Early Effects of Anti-Insulin Serum on Hepatic Metabolism of Plasma Free Fatty Acids in Dogs

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SUMMARY

Hepatic metabolism of free fatty acids was studied in six anesthetized fasted dogs before and for several hours after the abrupt induction of insulin deficiency. This was produced by injecting guinea pig anti-insulin serum in amounts capable of binding all circulating insulin for several hours. During each study, albumin-bound-1-C-14-palmitate and I-131-rose bengal were infused at a constant rate and serial samples of arterial, portal and hepatic venous blood were obtained through appropriate intravascular catheters. Maximal changes were observed three to four hours after administration of anti-insulin serum. Mean arterial concentrations of glucose, free fatty acids, glycerol and triglyceride fatty acids of very low density lipoproteins increased twofold and that of ketone bodies, fourfold. Hepatic extraction ratio of free fatty acids was unchanged after anti-insulin serum so that the hepatic uptake of free fatty acids approximately doubled. During the control period, about 25 per cent of free fatty acids removed by the liver were converted to CO_2, 11 per cent to triglyceride fatty acids of very low density lipoproteins and 25 per cent to ketones (assuming that ketones derived entirely from FFA). After anti-insulin serum, corresponding values were 14 per cent, 26 per cent and 51 per cent. The increased relative and absolute conversion of free fatty acids to ketones resembles that observed in chronically diabetic dogs withdrawn from insulin for forty-eight hours. However, the increased conversion of free fatty acids to triglyceride fatty acids of very low density lipoproteins observed in short-term deficiency of insulin contrasts with the greatly depressed conversion observed after two days of insulin lack. DIABETES *21*:280-88, May, 1972.

In previous work from this laboratory, it was shown that hepatic metabolism of free fatty acids (FFA) in dogs is greatly altered when a state of chronic diabetes mellitus is produced by pancreatectomy. In the diabetic animals, larger amounts of FFA were taken up by the liver and a greater fraction of FFA was converted to ketones, while their secretion in triglyceride fatty acids of very low density lipoproteins (VLDL-TGFA) was inhibited.[2] An important implication of these data is that the hyperlipemia encountered in such pancreatectomized animals is not a consequence of increased hepatic production but is primarily the result of a defect in removal of VLDL-TGFA in peripheral tissues.

In the studies reported here, we have shown that the diabetic state accompanying short-term deficiency of insulin produced by injection of anti-insulin serum (AIS) is characterized by a different pattern of hepatic metabolism of FFA. The changes observed in the chronic state of diabetes, therefore, do not appear to result solely from the immediate changes in hepatic influx of metabolites or rapidly occurring changes in hepatic enzymic activities.

MATERIALS AND METHODS

Experiments were performed on six normal mongrel male dogs weighing 18 to 26 kg. and maintained on a diet composed approximately of 75 per cent protein and 25 per cent fat. About four weeks before the experiment, through a subcostal incision, the vena cava was partially ligated immediately below the diaphragm and above the renal veins. This allowed the development of venous collateral circulation. One week before the experiment, a midline laparotomy was performed, the vena cava was completely ligated and a small catheter of Teflon was placed into the portal vein through a

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branch of the splenic vein so that the tip lay just below the bifurcation of the portal vein as it entered the liver. The free end of the catheter was passed subcutaneously and brought through the skin at the nape of the neck. The catheter was kept patent by flushing it daily with 0.15 M sodium chloride solution (saline). No anticoagulant was used. All animals were eating for at least three to four days but food intake was variable. Food was removed twenty-four hours before the study.

On the day of the experiment, anesthesia was induced with 20 to 30 mg./kg. of sodium pentobarbital and maintained by further small doses as needed to prevent shivering and to maintain heart rate below 120. A catheter of Teflon filled with Renografin was placed into the vena cava through an incision in the right jugular vein and its tip was placed under fluoroscopy about 0.5 cm. above the diaphragm. Blood sampled at that level represents almost exclusively hepatic venous (HV) blood because of the complete interruption of the vena cava below the diaphragm. This procedure minimizes the frequent production of thrombi encountered when catheters are placed in the hepatic vein itself for many hours in animals receiving no anticoagulants. A catheter of polyethylene was inserted into a femoral artery. Animals were intubated and ventilated artificially at a rate to maintain pH and tensions of O_2 and CO_2 of arterial blood in the normal range. Saline containing albumin-bound 1-C-14-palmitate and I-131-rose bengal (about 1 μCi/ml. for each isotope was infused at a constant rate of 0.25 ml./min. into a peripheral vein throughout the study. After a control period of two to three hours, 25 ml. of guinea pig AIS* (insulin binding potency of 3.56 to 3.65 U./ml.) was injected intravenously. Simultaneous samples of blood were obtained periodically from the artery, portal vein and hepatic vein during the control period and for three to four hours following injection of AIS. No apparent adverse effects of this injection were observed.

Samples were analyzed for glucose, FFA, C-14-FFA, glycerol, acetoacetate (AcAc), β-hydroxybutyrate (βOHB), TGFA and C-14-TGFA (total and fraction in VLDL) and labeled bicarbonate according to methods described elsewhere.[2] In addition, I-131-radioactivity was estimated in samples of arterial and hepatic venous blood plasma and flows were determined according to Combes.[3] At the end of the study, samples of the liver were taken from each of four lobes and analyzed for content of lipids and lipid-C-14.[2]

CALCULATIONS*

1. Net inflow transport of FFA $=$

$$\frac{\text{infusion rate of palmitate-C-14}}{\text{specific activity arterial FFA}}$$

2. *Hepatic metabolism.*

(a) Assuming that 80 per cent of the blood entering the liver is derived from the portal vein and 20 per cent from the hepatic artery,[2] hepatic input (i) of metabolite or C-14 was calculated as $(p \times 0.8) + (a \times 0.2)$.

(b) Extraction fraction $= \frac{i-v}{i}$ where i is the concentration of blood entering the liver and v the concentration in HV.

(c) Hepatic uptake of FFA $=$ concentration of FFA in plasma entering the liver $\times$ extraction fraction of C-14-palmitate $\times$ hepatic plasma flow.

(d) Hepatic uptake of glycerol $=$ (concentration in blood entering the liver — concentration in HV) $\times$ hepatic blood flow.

(e) Hepatic production of ketones, glucose, C-14-TGFA and C-14-O_2 $=$ [blood (plasma) concentration in HV — concentration in blood (plasma) entering the liver] $\times$ hepatic blood (plasma) flow.

(f) Net inflow transport of TGFA from liver $=$ $\frac{\text{hepatic output of C-14-TGFA}}{\text{hepatic uptake of C-14-FFA}} \times$ hepatic uptake of FFA.

3. *Extrahepatic splanchnic (EHS) metabolism.*

(a) Extraction fraction $= \frac{a-v}{a}$ where a is the concentration in artery and v the concentration in the portal vein.

(b) Uptake of FFA $=$ concentration in arterial plasma $\times$ extraction fraction of C-14-palmitate $\times$ portal vein plasma flow.

(c) Production of FFA $=$ [(concentration in portal vein plasma — concentration in arterial plasma) + (concentration in arterial plasma $\times$ extraction fraction of C-14-palmitate)] $\times$ portal vein plasma flow.

(d) Uptake of ketones, glycerol and glucose $=$ (arterial blood concentration — portal vein blood concentration) $\times$ portal vein blood flow.

(e) Production of C-14-O_2 $=$ (concentration in

*Generously provided by Dr. Peter H. Wright, Department of Pharmacology, School of Medicine, Indiana University, Indianapolis, Indiana.

*Nomenclature used here is that recommended by Task Group on Tracer Kinetics of International Commission on Radiation Unit.[15]

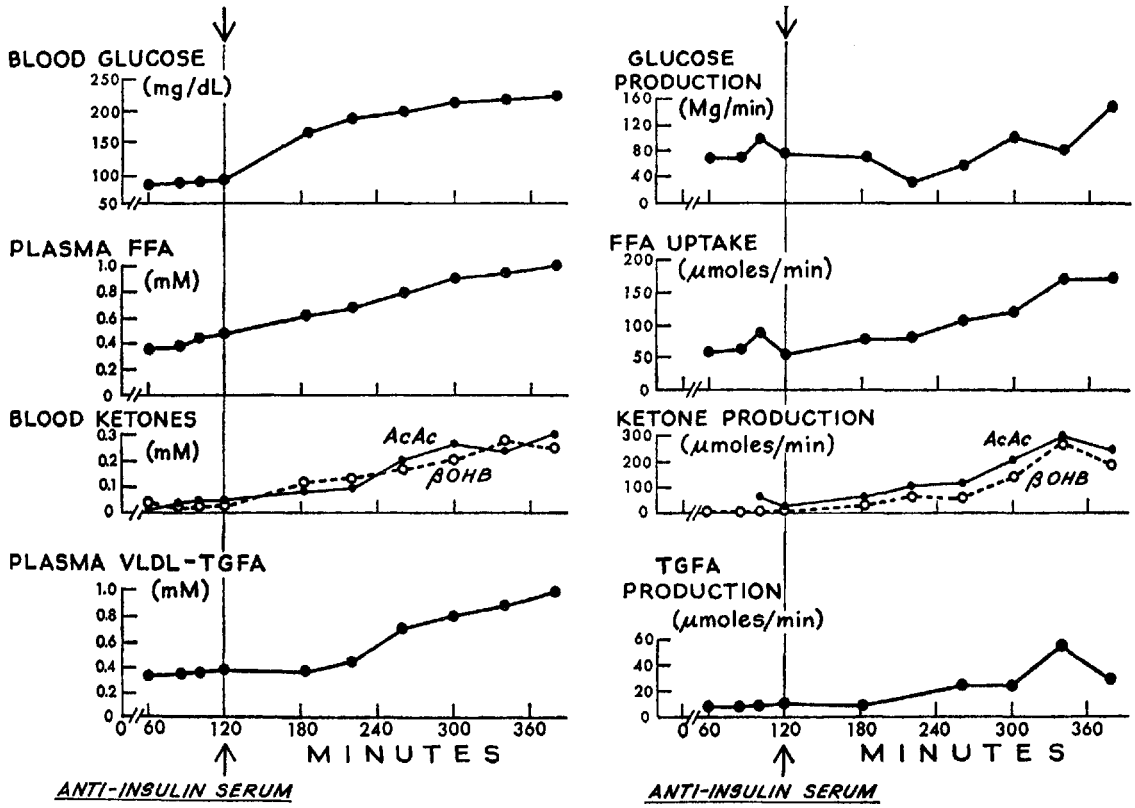

FIG. 1. Changes in concentration and hepatic transport of major metabolites before and after injection of anti-insulin serum in dog 68.

portal vein blood — concentration in arterial blood) × portal vein blood flow.

4. Average values for the control period were derived from four samples and for the AIS period from three samples obtained three to four hours after administration of AIS. Statistical significance of differences between the two periods was evaluated by paired *t* test.

RESULTS

Arterial concentration of metabolites (figure 1 and table 1)

Values for various metabolites did not vary systematically during the basal period and were in the accepted normal range. All concentrations increased after AIS. Changes in glucose, ketones, FFA and glycerol were apparent after sixty minutes, while increases in plasma TGFA occurred later (figure 1). Maximal changes were observed three to four hours after the administration of AIS. The magnitude of the hyperglycemia varied among animals and, for a given animal, the magnitude of change for various metabolites was variable. On the average, levels of FFA, glucose, glycerol and VLDL-TGFA increased twofold and ketone levels, fourfold. Increase in VLDL-TGFA, which is the fraction containing newly secreted triglyceride, accounted entirely for the increase observed in total TGFA of plasma.

Hepatic metabolism of FFA

Hepatic blood flow averaged 1,175 ml./min. during the control period and did not differ systematically after AIS (average 1,050 ml./min.). As shown in table 2, extraction fraction of palmitate-1-C-14 by the liver averaged 0.19 during the control period. After AIS, the extraction fraction was unchanged and the uptake of FFA by the liver paralleled the increase in concentration of FFA. Net transport and hepatic uptake of FFA increased almost twofold on the average and the liver took up about 40 per cent of transported FFA in both metabolic states. An average of 11 per cent of the 1-C-14-palmitate removed by the liver during the control period appeared in hepatic venous triglycerides. This fraction increased to 26 per cent after AIS. The net inflow transport from liver of TGFA derived from FFA was conse-

TABLE 1

Arterial concentration of metabolites*

Dog no.	Weight	Plasma FFA	Plasma TGFA	Plasma VLDL-TGFA	Blood AcAc	Blood βOHB	Blood Glycerol	Blood Glucose
	kg.	mM	mM	mM	mM	mM	mM	mg./dL
61	18	0.48	1.27	—	0.074	0.060	0.047	56
		0.56	1.22	—	0.109	0.097	0.077	98
63	22	0.36	1.42	—	0.024	0.032	0.046	90
		0.69	1.75	—	0.046	0.067	0.118	174
66	23	0.38	1.11	0.40	0.050	0.030	0.060	87
		0.85	1.70	0.95	0.300	0.220	0.131	227
68	26	0.42	—	0.37	0.047	0.030	—	90
		0.97	—	0.90	0.272	0.244	—	219
69	19	0.20	1.20	0.71	—	0.040	0.015	82
		0.45	2.25	1.73	0.075	0.130	0.047	100
71	22	0.32	0.67	0.27	0.015	0.025	0.043	102
		0.76	0.88	0.43	0.057	0.081	0.130	210
Mean ± S.E.M.	22	0.36 ± 0.04	1.13 ± 0.13	0.44 ± 0.10	0.042 ± 0.010	0.036 ± 0.005	0.042 ± 0.007	84 ± 6
		0.71 ± 0.08	1.56 ± 0.24	1.00 ± 0.27	0.143 ± 0.046	0.140 ± 0.040	0.101 ± 0.016	171 ± 23
p		<0.01	>0.05	<0.05	>0.05	<0.05	<0.01	<0.01

*In this and other tables: above = mean of four values obtained during the second hour of control period; below = mean of three values obtained during the third or fourth hour following administration of AIS.

quently increased, averaging 0.26 μmoles/min.•kg. during the control period and 1.21 μmoles/min.•kg. after AIS (table 2). During the last hour of the experiments (table 3), the specific activity (SA) of arterial VLDL-TGFA amounted to 76, 81 and 92 per cent of that of HV FFA (equivalent to that entering the liver) in three of the four dogs in which these measurements were made (dogs 66, 68 and 71). In these animals, most of the VLDL-TGFA was, therefore, derived from circulating FFA. In the remaining animal (dog 69) SA

TABLE 2

Metabolism of FFA, TGFA and glycerol

Dog no.	FFA Net inflow transport	FFA Hepatic extraction fraction of C-14-palmitate	FFA Hepatic uptake	FFA Hepatic uptake / Net inflow transport	TGFA Production of C-14-TGFA / Uptake of C-14-FFA	TGFA Net inflow transport	Glycerol Hepatic uptake	Glycerol Hepatic extraction fraction
	μmoles/min. • kg.		μmoles/min. • kg.			μmoles/min. • kg.	μmoles/min. • kg.	
61	7.6	0.16	1.2	0.16	0.08	0.10	0.94	0.72
	6.1	0.16	2.0	0.33	0.31	0.63	2.92	0.79
63	5.5	0.20	2.2	0.40	0.03	0.06	—	—
	11.6	0.26	5.3	0.46	0.24	1.27	2.58	0.46
66	7.5	0.30	2.8	0.37	0.18	0.49	1.73	0.64
	13.1	0.24	5.1	0.39	0.22	1.12	3.88	0.66
68	10.3	0.18	3.2	0.31	0.13	0.42	—	—
	17.2	0.18	5.5	0.32	0.22	1.22	—	—
69	5.4	0.12	1.6	0.30	0.16	0.25	0.66	0.59
	8.4	0.18	4.8	0.57	0.29	1.40	1.49	0.48
71	6.1	0.21	3.4	0.56	0.07	0.24	1.29	0.44
	10.8	0.24	5.2	0.48	0.31	1.61	2.40	0.47
Mean ± S.E.M.	7.1 ± 0.75	0.19 ± 0.03	2.4 ± 0.36	0.35 ± 0.05	0.11 ± 0.02	0.26 ± 0.07	1.15 ± 0.23	0.60 ± 0.06
	11.2 ± 1.57	0.20 ± 0.02	4.6 ± 0.57	0.42 ± 0.04	0.26 ± 0.02	1.21 ± 0.14	2.65 ± 0.39	0.57 ± 0.07
p	<0.05	>0.05	<0.01	>0.05	<0.01	<0.001	<0.02	>0.05

TABLE 3

Specific activities of fatty acids at termination of experiments

Dog no.	Minutes	Hepatic venous FFA*	Arterial VLDL-TGFA	Arterial plasma TGFA	Hepatic TGFA
61	307	1,860	—	610	330
63	360	1,718	—	963	775
66	380	1,505	1,150	768	760
68	370	959	780	—	665
69	380	3,700	1,640	1,540	1,185
71	285	1,078	987	640	852

* Mean values (cpm/μmole) during last hour of study.

in VLDL-TGFA was only 44 per cent that of HV FFA. SA of plasma total TGFA was always lower than that of TGFA on the VLDL fraction. On the average, AIS caused a fourfold increase in production rate of ketones from the liver (table 4). Ketone production was equivalent to 25 per cent of the uptake of FFA during the control period and to 51 per cent after AIS. As discussed below, these values may overestimate the conversion of FFA to ketones. AcAc accounted for 67 per cent of total ketone production during the control period and 57 per cent after AIS. Approximately 25 per cent of C-14-FFA taken up by the liver was converted to C-14-O_2 during the control period; this fraction fell to an average of 14 per cent after AIS. As discussed later, these are minimum values for complete oxidation of FFA. On the assumption that all AcAc and βOHB produced was derived from FFA and that hepatic metabolism of palmitate and total FFA are equivalent, a mean of 71 per cent of palmitate was converted to secreted products while 39 per cent was stored in hepatic lipids during the period of study (table 5).

Hepatic metabolism of glucose and glycerol

After AIS, hepatic production of glucose increased in four of the six animals tested (table 4) and was unchanged in the remaining two. One of these two (dog 69) had a slight and the other (dog 71) a pronounced hyperglycemic response (table 1). Hepatic extraction fraction of glycerol was not modified by AIS and varied between 0.44 and 0.79. Glycerol uptake at the end of the AIS period was about twice that in the control period (table 2) as a result of the increase in glycerol concentration (table 1).

Extrahepatic splanchnic metabolism (tables 6 and 7)

The extrahepatic splanchnic region extracted about 10 per cent of the incoming 1-C-14-palmitate during both periods. Uptake of FFA doubled after AIS. Production rates did not differ systematically from rates of uptake and also doubled after AIS. Production rates of

TABLE 4

Hepatic production of glucose, ketones and C-14-O_2

Dog no.	Production of glucose	Production of ketones	Production of ketone carbon / Uptake of FFA carbon	Fraction of palmitate oxidized to CO_2
	mg./min. • kg.	μmoles/min. • kg.		
61	−0.2	2.07	0.41	—
	1.0	5.64	0.68	—
63	3.7	2.55	0.28	—
	7.2	7.85	0.35	—
66	2.0	2.09	0.18	0.34
	4.1	7.28	0.33	0.21
68	3.1	2.16	0.16	0.15
	4.2	15.75	0.68	0.07
69	7.4	2.30	0.35	0.30
	7.4	15.75	0.78	0.16
71	4.8	1.63	0.11	0.22
	4.7	5.15	0.24	0.11
Mean ± S.E.M.	3.5±1.1	2.13±0.13	0.25±0.05	0.25±0.04
	4.8±1.0	9.56±1.99	0.51±0.09	0.14±0.03
p	> 0.05	< 0.02	< 0.02	< 0.01

FFA were equivalent to about 20 per cent of net arterial transport of FFA during both periods. Fraction of 1-C-14-palmitate oxidized to CO_2 increased consistently after AIS in the three animals so studied. The extraction fraction of ketones tended to decrease slightly after AIS. However, in dog 66, it fell from 0.20 to 0.04. Relative to the hepatic production rate, arterial concentration rose considerably more in this animal than in the others (tables 1 and 4). On the average, extrahepatic splanchnic uptake of ketones was about 40 per cent of hepatic production before and 24 per cent after AIS. The frac-

TABLE 5

Recovery of palmitate-C-14 extracted by the liver

Dog no.	Hepatic content of TGFA	Fraction of palmitate-C-14 stored in hepatic lipids	Fraction of palmitate oxidized and secreted as VLDL-TGFA	Total per cent recovered
	μmoles/gm.			
61	31.9	0.65	0.69	134
63	7.7	0.24	0.51	75
66	18.5	0.38	0.75	113
68	9.2	0.39	0.69	108
69	13.8	0.28	1.06	134
71	9.1	0.37	0.56	93
Mean ± S.E.M.	15.0±2.8	0.39±0.06	0.71±0.08	109±8

TABLE 6

Extrahepatic splanchnic metabolism of FFA

Dog no.	Extraction fraction of palmitate-C-14	Uptake	Production	Production / Net inflow transport	Fraction of palmitate-C-14 oxidized to CO_2
		μmoles/min. • kg.	μmoles/min. • kg.		
61	0.09	0.56	0.45	0.06	—
	0.14	1.15	0.99	0.16	—
63	0.07	0.50	0.94	0.17	—
	0.07	1.12	2.42	0.21	—
66	0.05	0.35	0.35	0.05	—
	0.05	0.91	0.98	0.07	—
68	0.11	1.14	2.08	0.20	0.37
	0.11	2.77	3.26	0.19	0.45
69	0.14	1.31	1.77	0.33	0.18
	0.07	1.44	2.16	0.26	0.70
71	0.11	1.19	1.79	0.29	0.19
	0.14	2.48	3.32	0.31	0.46
Mean±S.E.M.	0.10±0.01	0.84±0.17	1.23±0.31	0.18±0.05	0.25±0.06
	0.10±0.02	1.65±0.32	2.19±0.42	0.20±0.03	0.54±0.08
p	>0.05	<0.02	<0.01	>0.05	>0.05

tional uptake of AcAc was about twice that of βOHB in both metabolic states. Net changes of glycerol were inconsistent in several animals during the course of the study, suggesting that uptake as well as release occurs in this region. On the average, there was net uptake with no systematic changes after AIS. Extraction fraction of glucose was decreased after induction of insulin deficiency. However, owing to the increased arterial concentration, absolute uptakes were maintained. Extrahepatic splanchnic uptake of glucose averaged 43 per cent of hepatic production before and 35 per cent after AIS.

DISCUSSION

Elevation in arterial concentrations of glucose, FFA, glycerol, ketones and TGFA have been observed repeatedly in animals treated with AIS.[4-7] Production of a fatty liver has also been described but this occurs slowly; three to four hours after AIS the livers of our animals had still a normal content of triglycerides.[8]

TABLE 7

Extrahepatic splanchnic metabolism of various metabolites

Dog no.	Ketones		Glycerol	Glucose	
	Extraction fraction	Uptake	Uptake	Extraction fraction	Uptake
		μmoles/min. • kg.	μmoles/min. • kg.		mg./min. • kg.
61	0.27	0.64	0.77	0.031	0.33
	0.28	1.17	0.93	0.027	0.68
63	0.29	0.80	0.67	0.022	1.00
	0.24	1.04	0.43	0.016	1.04
66	0.20	0.54	−0.06	0.026	0.72
	0.04	0.78	−0.59	0.012	0.88
68	0.34	1.07	—	0.050	1.85
	0.28	5.46	—	0.023	1.87
69	0.34	1.47	0.37	0.052	2.75
	0.28	3.81	0.00	0.043	2.80
71	0.30	0.65	0.05	0.043	2.33
	0.27	1.41	0.48	0.035	2.79
Mean ± S.E.M.	0.29±0.02	0.86±0.14	0.36±0.16	0.037±0.005	1.50±0.39
	0.23±0.04	2.28±0.78	0.25±0.26	0.026±0.005	1.68±0.39
p	<0.05	>0.05	>0.05	<0.05	>0.05

Dog 61 responded only slightly to AIS. This animal was also the most "fasted" one. His food intake was low during the few days before the experiment, his basal glycemia was the lowest and his plasma concentration of FFA and ketones the highest of all the animals studied (table 1). He also had the most triglycerides in his liver at the end of the study. This absence of response to AIS in some animals in relation to duration of fasting has been observed by several authors.[6,7] Among the other animals, dog 69 had a small hyperglycemic response but changes in FFA, ketones and blood triglycerides were of average magnitude. In this animal, a rather high basal glucose production did not increase after AIS (table 4).

Possible modifications of the abdominal circulation resulting from the ligation of the vena cava have to be taken into account in understanding our experimental results: (1) Hepatic blood flows were higher than those usually reported, probably as a consequence of the development of caval-portal collaterals. This might have increased the fraction of the hepatic blood flow derived from the portal vein by comparison with that derived from the hepatic artery. However, for all the substrates studied (excepting extrahepatic production of FFA), contribution of the liver to uptake or production considerably exceeded that of the extrahepatic region. Therefore, differing estimates of distribution of inflowing blood to the liver have only minor influence on calculated hepatic fluxes. For example, were the fraction of hepatic blood flow contributed by the portal vein 95 per cent rather than 80 per cent, our estimates of hepatic extraction of palmitate-C-14 would decrease by less than 10 per cent. (2) We assumed in our calculations that blood drawn from the inferior vena cava above the diaphragm represented HV blood. However, some contamination by collateral circulation through newly opened diaphragmatic veins might have existed but is likely to be small; indeed the extraction fraction of glycerol (which is mainly taken up by the liver) averaged 0.60 which is only 10 per cent lower than values observed in intact dogs.[2] (3) The region drained by the portal vein may not consist entirely of extrahepatic splanchnic organs. Part of the blood draining the hindquarters probably contributed to portal blood through caval-portal collaterals. Therefore, rates of production or uptake of metabolites in the extrahepatic splanchnic region are likely to be overestimated during both periods of the experiments.

These modifications in blood distribution should not preclude a correct interpretation of the effects of AIS on splanchnic metabolism of FFA since each dog served as its own control.

Hepatic metabolism of FFA was greatly modified after AIS. Plasma concentrations of FFA increased twofold, leading to a proportional increase in hepatic uptake. Concomitantly, there was a twofold increase in the fraction of C-14-palmitate released as C-14-TGFA, so that the estimated production rate of TGFA was increased fourfold. These calculations can be considered only approximations, however. First, the fractional conversion of FFA to TGFA and production rate of VLDL-TGFA during the control period may have been underestimated somewhat because complete equilibration of pools between precursor and product had not yet occurred.[9] Second, the production rates of VLDL-TGFA might also be underestimated after AIS if secreted VLDL-TGFA were not derived entirely from circulating FFA. This could be the case, for instance, if lack of insulin stimulated an hepatic lipase so that the SA of the hepatic fatty acyl CoA pool was lower than that of entering FFA. In accord with this possibility, the average SA of VLDL-TGFA was only 74 per cent that of HV FFA at the end of the study (table 3) while that of hepatic TGFA was lower still. Finally, it should be added that comparison of SA of precursor (FFA) and product (VLDL-TGFA) cannot be made precisely since SA of FFA fell during the AIS period as a result of enhanced lipid mobilization from adipose tissue. However, from the substantial increase in both hepatic uptake of FFA and fractional conversion of FFA to VLDL-TGFA, there can be little doubt that the livers of our dogs secreted considerably more VLDL-TGFA after injection of AIS.

The situation was quite different in the chronically diabetic dogs studied by Basso and Havel.[2] In these animals, the fraction of C-14-palmitate released as TGFA was extremely small and calculated production rates of TGFA were lower than in normal controls. In both acutely and chronically diabetic animals, about 60 per cent of C-14-palmitate removed by the liver was converted into lipids (stored + secreted). This indicates that esterification processes are equally active in both types of diabetes. Thus, it appears that deficiency of insulin gradually leads to impairment of synthesis and secretion of TGFA in VLDL. This might be caused by inhibition of apolipoprotein synthesis as suggested by the studies of Wilcox et al. with perfused livers from alloxan diabetic rats.[10] The reason for the apparent increase in fractional conversion of FFA into VLDL-TGFA after AIS is not evident. As indicated above,

this apparent difference may be related in part to variable equilibration between hepatic "storage" pools of TGFA and the precursor pool leading to synthesis of VLDL-TGFA. Using an experimental approach similar to ours in studying hepatic metabolism of FFA, Spitzer et al. observed that two days after alloxanization, livers of diabetic and control dogs converted similar fractions of FFA into circulating TGFA.[11]

Our results indicate that the hyperlipemia observed in AIS-treated dogs was at least partly the result of increased production of VLDL. An approximately fivefold increase in net inflow transport after AIS was accompanied by a two- to threefold increase in concentration of VLDL-TGFA. Although levels of VLDL-TGFA were still rising slowly, the data suggest that impaired peripheral uptake of TGFA did not contribute substantially to the hyperlipemia during the period of study.

Oxidative metabolism of FFA was also markedly influenced by acute insulin deficiency. By comparison with the control period, AIS inhibited hepatic fractional conversion of FFA to CO_2 and enhanced the production of ketones. As discussed elsewhere,[2] values for hepatic conversion of FFA to CO_2 are minimal estimates because some C-14 in acetyl coenzyme A entering the tricarboxylic acid cycle is diverted into the pathway of gluconeogenesis by a process of isotopic exchange.[12] This process may be enhanced after AIS because of increased gluconeogenesis. Another problem arises in estimating conversion of FFA to ketones. Most ketones are probably derived from circulating FFA during the control period.[2] However, this may not be the case after AIS. Indeed, in one animal tested in this regard (dog 69), the SA of carbon atoms of AcAc at the end of the experiment was only 40 per cent that of HV FFA. This dog was also unusual in that the SA of VLDL-TGFA was much lower than that of HV FFA. Nevertheless as with VLDL-TGFA, some of the ketones secreted during the AIS period are probably derived from sources other than plasma FFA. Thus, the increase in fractional conversion of FFA to ketones after AIS is probably somewhat smaller than the twofold increase suggested from the data in table 4. A similar conclusion was reached in studies of chronically diabetic dogs.[2] This rapidly occurring change in hepatic metabolism of FFA may reflect the operation of a mechanism for disposal of FFA which do not undergo esterification in such a way as to maintain production of energy (ATP formation) in the normal range when influx of FFA increases. Such a regulatory mechanism has been suggested by Mayes and Felts from results with livers perfused with increasing amounts of FFA.[13] In this regard, it should be mentioned that although fractional conversion of FFA to CO_2 was reduced after AIS, absolute production of CO_2 from FFA remained unchanged.

Evaluation of changes in metabolism of FFA in the extrahepatic splanchnic region after AIS must take into account the fact that this area is composed of a variety of tissues, including adipose tissue. The simultaneous increases in uptake and production of FFA should be interpreted on this basis. It should also be noted that, for reasons outlined before, rates of production or uptake of metabolites in the extrahepatic splanchnic region are likely to be overestimated during both periods of the experiments. A rise in fractional oxidation of FFA, as observed here, has also been observed by Spitzer et al.[11] in alloxan diabetic dogs but was not apparent in the pancreatectomized animals studied by Basso and Havel.[2] An interesting observation was the observed decrease in extraction fraction of ketones (table 7). This could result from two different factors: the increase in arterial concentration of ketones and the insulin deficiency per se. We have observed that the rise in ketone concentration produced by infusions of AcAc into normal animals is accompanied by reduction of the extraction fraction in various areas, including the extrahepatic splanchnic bed.[14] We also found that, for a given ketone concentration, pancreatectomized dogs had a lower rate of transport than normal dogs. It is possible that hyperketonemia after AIS was to some extent the result of decreased utilization. However, with the exception of dog 66, the fall in extraction fraction was much less marked in the present study than in chronic insulin deficiency. In the three chronically diabetic dogs studied by Basso and Havel,[2] the average ketone production rate was 10.7 μmoles/min. • kg. and corresponding concentration was 0.9 μmoles/ml., whereas in our AIS-treated animals, similar ketone production rates were observed (average 9.6 μmoles/min. • kg.) with much lower concentration (0.3 μmoles/ml.).

The extraction fraction for glucose in the extrahepatic splanchnic region fell after AIS, indicating a reduction in peripheral glucose uptake in insulin-deficient animals. This is further substantiated by the following data: (1) two out of the six dogs exhibited no change in hepatic glucose output and nevertheless became hyperglycemic; (2) in most animals (as in the experiment shown in figure 1), the level of glucose began to rise before an increase in hepatic glucose output could be detected. Thus, both an increase in hepatic glucose production and inhibition of peripheral removal participate

in the hyperglycemic response after AIS. This is in agreement with earlier findings of Franckson et al.[7]

In conclusion, dogs rendered abruptly insulin-deficient by injection of AIS differ from chronically diabetic animals with respect to hepatic metabolism of FFA. In both cases, hepatic uptake of FFA is increased and fractional conversion to ketones is stimulated. Similar amounts of FFA taken up are esterified to TGFA but in chronically diabetic dogs their release as VLDL-TGFA is strongly inhibited whereas it is enhanced in acutely insulin-deficient animals. The mechanism of the hyperlipemia observed in these stages of uncontrolled diabetes is thus entirely different. While it is the result of decreased peripheral removal in chronically diabetic dogs, increased hepatic production appears to be the major causative factor in animals given AIS.

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