Studies of the Metabolic Effects of Acute Insulin Deficiency

I. Mechanism of Impairment of Hepatic Fatty Acid and Protein Synthesis

R. K. Kalkhoff, M.D., and D. M. Kipnis, M.D., St. Louis

SUMMARY

(1) Acute insulin deficiency was produced in rats by the intravenous administration of potent anti-insulin guinea pig serum. Plasma glucose and nonesterified fatty acid (NEFA) levels rapidly increased to an excess of 350 mg. per cent and 1,200 μEq./L. respectively, and were kept elevated by repeated injections of anti-insulin serum.

(2) Hepatic fatty acid synthesis was determined by measuring the incorporation of acetate-1-C-14, acetyl-1-C-14 CoA, and malonyl-1,3-C-14 CoA into fatty acid by cell-free liver homogenates. Within 90 min. after the induction of acute insulin deficiency, acetate and acetyl CoA incorporation were markedly decreased, but malonyl CoA conversion to fatty acids was unaffected, indicating a block in lipogenesis at the acetyl CoA carboxylase step.

(3) The acute inhibition of carboxylase appeared to be a feedback mechanism since significant increases in hepatic tissue NEFA were observed when fatty acid synthesis decreased, and similar patterns of inhibition could be reproduced in normal rat liver homogenates by addition of either palmitate or palmityl CoA.

(4) Hepatic protein synthesis, measured by the incorporation of lysine-U-C-14 did not become impaired until three hours after the induction of acute insulin deficiency. The delayed onset of inhibition and the failure to observe impairment of protein synthesis following the in vitro addition of palmitate, suggest that the factor(s) responsible for impaired hepatic protein synthesis following acute insulin deprivation differ from those causing impaired lipogenesis under similar conditions.

(5) In contrast to previous reports using chronically insulin deprived animals (alloxan diabetes), the protein synthesizing activity of both soluble and microsomal fractions was impaired following acute insulin deprivation. DIABETES *15*:443-50, July, 1966.

Hepatic protein and fatty acid synthesis are markedly impaired in the insulin deficient animal.[1,2] It is not clear, however, whether these disturbances reflect a direct action of insulin on the mechanisms of protein and fatty acid synthesis, or are secondary to alterations in other metabolic pathways either in the liver or other insulin responsive tissues (e.g., adipose tissue).

The parenteral administration of potent insulin antiserum produces acute insulin deficiency in a wide variety of animal species.[3] Not only is one able to define accurately the time of onset of insulin deficiency with this procedure, but interpretation of the metabolic alterations resulting from hormone deficiency is not complicated by the toxic, stressful and nutritional disturbances associated with the two commonly used methods for producing insulin deficiency, namely, pancreatectomy and alloxan administration. In the present study, the sequential changes in hepatic fatty acid and protein synthesis were determined following induction of acute insulin deficiency by administration of anti-insulin serum.

The results to be presented indicate that hepatic lipogenesis is rapidly impaired following acute insulin deprivation. The defect in fatty acid synthesis reflects a feedback inhibition of acetyl CoA carboxylase activity secondary to the accumulation of long chain fatty acids and their acyl CoA derivatives. Depression of protein synthesis, on the other hand, requires a significantly longer period of hormone deprivation to become manifest and is not caused by the same factors responsible for the impairment of lipogenesis.

MATERIAL AND METHODS

Insulin antiserum. Guinea pig insulin antiserum (AIS) to porcine insulin was prepared by the method of Moloney and Coval.[4] The neutralizing capacity of the pooled insulin antiserum used in this study was 2 to 4 U. of porcine insulin per milliliter as determined by both the rat epididymal fat pad assay[5] and immunochemical titration.[6]

Animals. Fed male Sprague-Dawley rats (Holtzman Rat Company, Madison, Wis.), weighing 175-200 gm. and fed ad libitum were used in all studies. Sucrose (10

From the Department of Medicine, Washington University School of Medicine, St. Louis, Missouri.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/15/7/443/341074/15-7-443.pdf by guest on 23 May 2024

per cent) was added to the drinking water 24 hrs. before the animals were killed.

Chemicals. L-lysine-U-C-14 (sp. act., 51 mc./mmole) was purchased from the Volk Radiochemical Company, Chicago, Ill. Na acetate-1-C-14 (sp. act., 2 mc./mmole), acetyl-1-C-14 CoA (sp. act., 40 mc./mmole) and malonic acid-1-C-14 (sp. act., 1.57 mc./mmole) were obtained from New England Nuclear Corp., Boston, Mass. For initial studies with malonyl-1,3-C-14 CoA, a preparation (sp. act., 3.9 mc./mmole) was used (gift of Dr. S. Wakil, Duke University School of Medicine). For subsequent experiments, malonyl-1,3-C-14 CoA was synthesized in this laboratory from malonic acid-1-C-14 (sp. act., 0.63 mc./mmole) by the method of Brady.[7] Palmityl CoA, prepared by the method of Kornberg and Pricer,[8] was a gift of Dr. R. Bressler, Duke University School of Medicine.

Experimental procedures. Two rats, matched in weight ($\pm$ 5 gm.), were used in each experiment. One rat was injected intravenously in the tail vein with 2 ml. anti-insulin serum every 90 min., the number of injections depending upon the duration of the study; the other animal served as the control and received nonimmune guinea pig serum in the same dosage schedule. At the termination of the experiment, the animals were placed under light ether anesthesia, the abdomen opened, a lobe of liver isolated with a cup fashioned with aluminum foil and frozen in situ with Freon-12 previously cooled to —150° C. in liquid nitrogen.[9] The frozen lobe was excised and stored at —70° C. for subsequent determination of tissue nonesterified fatty acids (NEFA). Glycolytic and Krebs cycle intermediates and pyridine nucleotides were also measured, and the results of these studies are reported in a separate paper.[10] After obtaining a blood sample from the inferior vena cava for determination of plasma glucose and nonesterified fatty acids, the remainder of the liver was removed and homogenized in three volumes of ice-cold 0.3 M sucrose-phosphate buffer, *p*H 7.6 as described by Zamecnik.[11] Homogenization and all subsequent manipulations of tissue extracts were carried out at 4° C. Most of the experiments in this study were done with 800 x g supernatant fractions. Mitochondria-free preparations were obtained by centrifuging at 10,000 x g for 20 min.

Fatty acid synthesis was determined by measuring the incorporation of acetate-1-C-14, acetyl-1-C-14 CoA and malonyl-1,3-C-14 CoA into fatty acids by liver supernatant fractions incubated for 2 hrs. in air at 38° C. The incubation mixture contained one milliliter of liver homogenate, 45 μmoles $KHCO_3$, 1.6 μmoles TPN, 32 μmoles K citrate, and 3.2 μmoles Na acetate made up to a final volume of 1.6 ml. Radioactive precursors were added in the following amounts per ml. incubation medium: 0.26 μmole acetate-1-C-14 and 0.05 μmole acetyl-1-C-14 CoA. Since two preparations of malonyl-1,3-C-14 CoA with different specific activities were used in this study, the amounts used will be recorded in the description of each experiment. The incubation was stopped in 2 hrs. by the addition of alcoholic KOH and followed by hydrolysis, acidification and hexane extraction.[12] Extracts were evaporated to dryness, dissolved in 1 ml. of hexane and duplicate 0.2 ml. aliquots transferred to planchets, dried and counted in a micromil gas flow counter. Results are expressed as cpm in total hexane extractable lipid.

Protein synthesis was measured by determining the incorporation of 0.04 μmole lysine-U-C-14 into protein using the same incubation mixture described above except for the exclusion of K citrate and Na acetate. Incubations were carried out at 38° C. in room air and stopped after one hour by the addition of 10 per cent cold trichloracetic acid. The protein fraction was repeatedly washed and dried as previously described.[13] Between 4 to 6 mg. of dried protein powder was dissolved in one milliliter of Hyamine, to which was added 10 ml. of scintillation liquid,* and assayed for radioactivity in a liquid scintillation counter. Quenching was corrected with internal standards. Results are expressed as cpm./mg. protein.

Two procedures were used for the addition of Na palmitate to the liver incubation mixture. One method involved the extraction of pre-existing nonesterified fatty acids (NEFA) from human serum albumin by the isooctane extraction procedure of Goodman[14]; a 15 per cent solution of this albumin preparation in Krebs-Ringer-phosphate buffer had a NEFA concentration of 109 μmoles/L. (low NEFA albumin). A portion of this preparation was complexed with Na palmitate according to the procedure of Bowman[15] and had a NEFA concentration of 6,000 μEq./L. as a 15 per cent solution (high NEFA albumin). The second procedure was to dissolve the Na palmitate in absolute alcohol in a concentration of 0.16 M. Aliquots of this solution (20 μl) were then added to the incubation mixtures; identical quantities of absolute alcohol were added to the control flasks.

---

*Five grams of PPO (2,5-Diphenyloxazole) and 300 mg. of dimethyl POPOP 1,4-bix-2(4-methyl-5-phenyloxazolyl)-benzene dissolved in one liter of toluene.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/15/7/443/341074/15-7-443.pdf by guest on 23 May 2024

Chemical determinations. Blood glucose was determined by the Somogyi-Nelson procedure,[16] plasma nonesterified fatty acids by the colorimetric method of Duncombe,[17] using the Dole extraction procedure,[18] and protein by the method of Lowry.[19] Frozen liver samples were pulverized in liquid nitrogen at —150° C., and lipid extracted according to the method of Dole.[18] The NEFA fraction was separated by thin layer chromatography on silica gel using the following solvent system: heptane, ethyl ether, methanol and acetic acid (90:20: 3:2). NEFA fractions were eluted with chloroform and measured colorimetrically.[17]

RESULTS

Effect of guinea pig anti-insulin serum (AIS) on plasma glucose and nonesterified fatty acids (NEFA). The plasma glucose and NEFA levels increased rapidly following a single injection of 2 ml. of anti-insulin serum, reaching maximum levels within 90 min. and returning slowly to baseline values over the next three hours. To maintain absolute insulin deficiency for longer periods, immune serum was repeatedly injected intravenously at 90 min. intervals. Under these conditions, plasma glucose remained above 400 mg. per 100 ml. and plasma NEFA in excess of 1,000 μEq./L. throughout the experimental period (figure 1). Control rats receiving nonimmune guinea pig serum in a similar dosage schedule showed no significant change in plasma glucose or NEFA level from baseline values.

Time of appearance of impaired hepatic protein and fatty acid synthesis following administration of AIS. Ninety minutes after the injection of AIS, fatty acid synthesis, measured by acetate-C-14 incorporation into fatty acids (figure 2) was less than 30 per cent (4,050 ± 1,010 cpm) of control values (14,118 ± 2,314 cpm). In contrast to this striking depression, protein synthesis in AIS-treated rats at this time interval (563 ± 97 cpm/mg.) was not significantly different from the control group (596 ± 187 cpm/mg.). Three hours after the induction of acute insulin deficiency, however, both fatty acid and protein synthesis were markedly impaired. Acetate-1-C-14 incorporation was 20 per cent (3,336 ± 720 cpm) of control levels and lysine-U-C-14 incorporation was less than 33 per cent (222 ± 42 cpm/mg.) of baseline (672 ± 169 cpm/mg.). Similar results with respect to fatty acid synthesis were also obtained with 10,000 x g supernatant fractions (table 1).

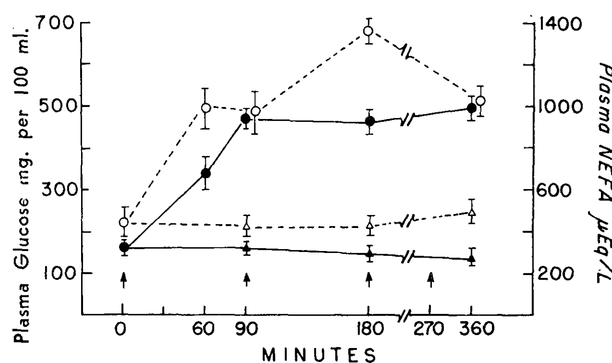

FIG. 1. The effect of guinea pig anti-insulin serum (AIS) on plasma glucose and NEFA levels in the fed rat. AIS (2 ml.) was injected intravenously at 90 min. intervals (indicated by arrows); control rats received nonimmune guinea pig serum in a similar dosage schedule. All values represent mean ± SEM of five to nine individual experiments. ●—● glucose-AIS; O—O NEFA-AIS; ▲—▲, glucose-control; △—△, NEFA-control.

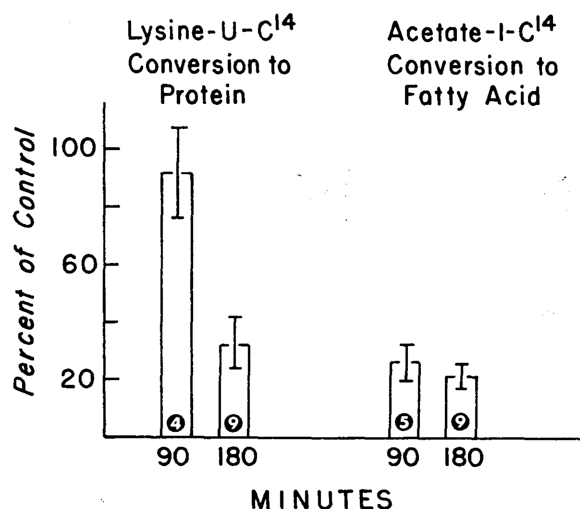

FIG. 2. Time of onset of impaired protein and fatty acid synthesis in 800 × g liver supernatant fractions of rats receiving anti-insulin serum. Abscissa represents time in minutes following initial AIS injection. Values are expressed as per cent of control level (mean ± SEM).

Effect of acute insulin deficiency on incorporation of precursors into fatty acids. The incorporation of both acetate-1-C-14 and acetyl-1-C-14 CoA into fatty acids was depressed approximately 80 per cent within 90 min. after the injection of AIS (figure 3). Three hours after acute insulin deprivation, acetate and acetyl CoA incorporation was depressed greater than 90 per cent, whereas malonyl CoA incorporation was not significantly affected. The incorporation of malonyl-1,3-C-14 into fatty acids also became impaired, however, when the diabetic state was prolonged for six hours.

The effect of palmitate on incorporation of precursors into fatty acids by liver homogenates. The addition of

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/15/7/443/341074/15-7-443.pdf by guest on 23 May 2024

TABLE 1

Incorporation of radioactive precursors into fatty acids by liver supernatant fractions obtained from normal and acutely insulin deprived rats. Animals were studied 180 min. after initial injection of anti-insulin serum (AIS) or nonimmune control serum (Cont.). Values represent mean of three experiments and are expressed as cpm in total hexane extractable lipid.

Supernatant fraction	Incorporation into Fatty Acids					
	Acetate-1-C-14		Acetyl-1-C-14		Malonyl-1,3-C-14 CoA*	
	Cont.	AIS	Cont.	AIS	Cont.	AIS
	cpm		cpm		cpm	
800 × g	9,348	2,275	9,150	970	6,800	5,900
10,000 × g	9,760	1,665	9,400	775	7,350	7,260

*0.4 μmoles malonyl-1,3-C-14 (sp. act., 0.63 mc./mole)/ml. incubation medium.

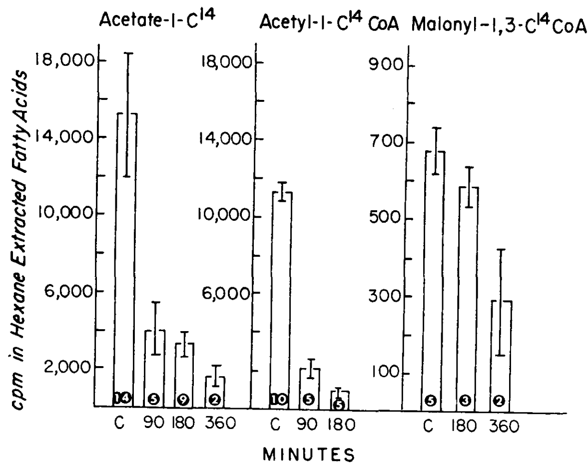

FIG. 3. Effect of acute insulin deficiency on the incorporation of precursors into fatty acids in 800 × g rat liver supernatant fractions. Abscissa represents time in minutes following initial injection of anti-insulin serum. Control values were obtained from animals receiving nonimmune guinea pig serum in a dosage schedule similar to AIS-treated animals. All values are expressed as mean ± SEM; number of experiments indicated at base of each bar. 0.013 μmole malonyl-1,3-C-14 CoA (sp. act., 3.9 mc./mmole) was used in this study.

palmitate, either complexed to albumin or as the free acid in absolute alcohol, in a concentration of 1.0 μEq./ml. of incubation mixture, inhibited acetate-1-C-14 and acetyl-1-C-14 CoA incorporation into fatty acids greater than 80 per cent whereas no inhibition of malonyl-1,3-C-14 CoA conversion to fatty acids was observed (figure 4). The effect of varying concentrations of Na palmitate and palmityl CoA on fatty acid synthesis was also assessed (table 2). Neither the free fatty acid nor its CoA derivative significantly affected malonyl CoA incorporation into fatty acids under conditions where acetyl CoA uptake was inhibited > 95 per cent. The in vitro addition of palmitate did not affect protein synthesis. Lysine-U-C-14 incorporation into protein was not altered at concentrations as high as 5.0 μEq./ml. of

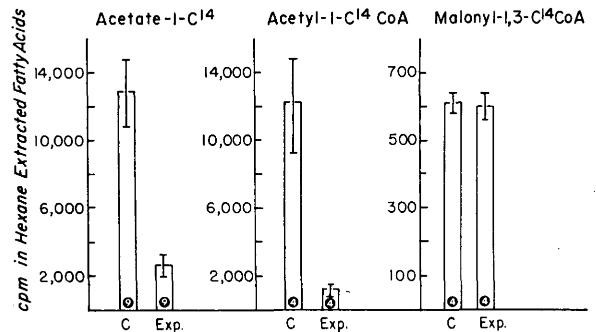

FIG. 4. The effect of Na palmitate on the incorporation of precursors into fatty acids in 800 × g rat liver supernatant fractions. Na palmitate (dissolved in absolute alcohol) was added to incubation mixtures in a final concentration of 1 μEq./ml. (Exp); a similar volume of absolute alcohol was added to control incubation mixtures (C). Values expressed as mean ± SEM; number of experiments indicated at the base of each bar; 0.013 μmole malonyl-1,3-C-14 CoA (sp. act., 3.9 mc./mmole) was used in this study.

incubation mixture (figure 5).

Hepatic tissue levels of nonesterified fatty acids in control and AIS treated rats. The NEFA content of pooled frozen liver specimens obtained from 9 AIS treated rats that were diabetic for a period of 180 min. was 584 ± 49 mμEq./gm. wet weight (table 3). The corresponding mean plasma NEFA concentration was 1,379 ± 65 μEq./L. Nine control animals, receiving nonimmune guinea pig serum in the same dosage schedule, had hepatic NEFA levels of 231 ± 35 mμEq./gm. wet weight and plasma concentrations of 432 ± 34 μEq./L.

Effect of acute insulin deficiency on the incorporation of lysine-U-C-14 into protein by soluble and microsomal liver fractions. Microsomal pellets were washed three times with sucrose-phosphate buffer and gently homogenized in 1.0 ml. of buffer per gm. wet weight of original liver, resulting in a suspension containing approximately 6 mg. of protein/ml. The 100,000 x g

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/15/7/443/341074/15-7-443.pdf by guest on 23 May 2024

TABLE 2

Effect of palmitate and palmityl CoA on fatty acid synthesis in 800 × g liver supernatant fractions obtained from normal rats. Values represent mean of three experiments and are expressed as cpm in total hexane-extractable lipid.

Addition	Incorporation into fatty acid Acetyl-1-C-14 CoA cpm	Malonyl-1,3-C-14 CoA* cpm
None	13,425	11,225
Palmitate (in alcohol)		
1 μEq./ml.	4,932	10,002
10 μEq./ml.	1,577	10,800
Albumin-fatty acid complex		
5 μEq./ml.	2,160	11,410
Palmityl CoA		
125 mμMoles/ml.	4,020	10,650

*0.14 μmole malonyl-1,3-C-14 (sp. act., 0.63 mc./mmole).

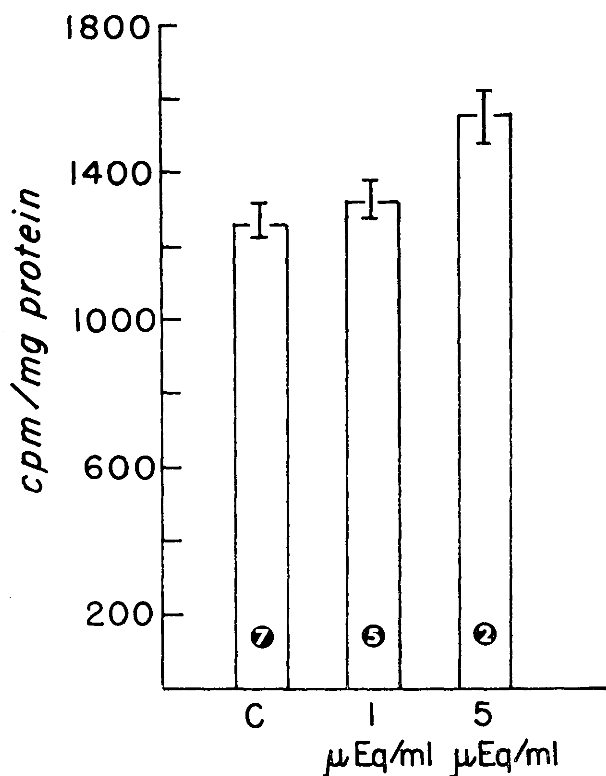

FIG. 5. The effect of Na palmitate on the incorporation of lysine-U-C-14 into protein in 800 × g rat liver supernatant fractions. Na palmitate (dissolved in absolute alcohol) was added to incubation mixtures in final concentrations of 1 and 5 μEq./ml.; a similar volume of absolute alcohol was added to control incubation mixture (C). Values expressed as mean ± SEM; number of experiments at the base of each bar.

supernatant fraction (soluble fraction) contained 14 to 16 mg. of protein/ml. To determine the effects of acute insulin deficiency on the capacity of the reconstituted soluble and microsomal fractions to incorporate lysine-U-C-14 into protein, it was first necessary to establish the relative quantities of these two fractions which, when combined, sustain maximal rates of protein synthesis (figure 6). Since similar results were obtained with both normal and diabetic preparations, the incubation mixture used routinely for subsequent studies contained 0.4 ml. of microsomal fraction, and 0.6 ml. of soluble fraction as well as the various cofactors and salts described in the Methods section.

TABLE 3

Effect of acute insulin deficiency on plasma and hepatic tissue concentrations of nonesterified fatty acids. Plasma and liver specimens were obtained 180 min. after the first intravenous injection of either nonimmune serum (control) or anti-insulin serum (AIS). Number of animals in each group indicated in parentheses. Plasma and tissue NEFA values represent mean ± SEM.

	Plasma NEFA μEq./L.	Hepatic tissue NEFA mμEq./gm. wet weight
Control(9)	432±34	231±35
AIS (9)	1,379±65	584±49

Lysine incorporation into protein in the reconstituted diabetic system (table 4) was approximately 27 per cent of control values. The addition of normal soluble fraction to diabetic microsomes resulted in a moderate increase in protein synthesis, but total incorporation was still less than 60 per cent of the control value. The addition of soluble fraction obtained from liver of acutely insulin deprived animals reduced amino acid incorporation by normal microsomes to 55 per cent of normal.

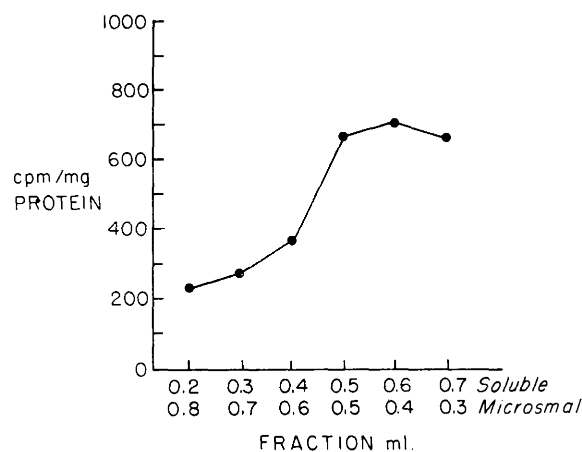

FIG. 6. The effect of varying ratios of soluble and microsomal fractions of rat liver on lysine-U-C-14 incorporation into protein. The 100,000 × g soluble fraction contained 14-16 mg. protein/ml. and microsomal fraction, 4-6 mg. protein/ml. Appropriate cofactors and salts added in a final volume of 1.7 ml. as described in the Methods Section.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/15/7/443/341074/15-7-443.pdf by guest on 23 May 2024

TABLE 4

Effect of acute insulin deficiency of three hours' duration on incorporation of L-lysine-U-C-14 into protein by reconstituted soluble and microsomal fractions of liver; 0.6 ml. of 100,000 × g supernatant fraction (14-16 mg. protein/ml.) and 0.4 ml. microsomal suspension (4-6 mg. protein/ml.) were combined. Cofactors and salts were added to a final volume of 1.7 ml. Values represent cpm/mg. protein.

Cell Fractions	Experiment No. 1	2	3	4	5	Mean ± SEM	Per cent decrease	P
Normal supernatant + Normal microsomes	1,365	414	1,357	628	1,266	1,000±201	—	—
Diabetic supernatant + Diabetic microsomes	533	247	112	200	253	269± 71	73.3	<0.01
Normal supernatant + Diabetic microsomes	787	359	464	541	812	593± 89	41.1	<0.01
Diabetic supernatant + Normal microsomes	971	336	488	489	471	551±109	45.2	0.01

DISCUSSION

Wieland and co-workers[21] suggested that the impaired hepatic synthesis of fatty acids in chronic alloxan diabetic rats is a consequence of decreased enzyme synthesis, since the defect could be corrected by the in vitro addition of acetyl CoA carboxylase and malonyl synthetase. These results differ in part with those of Gibson et al.[22] who reported that carboxylase activity was increased in the livers of alloxan diabetic rats and that the primary enzymatic defect in lipogenesis in these animals was at the malonyl synthetase step.

The present study indicates that the mechanism of impaired lipogenesis associated with acute insulin deficiency differs with that reported for chronic alloxan diabetic rats in two respects: (a) malonyl synthetase activity is not depressed, and (b) the rapidity (within 90 min.) with which decreased acetyl CoA carboxylase activity appears suggests that inhibition of enzyme activity rather than depressed enzyme synthesis and concentration is the immediate cause of impaired lipogenesis.

Bortz and Lynen[37] have recently demonstrated that long chain fatty acid acyl CoA derivatives inhibit acetyl CoA carboxylase and that the binding affinity of the enzyme for palmityl CoA is greater than for acetyl CoA. On the basis of these findings, it was suggested that a negative feedback mechanism operative at the acetyl CoA carboxylase step constitutes a major site of regulation of fatty acid synthesis. Our observations on the pattern of inhibition of lipogenesis produced by the addition of either palmitate or palmityl CoA to normal liver homogenates support this thesis. The concentration of palmityl CoA used in this study, 125 mμmoles/ml., is similar to the concentrations of this ester reported by Tubbs and Garland to be present in the livers of chronic alloxan diabetic rats.[26] The identical pattern of impaired lipogenesis observed in the acutely insulin deprived animal and seen in normal liver homogenates to which palmitate or palmityl CoA is added indicates that feedback inhibition of acetyl CoA carboxylase is responsible for the rapid onset of depressed fatty acid synthesis following acute insulin deficiency. Recent studies indicate that both free fatty acids[43] as well as long-chain acyl CoA derivatives[37] inhibit the carboxylase reaction, hence the inhibition by the in vitro addition of palmitate probably reflects an increased level of both of these substances. The proposal that the relative rates of fatty acid synthesis are inversely proportional to the tissue levels of fatty acids themselves[23-25,34] is supported by the results of this study and suggests that the tissue fatty acid acyl CoA level varies directly with the free fatty acid concentration.

The rapid mobilization of free fatty acids from adipose tissue and subsequent transport to the liver following acute insulin deprivation is undoubtedly an important factor responsible for NEFA accumulation in this tissue. The same mechanism is probably the initial cause of impaired lipogenesis observed during starvation,[27,28] following the administration of fat mobilizing hormones[29] or after fat feeding,[30,31] since all are associated with marked elevation of the plasma NEFA level. Another factor contributing to increased hepatic tissue

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/15/7/443/341074/15-7-443.pdf by guest on 23 May 2024

NEFA in acute insulin deficiency may be decreased rates of esterification of fatty acids to triglyceride secondary to a fall in α-glycerophosphate concentration. Recent studies in this laboratory have shown that the concentration of this intermediate in liver is decreased approximately 40 per cent within three hours after the administration of anti-insulin serum.[10] A third mechanism that may be involved in raising the hepatic NEFA level of AIS treated rats is increased hepatic lipolytic activity, since endogenous lipases in both adipose tissue[32] and striated muscle[33] have already been demonstrated to be functionally significant in promoting glyceride breakdown and NEFA formation. The relative importance of these three factors, however, remains to be elucidated.

Recently Robinson et al.[34] and Schweitzer[35] have reported that malonyl synthetase is also inhibited by long chain acyl CoA derivatives, but in contrast to the competitive nature of their inhibition of carboxylase, the mechanism of synthetase inhibition is non-competitive. In the present study malonyl CoA conversion to fatty acids was not impaired by acute insulin deficiency nor by the addition of palmitate or palmityl CoA to normal liver homogenates. This would indicate that in the normal liver, synthetase activity greatly exceeds that of acetyl CoA carboxylase. Similar conclusions have also been presented by Numa et al.[36] and Bortz and Lynen.[37] The latter authors, using more purified systems, have demonstrated that synthetase activity was not affected by the addition of acyl CoA derivatives under conditions that markedly inhibited carboxylase.

When insulin deficiency was prolonged for six hours, malonyl CoA incorporation into fatty acid was also depressed. In view of the prolonged time required for significant depression of synthetase activity to develop, it would seem more likely that this change reflected an absolute decrease in the enzyme content rather than product mediated feedback inhibition. Such a change in enzyme activity may be secondary either to impaired protein synthesis, which occurs about three hours after the induction of acute insulin deficiency, or to decreased substrate flow through the lipogenic pathway.

The delay in appearance of impaired hepatic protein synthesis in AIS-treated rats, in contrast to the rapid onset of impaired lipogenesis, indicates that different mechanisms are responsible for the impairment of these two biosynthetic pathways. Consistent with this interpretation is the failure to demonstrate any inhibition of protein synthesis following in vitro addition of palmitate at concentrations sufficient to inhibit lipogenesis by greater than 90 per cent. The factors responsible for the depressed protein synthesis of the acutely insulin deficient animal appear to reside in both the microsomal and soluble liver cell fractions. These results differ from the reports of Korner,[38] Robinson,[39] and Wool[40] who demonstrated that the decreased protein synthesis observed in chronically insulin deficient alloxan diabetic rats was completely attributable to changes in the microsomal fraction. Since it has been noted in the present study that the mechanism of impaired lipogenesis in the acutely insulin deprived animal differs from that observed in alloxan diabetic rats, it is conceivable that similar differences exist in the mechanisms responsible for the depressed protein synthesis in these two experimental models. At the present time we have no explanation for the observed defect in the supernatant fraction of AIS treated rats. The decreased ability of microsomes of both acutely insulin deficient and alloxan diabetic rats to incorporate amino acids into protein, however, may represent a similar defect. In view of the delayed time of onset of the microsomal defect (about three hours), the recent demonstration of insulin effects on nucleic acid synthesis,[41] and the reported half life of hepatic messenger RNA of about two hours,[42] it is tempting to speculate that the microsomal abnormality is secondary to the primary alterations in nucleic acid synthesis. Until additional experimental evidence relevant to these points is obtained, however, further discussion would appear unwarranted.

ACKNOWLEDGMENT

This study was supported in part by Grant AM-1921 of the National Institutes of Health, U.S. Public Health Service. Dr. R. K. Kalkhoff is Post-doctoral Research Fellow and Trainee, U.S. Public Health Service Grant AMO-5027.

The technical assistance of Mrs. I. Pommer is gratefully acknowledged.

REFERENCES

[1] Stetten, D., and Boxer, G. E.: Studies in carbohydrate metabolism. III. Metabolic defects in alloxan diabetes. J. Biol. Chem. *156*:271, 1944.

[2] Krahl, M. E.: Functions of insulin and other regulatory factors in peptide formation by animal cells. Recent Progr. Hormone Res. *12*:199, 1956.

[3] Armin, J., Grant, R. T., Wright, P. H.: Experimental diabetes in rats produced by the parenteral administration of anti-insulin serum. J. Physiol. *153*:146, 1960.

[4] Moloney, P. J., and Coval, M.: Antigenicity of insulin: Diabetes induced by specific antibodies. Biochem. J. *59*:179, 1955.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/15/7/443/341074/15-7-443.pdf by guest on 23 May 2024

[5] Sheps, M. C., Nickerson, R., Dagenais, Y. M., Steinke, J., Martin, D. B., and Renold, A. E.: Measurement of small quantities of insulin-like activity using rat adipose tissue. II. Evaluation of performance, J. Clin. Invest. *39*:1499, 1960.

[6] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: Two antibody system. Plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes *12*:115, 1963.

[7] Trams, E. G., and Brady, R. Q.: The synthesis of malonyl-C-14 coenzyme A. J. Am. Chem. Soc. *82*:2972, 1960.

[8] Kornberg, A., and Pricer, W. E.: Enzymatic synthesis of the coenzyme A derivatives of long-chain fatty acids. J. Biol. Chem. *204*:329, 1953.

[9] Burch, H. B., Lowry, O. H., and Von Dippe, P.: The stability of triphosphopyridine nucleotide and its reduced form in rat liver. J. Biol. Chem. *238*:2838, 1963.

[10] Kalkhoff, R. K., Hornbrook, K. R., Burch, H. B., and Kipnis, D. M.: Studies of the metabolic effects of acute insulin deficiency. II. Changes in hepatic glycolytic and Krebs-cycle intermediates and pyridine nucleotides. Diabetes *15*:451-56, July 1966.

[11] Zamecnik, P. C., and Keller, E. B.: Relations between phosphate energy donors and incorporation of labelled amino acids in proteins. J. Biol. Chem. *209*:337, 1954.

[12] Abraham, S., Matthes, K. J., and Chaikoff, I. L.: Fatty acid synthesis from acetate by normal and diabetic rat liver homogenate fractions. J. Biol. Chem. *235*:2551, 1960.

[13] Siekevitz, P.: Uptake of radioactive alanine in vitro into the proteins of rat liver fractions. J. Biol. Chem. *195*:549, 1952.

[14] Goodman, D.: Preparation of human serum albumin free of long-chain fatty acids. Science *125*:1296, 1957.

[15] Bowman, R. H.: The effect of long chain fatty acids on glucose utilization in the isolated perfused rat heart. Biochem. J. *84*:14P, 1962.

[16] Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. *153*:375, 1944.

[17] Duncombe, W. G.: The colorimetric determination of long-chain fatty acids. Biochem. J. *88*:7, 1963.

[18] Dole, V. P.: A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. *35*: 150, 1956.

[19] Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193*:265, 1951.

[20] Wakil, S. J.: Mechanism of fatty acid synthesis. J. Lip. Res. *2*:1, 1961.

[21] Wieland, O., Neufeldt, I., Numa, S., and Lynen, F.: On disorders in fatty acid synthesis in hunger and alloxan diabetes. II. Fatty acid synthesis in the liver of alloxan diabetic rats. Biochem. Z. *336*:455, 1963.

[22] Gibson, D. M., and Hubbard, D.: Incorporation of malonyl CoA in fatty acids by liver in starvation and alloxan-diabetes. Bichem. Biophys. Res. Communs. *3*:531, 1960.

[23] Langdon, R. G., in K. Bloch, ed.: Regulation of fatty acid synthesis. Lipid Metabolism, J. Wiley and Sons, New York, 1960, p. 255.

[24] Porter, J. W., and Long, R. W.: A study of the role of palmityl coenzyme A in fatty acid synthesis by the pigeon liver system. J. Biol. Chem. *233*:20, 1958.

[25] Masoro, E. J.: Biochemical mechanism related to the homeostatic regulation of lipogenesis in animals. J. Lip. Res. *3*:149, 1962.

[26] Garland, P. B., and Tubbs, P. K.: The long-chain fatty acid acyl CoA concentration in rat heart and liver. Biochem. J. *89*:25P, 1963.

[27] Boxer, G. E., and Stetten, D.: The role of thiamine in the synthesis of fatty acids from carbohydrate precursors. J. Biol. Chem. *153*:607, 1944.

[28] Bloch, K.: The biological synthesis of lipids. Cold Spring Harbor Symposia Quant. Biol. *13*:29, 1948.

[29] Perry, W. F., and Bowen, H. F.: Incorporation of acetate into fatty acids by liver slices of adrenalectomized rats. Endocrinology *56*:579, 1955.

[30] Masoro, E. J., Chaikoff, I. L., Chernick, S. S., and Felts, J. M.: Previous nutritional state and glucose conversion to fatty acids in liver slices. J. Biol. Chem. *185*:845, 1950.

[31] Bortz, W., Abraham, S., and Chaikoff, I. L.: Localization of the block in lipogenesis resulting from feeding fat. J. Biol. Chem. *238*:1266, 1963.

[32] Jungas, R. L., and Ball, E. C.: Studies on the metabolism of adipose tissue. XII. The effects of insulin and epinephrine on free fatty acid and glycerol production in the presence and absence of glucose. Biochemistry *2*:383, 1963.

[33] Garland, P. B., and Randle, P. J.: Effect of alloxan diabetes and adrenalin on concentrations of free fatty acids in rat heart and diaphragm muscles. Nature *199*:381, 1963.

[34] Robinson, J. D., Brady, R. O., and Bradley, R. M.: Biosynthesis of fatty acids: IV. Studies with inhibitors. J. Lip. Res. *4*:144, 1963.

[35] Schweizer, E.: Doctoral Dissertation. University of Munich, 1963.

[36] Numa, S., Matsuhashi, M., and Lynen, F.: Zur Stormung der Fettsauresynthese bei Hunger und Alloxan-Diabetes. Biochem. Z. *334*:203, 1961.

[37] Bortz, W., and Lynen, F.: The inhibition of acetyl CoA carboxylase by long-chain acyl CoA derivatives. Biochem. Z. *337*:505, 1963.

[38] Korner, A.: Alloxan diabetes and in vitro protein biosynthesis in rat liver microsomes and mitochondria. J. End. *20*:256, 1960.

[39] Robinson, W. S.: The relation of lipogenesis to reduced triphosphopyridine nucleotide generation and to certain enzyme activities in the liver of the totally depancreatized rat. Proc. Soc. Exp. Biol. & Med. *106*:115, 1961.

[40] Wool, I. G.: Incorporation of C-14-leucine into rat-liver ribosomes: Effect of soluble fraction from muscle of normal, diabetic and insulin-treated rats. Biochim. Biophys. Acta *68*: 411, 1963.

[41] Wool, I. G.: Insulin and incorporation of radioactivity into nucleic acid fraction of isolated diaphragm. Amer. J. Physiol. *199*:719, 1960.

[42] Trakatellis, A. C., Axelrod, A. E., Montjar, H.: Studies on liver messenger ribonucleic acid. J. Biol. Chem. *239*:4237, 1964.

[43] Korchak, S., and Masoro, E.: Free fatty acids as lipogenic inhibitors. Biochim. Biophys. Acta. *84*:750, 1964.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/15/7/443/341074/15-7-443.pdf by guest on 23 May 2024