Determination of Percent Incorporation of Radio Tracer(^{14}C-Acetate) in Newly Synthesized Macromolecules in Different Tissues of Streptozotocin Induced Diabetic Rat vis-control Rats

Shamima Nasrin Shahed[1*] and Nony Gopal Banik[2]

Department of Biochemistry[1], University of Dhaka, Dhaka-1000, BIRDEM[2], Shahbagh, Dhaka, Bangladesh.

Abstract

The effect of streptozotocin induced diabetes on the metabolic pattern of ^{14}C-acetate was investigated by IP injection of uniformly labelled ^{14}C-acetate and compared with sham controls. Significant metabolic changes were observed, synthesis of protein in SZID heart, liver, muscle and kidney was decreased by 99%, 97% and 99% and 98% respectively Synthesis of phospholipid, glycolipid and cholesterol of SZID brain was decreased by 67%, 62% and 90% respectively. Synthesis of glycogen in SZID liver and muscle was decreased by 34% and 99% while the incorporation of radio activity in newly synthesized lipid in adipose tissue, muscle and liver was depressed by 99%, 99% and 88% respectively.

Key Words : Macromolecules, ^{14}C-Acetate, Streptozotocin Induced Diabetic Rat (SZID).

Introduction

Acetyl CoA, active acetate, arises partly from oxidative decarboxylation of pyruvate and partly from beta oxidation of long chain fatty acids and several amino acids. There is no exaggeration to call active acetate a metabolite of cardinal importance.

The energy content of acetyl CoA, i.e. its free energy of hydrolysis is little higher than that of ATP and yet this reactive ester cannot be considered to be the universal reservoir of energy. Rather acetyl CoA is the reactive intermediate that provides the C2 fragment for so many conversion and synthesis. By being converted to acetyl CoA, the various intermediatary metetabolites enter the common terminal pathway of breakdown, known as the citrate cycle. Synthesis with active acetate, acetyl CoA is an important

Bangladesh Journal of Nutrition, Vol 14, December 2001. Institute of Nutrition and Food Science, University of Dhaka, Dhaka-1000, Bangladesh.

*Author for Correspondence

starting materials for biosynthetic reactions. First of all fatty acids can be formed from it. This is the major pathway followed during conversion of carbohydrate to fat. A second important anabolic pathway of acetyl CoA produces the isoprenoid lipids, especially the steroids. From acetyl CoA, aminoacids, protein and carbohydrate also are formed. Diabetes mellitus is a clinical syndrome characterized by a chronic state of hyperglycemia followed by chronic disorder. This state is characterized by a series of metabolic changes.

In insulin defiiency or in fasting, utilization of acetyle CoA by the ticarborylic acid cycle is depressed and also the synthesis of fatty acids. As a result, the tendency of the insulindeficient animal is to convert all its metabolically available resources into blood glucose, under these conditions acetyl CoA tends to be shunted into the formation of HMH CoA, leading to increased formation of ketone bodies and cholesterol. However, the amount of acetyl CoA, that can be shunted into cholesterol formation, is very limited. So the great bulk of the HMG CoA is converted into the ketone bodies, acetoacetic and 13-hydroxy nutyric acids. Thus, insulin deficiency causes profound alternations in a complex network of metabolic inter relationship.[1]

In the present study, we have seen the effect of streptozotocin induced hyperglycemia on the metabolism of ^{14}C-acetate. It was invesigated by IP injection of uniformely labelled ^{14}C-acetate. Six pair of rats was used and compared with sham control rats. This investigations include the areas involved in the synthesis of different macromolcules in the different tissues using the ^{14}C-acetate as the precursor molecules.

Materials and Methods

Animal and maintenance Long-cvans rats of black and white strain were used for this study. Adult animals between the ages of 75 to 120 days and weighing between 200 to 250 gms were selected. The experimental animals were obtained from the Animal Resources Branch, Diabetic Association, Shahabagh, Dhaka, Bangladesh. Each of the selected rat was housed in a screen bottomed cage (Geo H. Walsman Manufacturing Co. Baltimore, M.D) and maintained in a constant temperature environment with 12 hrs. of artificial light per 24 hours. The temperature of the room in which the rats were kept was 26°C ± 5°C. The rats were fed on a good quality based diet and water and libitum. The diet supplied for 7 days to each rat was approximately isocaloric, i. e. Approximately, 20 gms of diet per rat per day.[2]

Induction of diabetes : Diabetes was induced in the experimental rats by injecting streptozotocin. The drug (1ml/20 mg body wt) (1 ml contains : streptozotocin 100 mg and citric acid 22 mg) as administrered intraperitoneally after 24 hrs of fasting. The sham control rats were given equal amount of normal saline, ten pairs of rat was used for both cases. The degree of occurrence of diabetes was monitored by estimating blood glucose level using glucose strip in a reflectometer (Relemat-Labora Mannheim; GMBH fur labor-technik) and by wine analysis for detecting increased concentration of glucose by benedict reagent. One drop of blood was collected from a stab incision at the ventral position of the tail for the examination of blood glucose level. Blood glucose was estimated by modified Somgyi-Nelson method.[3]

Radiotracer Specification : The compound 14c), acetate (U-14c), specific actiity of 57-9 μci/mol (Code CF $_A$229, Batch 64) was supplied by ICN isotope and Nuclear Division, 2727 Campus Dive, Irving, U.S.A. Its purity was 99% as checked by paper chromatography.

Administration of Radiotracers : A tracer dose of 4μci/0.2ml acetate were administered IP following an overnight fasting rats.

Collection of tissue samples from rat : For the measurement of radioactivity after 24 hrs. of the IP injection of 0.2 ml of the radiotracer, the leg muscle, liver, kidney, heart, brain, pancreas and adipose tissues were excised from rats exsanguinated under ether anesthesia. The rats were anesthesized by an overdose of ether in a desiccator and were surgically treated with fine pair of scissors to dissect the tissues. These were used for the study of the percent incorporation of the radiolabelled substrate in the synthesize macromolecules of different tisues.

Processing and storage : Animal tissues excised for the isolation of different macromolecules were washed several times with ice-cold normal saline (0.9% Nacl). Experimental tissues were blotted dry and weighed accurately in an electrical balances (Mechaniki Zaklades Precyzjnej, Gdnask, Poland). The weighed tissues were wrapped in aluminium foil and stored in a deep freeze (ultralow revco. MC. West Columbia) at – 20°C unitl used.

Isolation of different macromolecule from different tissues

Only glycogen was isolated from liver and muscle.

TCA insoluble protein was isolated from liver, leg muscle, kidneys and heart.

Total lipid from liver, leg muscle and adipose tissue was extracted.

Brain lipids (Phospholipid, glycolioid and cholesterol) were isolated.

Sample preparation

In a different L-S counting vial the TCA precipitate, total lipid, and brain lipid isolated from different tissues were transferred quantitatively (1 to 5 mg) and dissolved in 0.1 ml chloroform. Isolated glycogen was transferred to and dissolved in d-water and tricholoroacetic acid. Insoluble protein was transferred to and added d-water and 0.1 ml 10% KOH and digestion was allowed to proceed at 50° – 60°C.

Measurement of radioactivity

Measurement of Radioactivity of all samples was done by the method as described in the ref.[4] All samples were counted thrice for one minute using a packard tricarb liquid scintillation spectrometer (Model No. 3255) with a teletype electric typewriter with automatic background substraction at BIRDEM. Results shown (count per min. CPM) are the mean of three counts, counting efficiency (CE) was determined by using an internal standard (% counting efficiency, between 86%-88%) and standard curve for varying degree of quench was constructed using the formula:

$$\% \text{ CE} = \frac{\text{CPM}}{\text{D.P.M.}} \times 100$$

(ii) Radioactivity Calculations :

Following formulations were used for radioactivity calculations :

% incoroporation of radioactivity per 100g tissue componnt CPM × TT Mmg × 100

$$= \frac{\text{CPM}}{\text{TWmg} \times \mu\text{ci}} \times \frac{100}{\% \text{ CE} \times 2'22 \times 10^6}$$

where, a) TWmg = tissue weiht in mg.

b) xuci = Microcurie injected

c) $\text{dpm} = \frac{\text{CPM} \times 100}{\% \text{ CE}}$

d) $2'22 \times 10^6$ is multiplied with × μci for converting μci into dpm,

(ii) 14c activity in terms of dpm per 100g tissue components.

$$= \frac{\text{CPM per 100g tissue component.}}{\% \text{ CE} \times 10} \times 100$$

% incorporation of radioactivity per 100g tissue component

$$= \frac{CPM \times TTMmg \times 100}{TWmg \times TWg \times 10^{3} \times x\mu i} \times \frac{100}{\% CE \times 2.22 \times 10^{6}}$$

where, TTM = total tissue macromolecules isolated in milligramme.

TM = tissue macromolecules taken for counting in mg.

TW = tissue weight (wet) in gram.

Results

Fig. 1-8 present the data for the conversion of acetate (u-^{14}c) into, (i) glycogen of liver and muscle, (ii) protein (TCA ppt) of liver, muscle, kidney and heart, (iii) total lipid of liver, muscle and adipose tissues, and (iv) phosphoipid, glycolipid and cholesterol of brain of SZID and SC rats 24 hours after IP injections of a dose of 4 μci. The percent incorporation of radioactivity in newly synthesized macromocules was depressed in heart, liver, muscle, kidney when compared with SC tissue components.

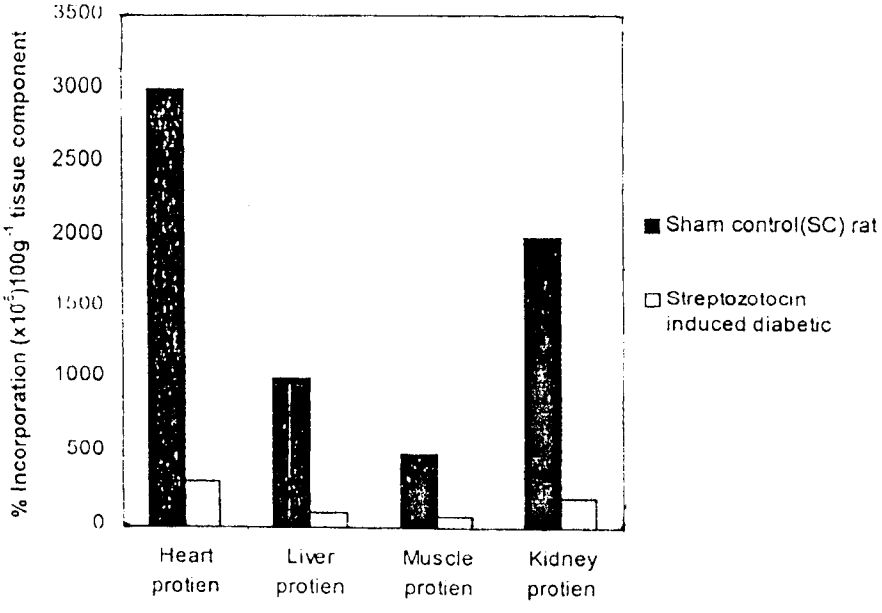

Fig. 3. % Incorporation of radioactivity in newly synthesized tissue protein after pulsing with uniformly labeled 14^{c} acctate

The protein synthesis was decreased by 99% ($P \leq 0.001$) in SZID heart protein and from SZID heart very minute quantity of protein was isolated. The synthesis of liver, muscle and kidney protein (TCA PP1) in streptozotocin diabetes was decreased by 97% ($P \leq 0.001$), 99% ($P \leq 0.001$) and 98% ($P \leq 0.001$) respectively as shown in fig. 3 and 4.

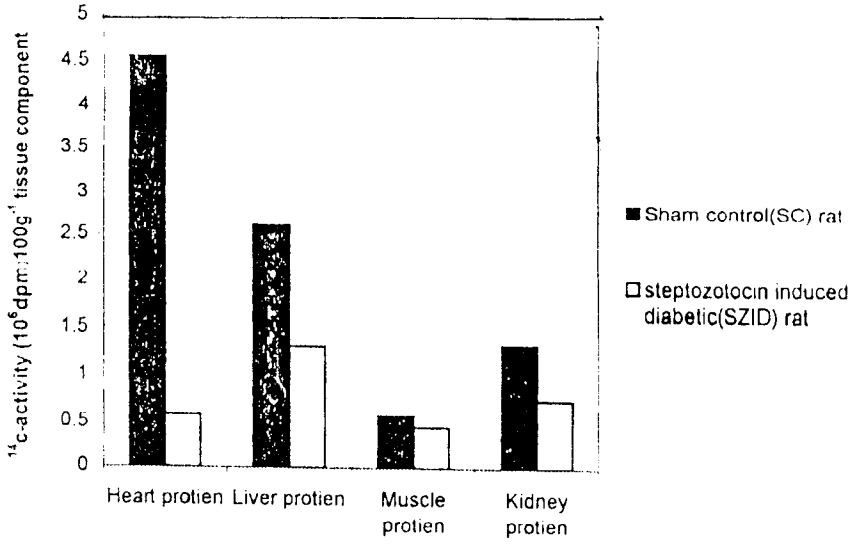

Fig. 4. Distribution of radioactivity in tissue component (Protein) after pulsing with uniformly labeled 14' acetate.

The SZID adipose lipid shows significantly higher radioactivity as shown in fig. 5 and 6 (+308%, $P \leq 0.001$), though synthesis of adipose lipid was decreased by 99% ($P \leq 0.001$) followed by muscle and liver lipid showing a decreased y 99% ($P \leq 0.001$) and 88% ($P < 0.001$) respectively.

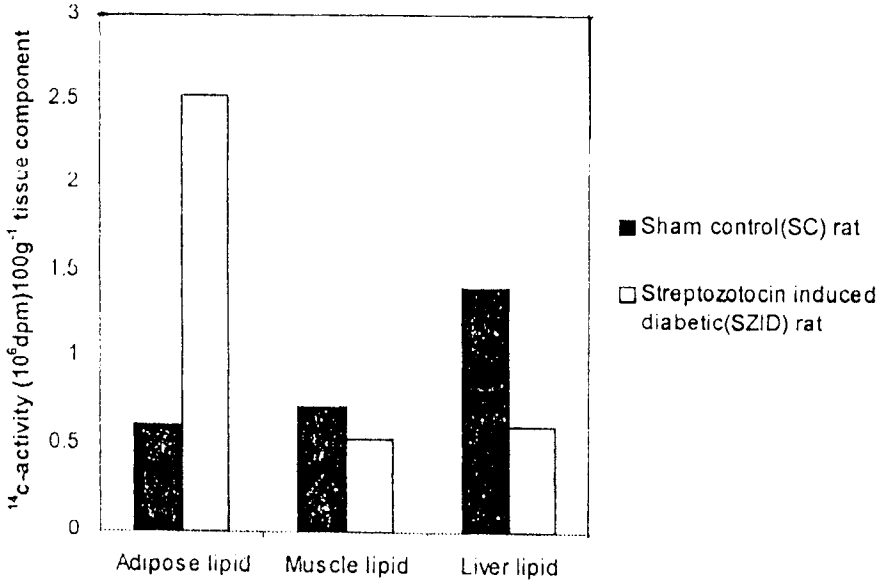

Fig. 5. % incorporation of radioactivity in newly synthesized tissue Lipid after pulsing with uniformaly labeled 14^c acetate.

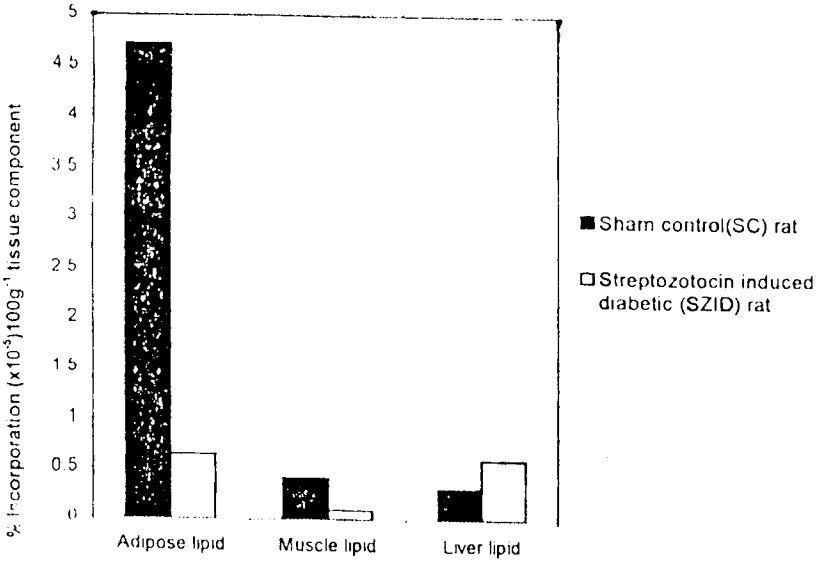

Fig. 6. % Distribution of radioactivity in tissue component (Lipid) after pulsing with uniformal labeled 14c acetate.

In synthesis of phospholipid, glycolipid and cholesterol of brain the uptake was decreased by 67% ($P \leq 0.001$), 62% ($P \leq 0.001$) and 90% ($P \leq 0.001$) respectively. This data resent in fig. 7 and 8.

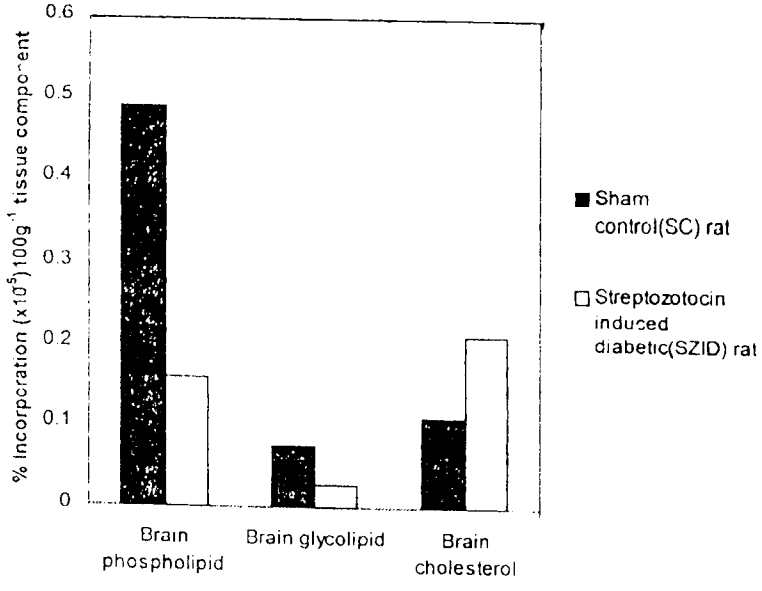

Fig. 7. % Incorporation of radioactivity in newly synthesized tissue Phospholipid, glycolipid and cholesterolafter pulsing with uniformly labeled 14^c acetate.

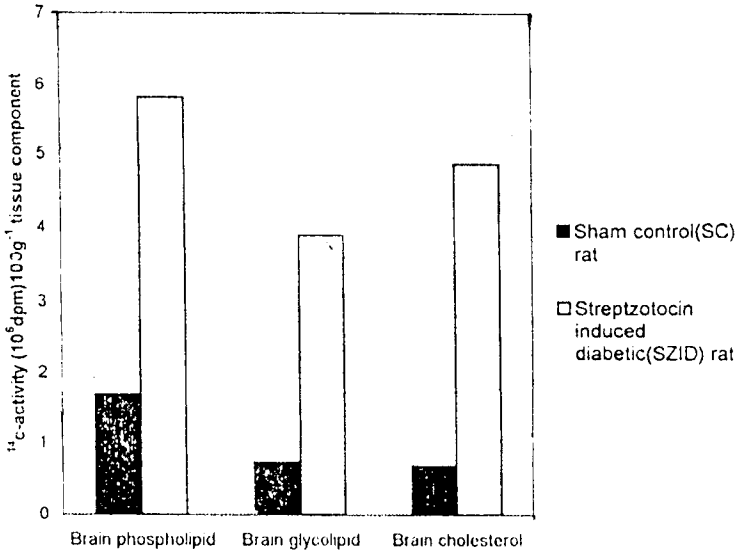

Fig. 8. Distribution of radioactivity in tissue component (Phospholipid, glycolipid, cholesterol) after pulsing with uniformaly labeled 14-c acetate.

Similarly, % imcorporation of radioactivity in newly synthesized liver and muscle glycogen in streptozotocin diabetes was decreased by 34% ($P \leq 0.001$) and 99% ($P \leq 0.001$) respectively. Distribution of radioactivity in tissue component was decreased in SC rat where as it increases in SZID rat shown in fig. 1 and 2.

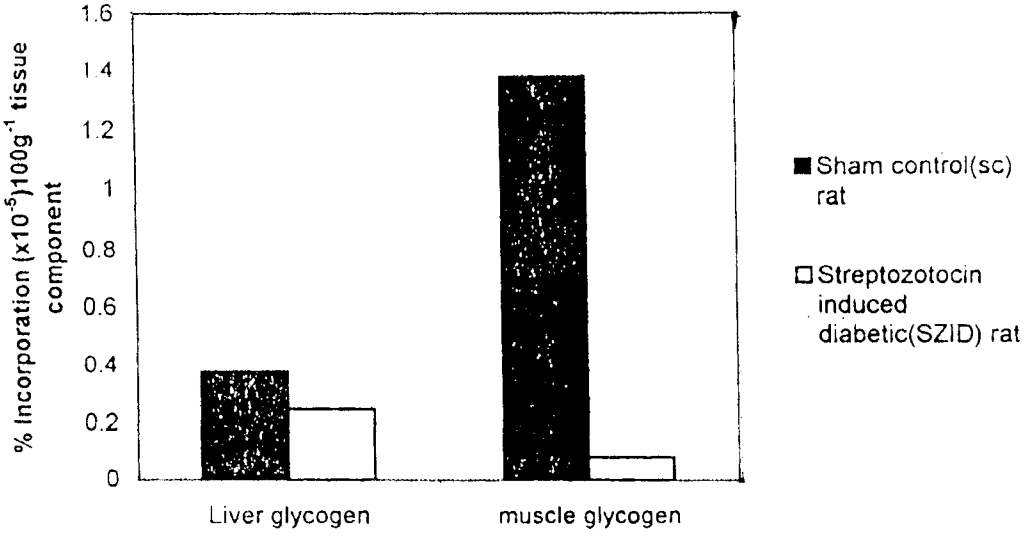

Fig. 1. % Incorporation of radioactivity in newly synthesized tissue glycogen after pulsing with uniformly labeled 14^c acetate.

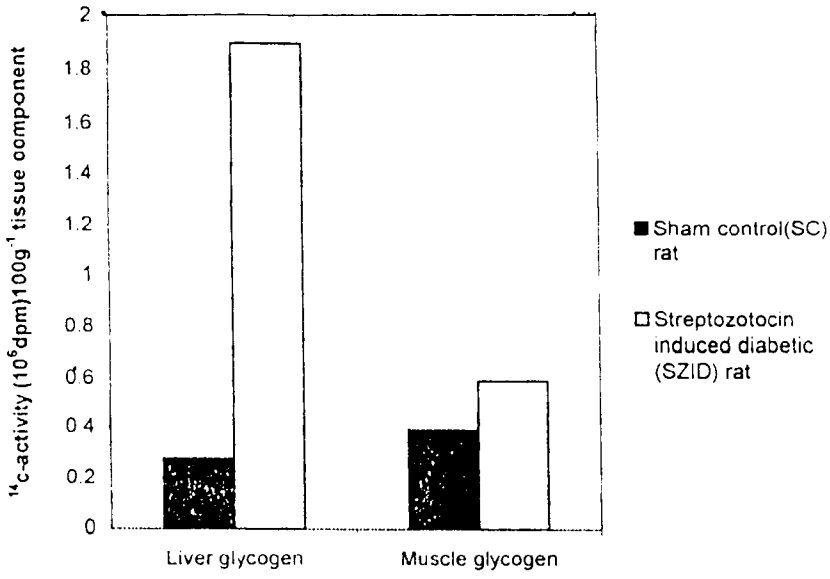

Fig. 2. Distribution of radioactivity in tissue component (Glycogen) after pulsing with uniformly labeled 14^c acetate.

Discussion

In all SZID tissues, a deaccelerated synthesis of labelled macromolecules from acetate (U-14c) indicates the serious problem of peripheral utilization of glucose and impairment of HMP shunt in diabetes.

Muscle and liver glycogen was detected in very low amount in case of acetate treated SZID rats. This indicates that though 14c acetate could be converted, but in very low amount. An impaired peripheral utilization of these precursors for glycogen biosynthesis by SZID rats may be indicated by these findings.

Villee *et al.*[5] reported that SZID rat muscle takes up less glucose and also acetate or metabolites of glucose than normal animals, and the extend of glycogenesis also decreases. The mechanism whereby insulin exerts its effect on the utilization of glucose or acetate by muscle has not been completely elucidated.[6]

J. W. CRAIG, L. S. Huans and J. Laiver[7] have demonstrated the activation of the enzyme glycogen synthesases by insulin in several tissues. This enzyme exists in both active and inactive forms, which are interconvertible by phosphorylation-dephosphorylation reactions. Dephosphorylated glycogen synthase is the active form and is recognized by the property of being independent of glucose-6-phosphate for activity.

In the course of comparative studies of some biochemical characteristics of fibroblasts cultured from non-diabctic and diabetic human subjects, an effect of insulin on the activity of the enzyme glycogen synthase was demonstrated by Cahill and his coworkers.[8] For these reasons, liver and muscle tissue of SZID rats could synthesize glycogen in very minutes quantities as compared to control.

Cahill[8] observed that insulin is the main regulator of protein metabolism. Hence insulin deficiency results in massive protein catabolism. This increased catabolism was confirmed by the observation of Martin and Wool[9] who showed circulating concentration of amino acid in diabetic liver. In diabetic animal, the polysomes become disaggregated and insulin in-vivo restores them to the normal aggregated form. Studies of Martin and Wool[9] indicate that insulin may act at the ribosomal level to increase the capacity of this organelles to translate information from m-RNA to the protein synthesizine machinery. A decreased protein synthesis as observed in our study, is in agreement with similar findings by previous investigators.[9,10]

Glucose is taken up b adipose tissue and its carbon atoms are incorporated into lipids. This process is directly stimulated by insulin.[11] It has recently been established that in adipose tissue, as in muscle, insulin accelerates the rate of glucose transport across the cell membrane. Glucose is degraded by both the EMP and HMS (Hexose monophosphate shunt), yielding acetyl-coA and reduced co-factors nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH). Together, these products allow for the subsequent de novo synthesis of fatty acids which proceeds by the sequential condensation of 2 carbon units, the last one being provided by malonyl COA which in turn arises from the carboxylation of acetyl COA in the presence of acetyl COA-carboxylase. This later reaction is rate limiting for fatty acid synthesis and thus a site of metabolic and possible endocrine regulation.[14] In SZID tissues, lipid synthesis from these precursors was perhaps directly hampered due to the lack of insulin. Perhaps, uniform labelling was also hampered as all SZID tissue showed a decreased uptake of ^{14}Ccarbon from acetate (U-^{14}C) their lipid components.

Brady[12] and other workers[13,14] have shown that lipogenisis is depressed in SZID tissues. With isotope technique, Chernick[15] demonstrated that insulin accelerates lipogenesis from carbohydrate. The process of fat formation from C_2 units require much energy and must be coupled to energy yielding reactions. It appears likely that a decrease in the normal rate of glucose utilization leads to a concomitant decrease in the energy available for lipogenesis.

Hypoinsulinemia decreased the ability of different diabetic tissues to utilize acetate (U-^{14}C) as a biosynthetic precursor for macromolecules as seen in these experiments (Fig. 1-8). Decreased extraction of C-14 label was noticed in the phospholipid, glycolipid and cholesterol of he brain of SZID rats as compared to control rats, Our finding also indicates that hypoinsulinemia markedly decreases the formation of cholesterol, phospholipid and glycolipid in brain.

In presnetly, findings with the use of uniformly labelled acetate clearly indicate that all carbons molecules of acetate are not required for the synthesis of macromaleculs. Perhaps, carbons of certain positions in the molecules are utilized for the synthesis of macromolecules. Further study with labelling of carbon at certain position may clear this. However, it is established by our findings that diabetes mellitus definitely shifts the metabolic pattern and also that mode of macromolecular conversion and capacity of different tissues to do it are variable.

References

1. Albert L. Lehninger. Carbohydrate Metabolism. Biochemistry, 6th edition 1972 : 36
2. Hillman L. Lipid Metabolism. Metabolism. 1975; 23 : 827.
3. Nelson N. A. J. Biol. Chem. 1944; 153 : 375.
4. Shahed SN and Banik NG. In Vivo Oxidation of Cholesterol in Streptozotocin Induced Diabetes Rat. Bangladesh J. Nutrition. 1999; 12 : 69-77.
5. Villee C A and Stadie W C. J. Biol. Chem. 1952; 195: 287.
6. Wool IG and Kurihara K. Proc. Nat. Acad. Sci. 1967; 58 : 2401.
7. Craig JW. Huans LS and Laiver J. Critical Reviews in Clinical Laboratory Sciences. 1971.
8. Cahill GF. Am. Physiological Society. Washington. 1972; 563.
9. Martin TE and Wool G. Proc. Nat. Acad. Sci. 1968; 60: 569.
10. Stadie WC. Physiological Reviews. 1954; 34 : 52.
11. Bradley RF. In. Joslin's diabetes Mellitus. 1971; 417, Philadelphia.
12. Brady RO and Parker KM. J. Biol. Chem. 1951; 193 : 459.
13. Stetten D and Boxer GE. J. Biol. Chem. 1944 ; 156 : 271.
14. Thompson GR. Circulation. 1996. 54. Suppl. 4(abstract).
15. Chernick SS. and Shaw W A. J. Biol. Chem. 1950; 186 ; 527-535.