a 5% solution of dextrose in saline subcutaneously 1 hr before the operation in order to offset the tendency of the anesthesia and operation to produce elevations in the plasma FFA concentration (4).

Arterial and venous samples were obtained at laparotomy in the manner previously reported (4). These were not obtained simultaneously, but rather in two groups; each group consisted of 2–3 venous samples and one arterial sample. Each group of samples was obtained rapidly so that not more than 2 min elapsed between the collection of any one venous sample and the arterial samples in the same group. The total of all seven samples was obtained within 5–8 min. Blood samples were prevented from clotting with heparin and placed immediately into an ice-water bath. They were centrifuged in a refrigerated centrifuge at 4°C for 10 min at 3000 rpm. The entire plasma layer was removed and single aliquots for TG or for FFA analyses were taken. Calculations of the A–V differences were made by subtracting the concentration of the substance in the venous sample from the concentration of that substance in the arterial sample collected within the same time period. The exact relative amounts of blood supplied to the liver by the hepatic artery and the portal vein are not known; therefore the plasma concentrations in the abdominal aorta, hepatic vein, and portal vein are listed as such in the tables. The hepatic A–V difference was obtained by subtracting the plasma TG concentration in the heptaic vein from the arithmetic mean of the TG concentration in the corresponding abdominal aorta and portal vein samples. For simplicity, only the mean hepatic A–V differences for each group of animals are listed in Tables 1 and 2.

Determinations

Plasma TG was measured by the method of Van Handel and Zilversmit (6); a single aliquot of plasma was analyzed in duplicate. In a series of ten plasma samples with a TG concentration ranging from 173 to 651 mg/100 ml, in which duplicate plasma aliquots were analyzed by this method, the maximum individual variation was ±2.4%. Plasma FFA was measured by the method of Dole (7). Urine was examined for the presence of FFA by the method of Trout, Estes, and Friedberg (8), and for TG by the same method used for plasma.

RESULTS

A–V Differences in Plasma TG in Control Rabbits

Ten rabbits received no adipokinetic substance. The plasma TG concentration in this group of animals ranged from 7 to 63 mg/100 ml (Table 1). The average A–V differences in plasma TG concentration across the five organs and tissues studied (Table 1) were all small, averaging from −2 to +3 mg/100 ml. The individual differences across the liver, kidney, perirenal fat pad, and intestines and mesentery were almost equally divided between plus values (indicating a decrease in concentration) and minus values (indicating an increase in concentration). Across the leg, three values were zero; of the five positive values, all were small.

A–V Differences in Plasma TG after Injection of Fraction H or Pituitary Extract

Twenty-eight rabbits each received a single subcutaneous injection of pituitary extract. Two of these animals were studied 6 hr after the injection; the plasma TG concentrations of these animals were 37 and 56 mg/100 ml respectively, and the A–V difference across each of the five organs was in the range of −6 to +6 mg/100 ml, a range similar to that in the control animals.

Nine rabbits (Nos. 11–19) were studied 8 hr after each had received a single subcutaneous injection of 3–5 mg of Fraction H. All animals had an elevated plasma TG concentration: 131–633 mg/100 ml, mean 252 (Table 2). There was a consistent increase (negative A–V difference) in the plasma TG concentration across the liver in these animals, with an average increase of 34 mg/100 ml. A consistent decrease in plasma TG concentration (positive A–V difference) across the perirenal fat pad averaged 26 mg/100 ml. Comparison of these values with the corresponding values of the control animals gave P values of < 0.001. Seven of the nine A–V differences across

TABLE 1 A–V DIFFERENCES IN PLASMA TRIGLYCERIDES ACROSS FIVE BODY AREAS IN CONTROL RABBITS

Rabbit No.	Arteriovenous Difference				Plasma Concentration		
	Fat Pad	Intestines and Mesentery	Leg	Kidney	Abdominal Aorta	Portal Vein	Hepatic Vein
	mg/100 ml				*mg/100 ml*		
1	0			−2	7		
		−5	+3		9	14	
2		+2			12	10	16
			0		10		
	−6			−8	12		
3		0			18	18	25
	−11		+3	−1	23		
4	0	+3	0	+5	13	10	29
					16		
5	0			+1	18		
		+1	+6		21	20	21
6	0		+3	+5	21		
		+6			30	24	19
7	0		+2	−4	33		
		+2			38	36	38
8		−6			34	40	35
9		−1			46	47	35
				+31	63		
10		+3	0		52	49	58
	+2			+1	55		
Mean A–V difference ± SEM	−2 ± 1.3	+1 ± 1.0	+2 ± 0.7	+3 ± 3.6	Mean* 29	31	31
					—	—	−2 ± 2.9

* Mean for those animals where an arterial, a portal, and a hepatic venous sample were obtained.

the intestines and mesentery, and six of the seven across the leg indicated decreases in TG concentration, while there were equal numbers of positive and negative values found across the kidney. The average A–V differences across each of these areas were not statistically different from the corresponding figures obtained in the control animals.

Eight rabbits (Nos. 20–27, Table 2) were studied 7–12 hr after each had received a single injection of 10 ml of pituitary extract. The average plasma TG concentration ranged from 198 to 787 mg/100 ml, mean 331. Again, there were consistent increases in plasma TG concentration across the liver and consistent decreases across the fat pad. The average change across the fat pad was highly significant ($P = 0.001$); the change across the liver was significant only at the 90% level because of the large standard error of the mean. The individual A–V differences across the other three areas were almost equally divided between increases and decreases, and the mean values were not statistically different from the control values.

The plasma TG concentration of nine rabbits (Nos. 28–36, Table 2) studied 16–18 hr after injection of pituitary extract ranged from 431 to 2343 mg/100 ml, reflecting the large variability in the extent of the hypertriglyceridemic response to pituitary extract, as well as the variability in its time of onset and duration. The mean A–V difference across the liver was −100 mg/100 ml, indicating a significant net movement of TG into the blood ($P < 0.01$). The difference across the fat pad was +52 mg/100 ml ($P < 0.1$). The mean differences across the other three areas were relatively small and were not statistically different from the controls.

Fate of the Renal Tissue Triglycerides

The foregoing data suggested that at least a portion of the triglycerides that accumulated in the liver during the first 16–18 hr following adipokinetic stimulation by pituitary extract was mobilized into the blood between 8 and 18 hr after the injection. In contrast, the triglycerides that accumulated in the kidney did not appear to be mobilized back into the blood as such; thus their fate was unclear. It is possible that they could be rehydrolyzed and mobilized into the blood as FFA, excreted in the urine as either TG or FFA, or metabolized within the kidney. Two experiments were done in order to explore further the fate of the triglycerides in the kidney. Table 3 contains the renal plasma FFA A–V differences for five rabbits studied 7–12 hr after a single injection of pituitary extract and four rabbits after Fraction H. During this time interval the FFA A–V difference was negative in eight of the nine animals, indicating that FFA were being mobilized from the kidney into the blood. Secondly, urine from animals at 12, 24, and 48 hr after injection of pituitary extract was examined for the presence of FFA

TABLE 2 A–V DIFFERENCES IN PLASMA TG ACROSS FIVE BODY AREAS IN RABBITS INJECTED WITH PITUITARY ADIPOKINETIC SUBSTANCES

Rabbit No.	Arteriovenous Difference: Fat Pad	Intestines and Mesentery	Leg	Kidney	Plasma Concentration: Abdominal Aorta	Portal Vein	Hepatic Vein
	mg/100 ml					*mg/100 ml*	
	8 hr after injection of Fraction H						
11	+2	−8		+6	131	139	142
12	+12	+7			167	160	189
			−8	−8	129		
13	+30	+18			191	173	189
				−9	162		
14	+8		+2	−8	186		
		+7			204	197	230
15		+8	+34		211	202	281
	+38			+36	236		
16		−6	+3		225	231	267
				−11	298		
17	+36		+18	+5	239		
		+43			248	205	293
18	+32	+16	+8		272	256	302
				+7	227		
19	+38	+2			611	609	632
			+64	+36	633		
				Mean*	252	241	281
Mean A–V difference ± SEM	+25 ± 5.2	+10 ± 5.1	+17 ± 9.4	+6 ± 8.8	—	—	−34 ± 7.8
P (vs. Control)	0.001	0.1	0.1	0.7	—	—	0.001
	7–12 hr after injection of pituitary extract						
20		+1	−3		198	197	288
	+24			+14	210		
21		−7	+16		237	244	273
	+2			+2	230		
22		+21		−8	257	236	280
23		−9	−41		418	427	462
	+27			−14	447		
24		+15			430	415	862
25		−58	−85		446	504	598
	+53			+87	531		
26	+20		−33	−8	645		
27	+30		+55	+27	787		
				Mean*	331	337	461
Mean A–V difference ± SEM	+26 ± 6.7	−6 ± 14.0	−15 ± 19.9	+14 ± 13.3	—	—	−127 ± 64.3
P (vs. Control)	0.001	0.7	0.4	0.4	—	—	0.1
	16–18 hr after injection of pituitary extract						
28	+69		−261	−236	2342		
29		+17	+38		1621	1604	1686
30		−2	+48		1620	1622	1806
	+6			+19	1574		
31		−112	−53		1364	1475	1438
	+271			+92	1402		
32	−26		−10	+56	1326		
33		+18	+30		1230	1212	1269
	+21			+116	1242		
34	+93		+57	+49	1039		
		−18			895	913	1073
35		−5	−24		594	599	575
	+9			−17	581		
36		−14	−2		452	466	668
	−29			−16	431		
				Mean*	1111	1127	1216
Mean A–V difference ± SEM	+52 ± 26.0	−17 ± 16.8	−20 ± 32.5	+8 ± 38.6	—	—	−100 ± 29.3
P (vs. Control)	<0.1>0.05	<0.4>0.3	0.5	0.9	—	—	<0.01>0.001

* Mean for those animals where an arterial, a portal, and a hepatic venous sample were obtained.

and TG. No urinary FFA or TG were found in any of these animals.

DISCUSSION

The consistent and substantial negative A–V differences found only across the liver in rabbits 7–18 hr after the parenteral administration of either pituitary extract or Fraction H are interpreted to indicate that the liver is the principal source of TG for the hypertriglyceridemia in these animals. Similarly, the consistent positive A–V differences found across the perirenal fat pad suggest that the plasma TG concentration is decreased principally in passage through adipose tissue. Blood flow across these organs was not measured and it is possible that a fall in blood flow in the treated animals could reduce the net amount of triglyceride per minute exchanging between the blood and these organs. However, a 12- to 75-fold decrease in blood flow would be required to negate completely the highly significant changes in A–V differences found in the treated and the control animals. Such a large decrease is unlikely. In fact, Rudman, Seidman, Brown, and Hirsch report a grossly visible hyperemia in the adipose tissue of rabbits given pituitary extract (2), which suggests that increased perfusion actually enhances the exchange of lipids between blood and adipose tissue in these animals. It is not known whether the arterial concentration of TG or the blood flow was or was not changing during collection of the sample (9), but it is considered unlikely that any such changes could have been sufficiently large to affect the interpretation of the data.

A–V differences across the intestines and mesentery, leg, and kidney showed considerable individual variation. Eight hours after injection of Fraction H, seven out of nine measurements across the intestines and mesentery and six out of seven across the leg were positive, but the difference between the mean values and the means of the controls was significant only at the 90% level. Nevertheless, this finding suggests that the adipose tissue that constitutes a portion of each of these two areas might be acting to reduce the TG content of the plasma. Across the kidney, the A–V differences were mostly small and almost equally divided between positive and negative values, suggesting that little or no TG was exchanged between blood and kidney. The A–V differences across these three areas showed even greater variation in sign and magnitude after administration of pituitary extract. No significant difference in procedure or technique could be found to account for the occasional large A–V difference, and so all values were included in calculating mean A–V differences. Comparison of these mean values with those of the control animals reveals little or no exchange of TG between any of these areas and the blood under these conditions. On the other hand, there is considerable biological variation in time of onset, magnitude, and duration of response to pituitary extract, and it is possible that each individual value has significance for its specific animal and conclusions drawn from mean values are therefore misleading. Most probably the pattern of net exchange of TG between the blood and areas of the intestines and mesentery, leg, and kidney after pituitary extract is similar to the pattern after Fraction H, but the variations in the data require further study.

The previous and present findings indicate that the FFA mobilized from rabbit adipose tissue by pituitary extract or Fraction H return to the adipose tissue by the same pathways utilized for the transport of endogenous FFA in untreated rabbits with normal plasma lipid levels. After an injection of pituitary extract, the large amounts of FFA circulating in the blood were taken up principally by the liver and kidney and converted to TG. After injection of tracer amounts of radioactive FFA into normal rabbits, Havel, Felts, and Van Duyne (10) found that the FFA were taken up principally by the liver and esterified completely within 2 min. Seven hours after injection of pituitary extract, the FFA taken up by the liver appear to be released into the blood in the form of TG in lipoproteins. Several investigators have reported that endogenous plasma TG are derived almost exclusively from the liver in the normal rabbit, rat, and dog

TABLE 3 A–V Difference in Plasma FFA across Kidney in Rabbits 7–12 Hr after Receiving Pituitary Extract or Fraction H

Pituitary Extract			Fraction H		
Rabbit No.	Abdominal Aorta	A–V Difference across Kidney	Rabbit No.	Abdominal Aorta	A–V Difference across Kidney
	μeq/liter			*μeq/liter*	
37	333	−145	42	762	−59
38	428	−98	43	669	−167
39	538	−150	44	1138	−284
40	655	−52	45	1216	−154
41	710	+112			
Mean	—	−67	Mean	—	−166

(10–12). Finally, the plasma TG concentration was reduced most consistently in passage through the perirenal fat pad. This is similar to the finding in normal refed rabbits given ^{14}C-labeled triglycerides in the form of very low density lipoproteins; 2 hr later, when a total of 70% of the injected dose was recovered, 54% was found in adipose tissue (10).

The TG A–V difference across the liver remained small for the first 6 hr after injection of pituitary extract, and the plasma concentration remained within the normal range in spite of the massive transport of FFA to the liver and the 8- to 12-fold increase in hepatic TG content that occurs during this time period (2). The reason for this delay in mobilizing appreciable amounts of TG from the liver into the blood is unknown. Perhaps a critical concentration of hepatic TG must be reached before large amounts of $d < 1.006$ lipoproteins are synthesized or released from the liver. Alternatively, the delay may be the time required for induction of the enzyme systems responsible for the formation of these lipoproteins. Once appreciable quantities of TG begin to move into the blood, the large A–V difference (−100 to −127 mg/100 ml) and the relatively rapid rate of perfusion of the liver [74 ml/min for 100 ml of liver tissue (13)] produce a rate of efflux which most probably is well in excess of the capacity of the adipose and other tissues to remove TG from the blood, and hypertriglyceridemia develops.

After adipokinetic stimulation with pituitary extract, the renal cortex, as well as the liver, took up large amounts of FFA and converted them into TG. However, unlike the liver, the kidney did not appear to release significant quantities of TG as such, probably because it lacks the ability to synthesize lipoproteins. It was shown previously that the renal TG are localized in the convoluted tubules of the cortex (4). Hollenberg and Horowitz have detected (14) a lipase in the renal cortex capable of hydrolyzing TG not already in the form of lipoproteins. The action of this enzyme on the TG in the convoluted tubules and subsequent mobilization of the FFA could account for the negative A–V difference in plasma FFA concentration found across the kidney 7–12 hr after injection of pituitary extract or Fraction H. Alternatively, this finding could be due to (*a*) mobilization of FFA stored as such in the kidney, or (*b*) release of FFA by hydrolysis of circulating TG, catalyzed by lipoprotein lipase located at the plasma membrane within the kidney. In any event, the rabbit kidney appears to act as a temporary repository of FFA during periods of rapid mobilization of FFA from adipose tissue.

The relative importance of this function in the control of lipid transport and metabolism is unknown. In fact, little is known in general of the significance of fat in the kidney. The proximal convoluted tubules of the normal cat and dog contain large droplets of lipid; these are said to be principally neutral fats, and lipid droplets are not unusual in the urine (15). There is no fat seen in the tubules or urine of the normal human being, but lipids have been demonstrated in the renal tubules of human patients with the nephrotic syndrome (16). Studies of the relationship between the kidney and the blood lipids are needed to determine whether the normal kidney plays a significant role in lipid metabolism or whether abnormalities of lipid metabolism can injure the kidney.

This work was supported in part by PHS Research Grants H-00052-16 and AM 07847-02 from the National Institutes of Health, and the Health Research Council of the City of New York under contract No. U-1017.

Manuscript received 19 March 1965; accepted 18 October 1965.

References

1. Rudman, D., and F. Seidman. *Proc. Soc. Exptl. Biol. Med.* **99:** 146, 1958.
2. Rudman, D., F. Seidman, S. J. Brown, and R. L. Hirsch. *Endocrinology* **70:** 233, 1962.
3. Friesen, H., R. J. Barrett, and E. B. Astwood. *Endocrinology* **70:** 579, 1962.
4. Hirsch, R. L., D. Rudman, R. Ireland, and R. K. Skraley. *J. Lipid Res.* **4:** 289, 1963.
5. Rudman, D., F. Seidman, and M. B. Reid. *Proc. Soc. Exptl. Biol. Med.* **103:** 315, 1960.
6. Van Handel, E., and D. B. Zilversmit. *J. Lab. Clin. Med.* **50:** 152, 1957.
7. Dole, V. P. *J. Clin. Invest.* **35:** 150, 1956.
8. Trout, D. L., E. H. Estes, Jr., and S. J. Friedberg. *J. Lipid Res.* **1:** 199, 1960.
9. Zierler, K. L. *J. Clin. Invest.* **40:** 2111, 1961.
10. Havel, R. J., J. M. Felts, and C. M. Van Duyne. *J. Lipid Res.* **3:** 297, 1962.
11. Borgstrom, B., and T. Olivecrona. *J. Lipid Res.* **2:** 263, 1961.
12. Havel, R. J., and A. Goldfien. *J. Lipid Res.* **2:** 389, 1961.
13. Bradley, S. E. In *Handbook of Physiology*, edited by W. F. Hamilton and P. Dow. Am. Physiol. Soc., Washington, D.C., 1963, Sect. 2, Vol. 2, Chap. 43, p. 1405.
14. Hollenberg, C. H., and I. Horowitz. *J. Lipid Res.* **3:** 445, 1962.
15. Bloom, F. *Pathology of the Dog and Cat.* American Veterinary Publications, Evanston, Ill., 1954, pp. 8–9.
16. Zimmer, J. G., R. Devey, C. Waterhouse, and R. Terry. *Ann. Internal Med.* **54:** 205, 1961.