Effects of Fat Mobilization on Liver Acetate Metabolism

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SUMMARY

In insulin deficiency, release of FFA from adipose tissue and increased mobilization of lipids to the liver are followed by excessive ketone body production and a reduction in the incorporation of C-14 from labeled acetate into fatty acids. These changes in liver metabolism may not be related to insulin lack per se, but rather due to a direct effect of an accumulation of lipids or acyl-CoA esters in the tissue. The effect of lipid mobilization was investigated using dichloroisoproterenol (DCI), an adrenergic blocking agent, which has been shown to raise the plasma FFA level in anesthetized dogs with no significant effect on the blood sugar and no apparent effect on plasma insulin levels. In vitro studies have shown that DCI does stimulate FFA release from adipose tissue of the rat or the dog, at a concentration of 10^{-5} molar, but will block the release normally produced by catecholamines if the concentration of DCI is 10^{-3} molar. The rapid and sustained elevation of plasma FFA level obtained in the rat following I.P. injection of DCI (50 mg./kg.) over six hours led to an increase in total liver lipids by about 15 mg./gm. liver weight, a 50 per cent increase in ketone production by liver slices and a rise in blood ketone level. Fatty acid synthesis from 2-C-14 sodium acetate was reduced to 25 per cent of the control.

An injection of insulin given to the DCI-treated rats one hour before sacrifice, and sufficient to produce hypoglycemia, reduced the plasma FFA level to normal, but did not influence incorporation of labeled acetate into fatty acids by the liver slices, or the blood ketone level.

The results suggest that mobilization of lipids is sufficient to produce changes in liver metabolism similar to those occurring in acute insulin deficiency. DIABETES *15*: 346-50, May, 1966.

The impaired lipid synthesis in diabetes mellitus and its alleviation by the administration of insulin to diabetic animals were first demonstrated about thirty years ago.[1,2] Spiro, Ashmore, and Hastings[3] showed later that liver slices obtained from alloxan-diabetic rats following insulin withdrawal, incorporate precursors into fatty acids at a reduced rate, and Renold et al.[4] demonstrated that lipogenesis was restored to normal twelve to twenty-four hours after the administration of insulin. The delay in the correction of lipogenesis following insulin treatment suggests that hepatic fatty acid synthesis is not directly controlled by insulin, but rather by some secondary effect of that hormone.

More recent experimental work indicates that increased mobilization of lipids to the liver, followed by an accumulation of the long-chain fatty acid esters of Co-enzyme A within the liver cells, may limit the rate of fatty acid synthesis and, at the same time, lead to an increased production of ketone bodies.[5,6] Accelerated release of fatty acids from peripheral adipose tissue with subsequent elevation of plasma free fatty acid (FFA) levels occurs in starvation, forced feeding with fat, and, more particularly, during the insulin deficiency of diabetes mellitus.[7] The synthesis of fatty acids within the liver may be directly controlled by the level of fatty acid-Co-enzyme A ester. Bortz and Lynen[8] have shown that the activity of acetyl-CoA carboxylase, an enzyme involved in fatty acid synthesis, is strongly inhibited by the in vitro addition of these esters. This inhibition appears to be the result of competition with acetyl-CoA for the active enzyme site. Wieland and Weiss,[9] and Tubbs[10] have further demonstrated an inhibitory effect of fatty acid-Co-enzyme A esters on the enzyme citrate-synthetase, which controls the condensation of oxaloacetate and acetyl-CoA to form citrate. An accumulation of fatty acid-Co-enzyme A esters might, therefore, lead to a reduction in the amount of acetyl-CoA oxidized by the citric acid cycle. As a result of both these inhibitory effects of the CoA-esters, the latter could divert the metabolism of acetyl-CoA toward ketone body formation, and reduce the rate of fatty acid synthesis.

Tarrant and Ashmore[11] followed the changes in glucose and lipid metabolism which arose following insulin withdrawal from alloxan-diabetic rats that had been controlled by insulin over a period of two weeks. The rise

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in plasma FFA ran parallel to the rise in blood glucose, and these were followed by elevation of liver lipids and blood ketones. A defect in lipogenesis by liver slices taken from rats made acutely insulin deficient by the injection of anti-insulin serum was demonstrated by Sweeney and Ashmore.[12] The serum was injected intravenously ninety minutes before the animals were killed, and the synthesis of long-chain fatty acids from C-14-labeled acetate was reduced to 25 per cent of the level reached in controls.

Insulin has been shown, under certain circumstances, to have a direct effect on hepatic lipogenesis in vitro,[13] but it may exert most of its effect indirectly by preventing release of fatty acids from peripheral adipose tissues.[14] Hormonal factors known to stimulate release of fatty acids from adipose tissue include epinephrine, ACTH, glucagon, growth hormone, thyrotropin, follicle stimulating hormone, and adrenal glucocorticoids.[15] These substances appear to act by accelerating triglyceride breakdown. Stimulation of fatty acid release both in vivo and in vitro can be reduced or even completely inhibited by insulin and adrenergic blocking agents. Ergotamine,[16] phenoxybenzamine,[17] dibenamine,[18] and phentolamine[19] have been shown to be effective, with some species variation.

Dichloroisoproterenol (DCI) is an adrenergic blocking agent antagonizing the action of epinephrine on blood pressure, rabbit ileum, and guinea pig tracheal chains.[20] It will also block the in vitro effect of epinephrine on fatty acid release from rat epididymal fat pads at a concentration of 10^{-3} molar.[21] However, at lower concentrations, DCI alone stimulates the release of fatty acids from adipose tissue, a maximum effect being observed at 4×10^{-5} molar. The presence of glucose in the incubation medium reduced the effect, probably by making available α-glycerophosphate. DCI, therefore, demonstrates some sympathomimetic effects, in addition to behaving as an adrenergic blocking agent. Fleming and Hawkins[22] termed this phenomenon "competitive dualism." When injected intravenously into anaesthetized dogs, no effect, or a slight increase, was obtained on the blood sugar, but a marked increase in plasma FFA occurred.[23] Figure 1 indicates that a rise in plasma FFA follows intraperitoneal injection of DCI (20 mg./kg.) into rats and is sustained for at least three hours. When administered to rats in doses up to 100 mg./kg., DCI is a potent lipolytic agent, giving a prolonged elevation of plasma FFA without any effect on blood glucose.

In the present study we have made use of the fat

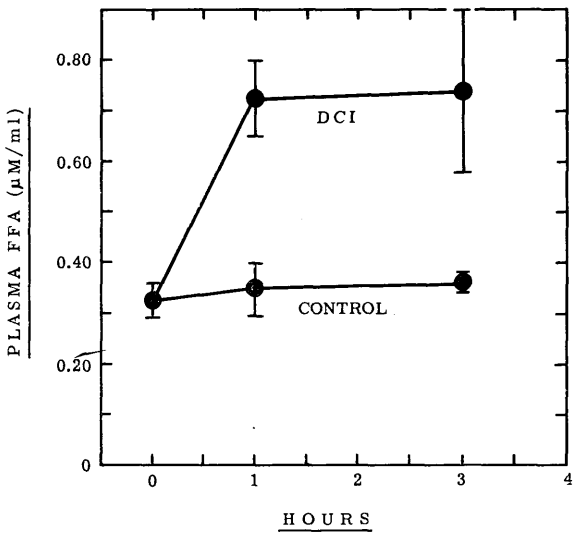

FIG. 1. Mean concentration (± S.D.) of free fatty acids in plasma of rats injected I.P. at zero time with DCI (20 mg./kg.) or saline (controls).

mobilizing property of DCI as an experimental tool, and the effects of an accumulation of lipids in the liver were observed by incubating liver slices with acetate-2-C-14-C. Modification of the action of DCI was attempted with insulin.

METHODS AND MATERIALS

The experimental studies were carried out on 200 to 400 gm. male albino rats of the Wistar strain, maintained on a 65 per cent carbohydrate diet and water ad libitum for at least three days prior to the experiments. DCI, dissolved in saline, was injected intraperitoneally in a dose of 50 mg./kg. in three divided doses during the six hours before sacrifice. A group of rats received, in addition, five units of soluble insulin subcutaneously one hour before they were killed. Control animals were injected with saline. The rats were killed by stunning and exsanguination, and the livers were rapidly removed and chilled before slicing. Blood taken at the time of sacrifice was analyzed for glucose,[24] FFA,[25] and ketones.[26] Total lipid[27] and fatty acid[4] contents of the livers were determined and 0.5 gm. was sliced with a Stadie-Riggs hand microtome. The liver slices were incubated for ninety minutes at 37° C. in 5 ml. Ringer-bicarbonate which contained 5 mM. sodium acetate-2-C-14. Following incubation, the medium was analyzed for ketone release[26] and C-14-O_2 production,[28] and the incorporation of C-14 into tissue fatty acids was estimated.[4]

TABLE 1

Levels of blood glucose, plasma FFA, blood ketones, and liver lipids six hours after injection of saline or DCI

Injection	Blood glucose mg./100 ml.	Plasma FFA μmoles/ml.	Blood ketones μmoles/ml.	Total liver lipids mg./gm. wet weight	Long-chain fatty acids mg./gm. wet weight
Saline	74.3±2.67	0.28±0.03	0.36±0.06	29.9±0.77	20.8±1.03
DCI	74.0±1.63	0.64±0.1	0.75±0.04	46.0±1.96	31.4±0.72

Values are means of nine observations ± S. E.

RESULTS

The results of the blood analyses and liver lipids are shown in table 1. There was no significant difference between the levels of blood glucose in the DCI-treated rats and the controls, but the plasma FFA level rose to more than twice the control levels after the injection of DCI ($p < 0.005$), demonstrating the potent lipolytic effect of DCI at the dose level used. The treated rats also had a twofold increase in the blood ketone level ($p < 0.001$). During the six-hour period between the first injection and sacrifice, the total liver lipids increased in the DCI-treated animals by a mean of 16 mg. per gm. of liver ($p < 0.001$), and there was a 10 mg. per gm. increase in the liver fatty acids ($p < 0.001$). Administration of DCI led to a 43 per cent increase in ketone production by liver slices, compared to the control slices ($p < 0.005$) (table 2). CO_2 production from acetate was reduced to 70 per cent ($p < 0.001$). The incorporation of C-14 from labeled acetate into fatty acids by the liver slices from DCI-treated rats was markedly depressed to 25 per cent of that observed in controls ($p < 0.001$).

During the hour between insulin injection and death, the plasma FFA level returned to normal (table 3), but the blood ketones were unaffected. Incorporation of C-14 from acetate into fatty acids and C-14-O_2 production from acetate-2-C-14 were not altered by the administration of insulin.

TABLE 2

Metabolism of liver slices from rats killed six hours after injection of saline or DCI

Injection	Incorporation of C-14 from acetate-2-C-14 into fatty acids cpm./gm. wet weight/ 90 minutes	C-14-O_2 production from acetate 2-C-14 cpm./gm. wet weight/ 90 minutes	Ketone release μmoles/gm. wet weight/ 90 minutes
Saline	26,300±1,700	16,600±640	4.48±0.14
DCI	7,400±980	11,600±290	6.41±0.53

Values are means of twelve observations ± S. E.

DISCUSSION

The fat-mobilizing property of dichloroisoproterenol is confirmed by these experiments, and, from the results, there appears to be some similarity between the effects of fatty acid mobilization following the administration of this compound, and the development of diabetic acidosis in acute insulin deficiency. In both situations accelerated release of FFA from adipose tissue, with

TABLE 3

Effect of insulin on DCI-treated rats (five units given subcutaneously one hour before sacrifice)

			Metabolism of liver slices	
Injection	Plasma FFA μmoles/ml.	Blood ketones μmoles/ml.	C-14-O_2 production from acetate-2-C-14 cpm./gm. wet weight/ 90 minutes	Incorporation of C-14 from acetate-2-C-14 into fatty acids cpm./gm. wet weight/ 90 minutes
DCI	0.65±0.06	0.69±0.1	10,500±1,100	3,400±1,600
DCI + insulin	0.26±0.04	0.69±0.09	11,900±1,400	2,500±460

Values are means of twelve observations ± S. E.

subsequent elevation of the plasma levels, is followed by increased fat deposition in the liver. In the diabetic animal, this accumulation of lipids is associated with an increased production of ketone bodies. Our results indicate that a similar process has occurred in the DCI-treated rats, though the degree of ketosis is not quite as marked as that occurring in the diabetic. The reduction of incorporation of C-14 from labeled acetate into liver fatty acids to one quarter of the control level after six hours is the same as has been shown to occur ninety minutes following the production of acute insulin deficiency by the administration of anti-insulin serum. Alteration in the size of acetyl-CoA pool in these livers could conceivably account for some apparent reduction. Similarly, the reduced C-14-O_2 production by the liver slices from DCI-treated animals could be a dilution effect, rather than the result of inhibition of acetyl-CoA condensation with oxaloacetate, this inhibition having been produced by an accumulation of fatty acid-CoA esters.[9] The sizes of the fatty acid-CoA and acetyl-CoA pools are not yet known, though various estimates have been made. Wieland and Weiss estimated the level of fatty acid-CoA and acetyl-CoA esters as about 15 mμ-moles each per gm. wet weight of normal rat liver,[29] whereas Tubbs and Garland showed a marked increase of fatty acid-CoA esters to 180 mμmoles per gm. wet weight in livers from rats fasted fourteen to forty-eight hours.[6] Until there is a precise method for measuring these substances and their rate of turnover intracellularly under physiological and experimental conditions, conclusions concerning alterations in pool size must be speculative. A direct effect of DCI on liver metabolism seems unlikely to account for the changes observed, since incubation of liver slices with DCI and acetate demonstrated no abnormalities of CO_2 production, ketone release, or fatty acid synthesis. The absence of any difference in blood glucose levels between the DCI-treated rats and controls suggests that DCI has no effect on plasma insulin levels.

The decrease in hepatic fatty acid synthesis and increased production of ketone bodies by the liver in insulin deficiency may not be related to an absence of insulin per se. Rapid mobilization of fatty acids from adipose tissue, which occurs in the absence of insulin, may be sufficient to produce these changes. Furthermore, livers from adrenalectomized diabetic animals demonstrate normal fatty acid synthesis, despite insulin lack.[30] However, the exact roles of insulin and lipid mobilization in this process are not completely clear. Hypophysectomized rats treated with anti-insulin serum do not show an increased plasma FFA level, but incorporation of acetate carbon into liver fatty acids is reduced.[12] That insulin may not be directly involved is indicated in the present experiments where insulin was also given to DCI-treated rats. Under these conditions a reduction to normal of the plasma FFA level was observed, whereas the blood ketone level was unchanged, and the incorporation of C-14 from acetate into fatty acids was unaltered. It seems likely that abnormal ketone and fatty acid production would continue until the liver had disposed of its accumulated fatty acid-Coenzyme A esters. These studies have demonstrated that the mobilization of lipids, brought about by DCI in the presence of apparently normal insulin levels, can produce metabolic changes, such as reduced incorporation of labeled acetate into fatty acids and increased production of ketones, which appear to be similar to those occurring in acute insulin deficiency.

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