1970
Nagoya J. med. Sci. 32: 261-280, 1970

STUDIES ON THE DIABETIC STATE OF RATS FED A HIGH FAT DIET FOR 400 DAYS

—A POSTULATED MECHANISM OF DISTURBED CARBOHYDRATE METABOLISM—

KAZUO KATSUMATA

3rd Department of Internal Medicine, Nagoya University School of Medicine
(Director: Prof. Kozo Yamada)

ABSTRACT

Three groups of male albino rats were fed with synthetic diets—high fat, low glucose (high fat diet group), high protein, low glucose (high protein diet group), and normal diets (normal diet group)—for 400 days, and the influence of these diets upon carbohydrate metabolism were examined. The following results were obtained.

1) Rats in the high fat diet group increased greatest body weight among the three groups, 400 g on the 200th day, and thereafter, continued to be obese.

2) Glucose tolerance curves and insulin sensitivity tests demonstrated significantly impaired carbohydrate metabolism of rats in the high fat diet group.

3) The decrease of glucose utilization and the increase of gluconeogenesis were observed in the tested liver slices of rats in the high fat diet group.

4) Glucose utilization and insulin sensitivity of adipose tissues and diaphragms of rats in the high fat diet group were significantly reduced compared to other groups.

5) The addition of papain in the medium reduced the incorporation of glucose-U-C^{14} into CO_2 of diaphragms of rats in high fat diet group and normal diet group but in the case of adipose tissues, although increased incorporation of radioactivity was observed in normal diet group, decreased incorporation was apparent on high fat diet group suggesting the presence of some different membrane structure of adipose tissue in high fat diet group.

6) In the pancreases of rats in the high fat diet group, hypertrophy of Langerhan's island and fibrosis of the exocrine glands were observed at the same time with the high level of insulin content of the pancreases.

From the above results the author concluded that the disorder in the carbohydrate metabolism of rats in the high fat diet group resembles the metabolic disarrangement of maturity onset type of human diabetes mellitus. The phenomenon can be comprehensive as an adaptation to the increased oxidation of fatty acids and the metabolic changes due to the accumulated lipid at the adipose tissue, and it is also partly consistent with Randle's theory on glucose-fatty acid cycle. The results suggest that high rate of fat in diet can aggrevate carbohydrate metabolism in rat as well as in the human being.

勝 又 一 夫
Received for publication July 2, 1968.

It is known that fat is one of the essential energy requirements but that an excessive intake of fat not only facilitates deposition of fat on the arterial wall and the liver, but also impairs carbohydrate metabolism. The pathogensis of the above mentioned fact has not yet been completely resolved.

One method to clarify its mechanism is to study animals fed a high fat diet. Gonzalenz C.[1] found that liver glucokinase activity was significantly lower in the rats fed high fat diet. On the other hand, Randle[2] reported that the excessive oxidation of fatty acid inhibited the uptake and oxidation of glucose in the muscles of animals.

The author studied this subject using rats fed various diets and found that feeding a high fat diet induced the inhibition of the glycolysis and the increase of the gluconeogenesis. The rats were fed with specially composed high fat diets for 100 to 400 days. It was also found from the experiments that distinct carbohydrate disarrangement occurred even in the early stage of the feeding of the rats, and that the accumulated lipid of the adipose tissue in high fat group induced the changes of it's membrane structure resulting in the disturbed carbohydrate metabolism.

MATERIALS AND METHODS

One hundred thirty-five male albino rats (Wistar strain) weighing on an average of 70 g each were divided into normal, high fat, and high protein groups which were designated by the diet compositions shown in Table 1. The normal group of 55 rats, the high fat group of 55 rats, and the high protein group of 25 rats were maintained on three kinds of synthetic diets containing a vitamin mixture of 0.1 ml per 10 g of diet. The rats were placed in individual metabolic cages in an air conditioned room at approximately 24°C, with ad libitum water supply and were weighed periodically.

TABLE 1. Composition of Diet

Composition of diet → Group ↓	Fat	Protein	Carbohydrate	Macculum salts*
Normal diet	10	24	62	4
High fat diet	48	24	24	4
High protein diet	10	62	24	4

* KJ 30 g, Fe citrat 100 g, $MgSO_4\ 7\,H_2O$ 500 g, Ca lactate 1,300 g, Na_2HPO_4 200 g, KH_2PO_4 1,100 g, KCl 150 g, NaCl 50 g, $CaHPO_4\ 2\,H_2O$ 500 g were mixed, and then 0.4 g of mixture was used in 10 g of diet.

Lipid composition was cotton seed oil and butter fat (60: 40, W/W). Protein was milk casein, carbohydarte was glucose. The numbers in the table represent weight % of each component in the diet.

TABLE 2. Composition of Vitamin Mixture

Kind of vitamin	Quantity	Kind of vitamin	Quantity
B_1	40 γ	Cholin	10 mg
B_2	60 γ	Inositol	3 mg
Nicotinic acid	200 γ	A	300 I.U.
Pantotenic acid	200 γ	D	30 I.U.
PABA	0.5 mg	B_6	60 γ
Biotin	1 γ	VE	1.2 mg
B_{12}	0.1 γ	VC	1.0 mg
Folic acid	5 γ	VK	0.1 mg

Vitamin contents in the table represent the final concentration in one-tenth ml. The final volume of the mixture was adjusted by adding distilled water.

The rats weighing less than 129 g were supplied with 10 g of food per body weight of 100 g, those weighing 130 to 150 g with 13 g of food, those weighing 151 to 200 g with 15 g of food, and those of more than 201 g with 17 g of food during the periods of experiment.

The glucose tolerance test of 7 rats in each group was performed on the 100th and 400th day and the insulin sensitivity test on the same rats on the 398th day. 1 ml of a 20% glucose solution was forcely given by stomach tube for the former test and 0.2 unit of regular insulin (obtained from the Shimizu Company) per kg of body weight was intraperitoneally injected for the latter test. For both tests, 0.1 ml of blood was taken from the tail veins of the rats, blood glucose were determined according to the method of Hagedron and Jensen. Another ten rats from each group were decapitated on 100th and 400th day and the livers were quickly removed. The total lipid of the livers was determined by the method of Bragdon[4], liver glycogen by the method of M. Dubois[7], and serum NEFA (none esterified fatty acid) by the method of Dole[5]. On the 105th day, another ten rats in the high fat and the normal diet groups were decapitated, and the diaphragms and the distal parts of the epidymal fat pats were immediately removed and rinsed with Krebs Ringer bicarbonate buffer containing 100 mg glucose. A 100 mg pieces of tissues of the distal part of the epididymal fat pats and 100 mg pieces of diaphragms were blotted and placed into separate vessels. The medium in the vessels was 2 ml of Krebs Ringer bicarbonate buffer, pH 7.4, with or without the insulin (obtained from the NOVO Company), or the papain (obtained from the WAKO Company) gassed with O_2 and CO_2 in the ratio of 95% to 5%, with an addition of 200 mg glucose per 100 ml of medium. When this insulin was present, 0.5 mU insulin per ml of medium was added. The vessels then incubated in the

metabolic shaker with constant agitation at 37.4°C for 2 hours. The determination of the glucose in the media containing adipose tissue or diaphragm was performed after 2 hour's incubation using the method of Hagedron and Jensen. The value of the control solution was determined in the media containing neither adipose tissue nor diaphragm. The difference between the glucose of the control and the experimental glucose was expressed as the amount of glucose uptake in mg per 100 ml of the medium, which represents the amounts of glucose uptake by the tissue.

In another series of experiments, 0.5 μC of glucose-U-C^{14} was added to each vessel containing adipose tissues and diaphragms and were incubated for 2 hours. Next, 0.2 ml of 10 N H_2SO_4 was added to the medium after which 0.4 ml of 1.8 N NaOH was added by injecting it into a plastic cup suspended in the vessels. The vessels were incubated for another hour to accelerate the collection of $C^{14}O_2$ evolved by the adipose tissues or diaphragms. The measurement of $C^{14}O_2$ was performed by a gas flow counter which counts $C^{14}O_2$ in the form of $BaC^{14}O_3$, and the data taken were corrected by self-absorption.

The incorporation of alanine-U-C^{14} into liver glycogen and blood glucose, that of glucose-U-C^{14} into liver glycogen and fat, and that of sodium acetate-1-C^{14} into liver fat were measured in each of 5 rats of the high fat and the normal diet groups. For this measurement, 10 μC of alanine-U-C^{14} per 100 g body weight was injected into the abdomens of these animals on the 110th day, and 4 hours after this injection, they were decapitated. For the measurement of C^{14} glycogen, the method of R. W. Gomery[6] was used to precipitate the glycogen. The amount of C^{14} in the liver glycogen was counted by gas flow counter, but part of it was used for measuring the value of glycogen by the method of M. Dubois[7]. As for blood glucose, it was precipitated as osazone using the method of Ashmore[8]. As for the measurement of liver fat, the method of Folch was used to extract fat and the gas flow counter was used also, but a part of the liver was used for the determination of total fat using the method of Bragdon[4]. The measured data of C^{14} was adjusted by self-absorption.

On the 115th day five rats of the normal and the high fat diet groups were decapitated and the livers were removed quickly. Then 200 mg slices of the liver were placed into individual vessels containing 2 ml of Krebs Ringer phospate or bicarbonate buffer (pH 7.4) gassed with O_2 and CO_2 in the ratio of 95% and 5%. Next, 0.5 μC of glucose-U-C^{14} or 0.5 μC of sodium pyruvate-U-C^{14} was injected into the vessels, and they were incubated at 37.4°C in the metabolic shaker with constant agitation. In this experiment the $C^{14}O_2$ evolved after the incubation of 2 hours was collected into a 1.8 N NaOH solution and then counted by the gas flow counter.

On the 400th day the rest of 15 rats from each group were killed by decapitation and the pancreas of each was removed quickly. The pancreatic insulin was extracted by the method of Best[9]. The amount of pancreatic

insulin in each group was determined using ten pancreases of each group by biological method[10]. These pancreases were put into a 10% formalin solution and then stained by the H.E. method. All experiments were performed 4 hours after food was removed from the cages.

RESULTS

1. Body weights

Fig. 1 shows the changes and the mean±S.E. of body weight by diet composition. The rats of the normal diet group weighed an average of 270 g on the 300th day but increased no more. The rats of the high protein diet group weighed an average of 330 g on the 400th day. However, the high fat diet group grew biggest and fastest, weighing 350 g on the 100th day and 400

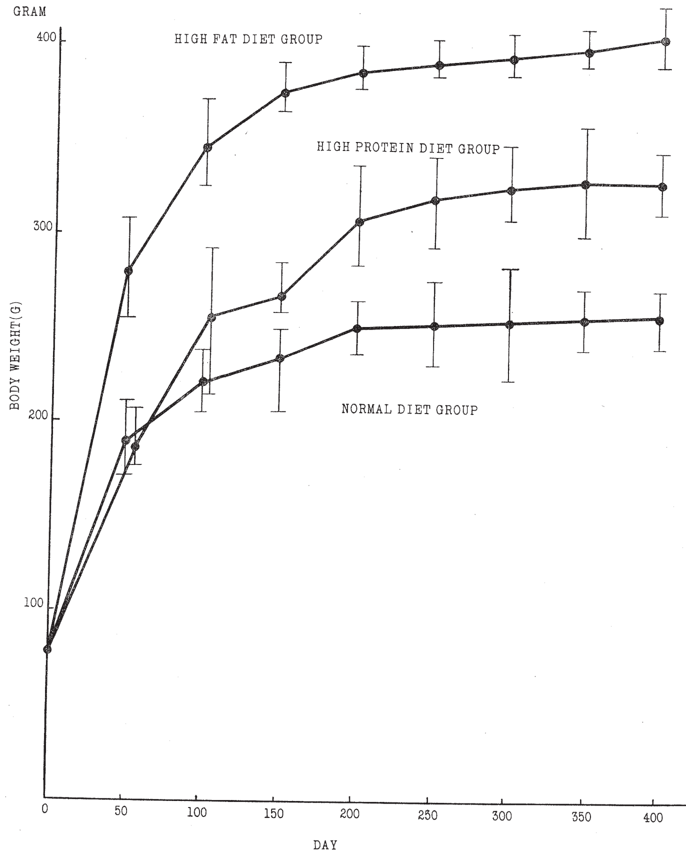


FIG. 1. Changes in body weight by the composition of diet. Each point and bar represents mean body weight±S.D. of seven rats in each group.

g on the 200th day, as seen in Fig. 1.

2. Glucose tolerance test and insulin sensitivity test

Fig. 2 shows blood glucose changes in the three groups during the period of the glucose tolerance test on the 100th day. As seen in Fig. 2, while the glucose tolerance of the high protein group and normal diet group was normal, that of the high fat diet group was disturbed. The remarkable change of G.T.T. by the high fat diet group was seen on the 400th day, as shown in Fig. 3–a. The insulin sensitivity of the high fat fed rats was low. No difference was seen in insulin sensitivity between the normal and the high protein fed rats, as shown in Fig. 3-b.

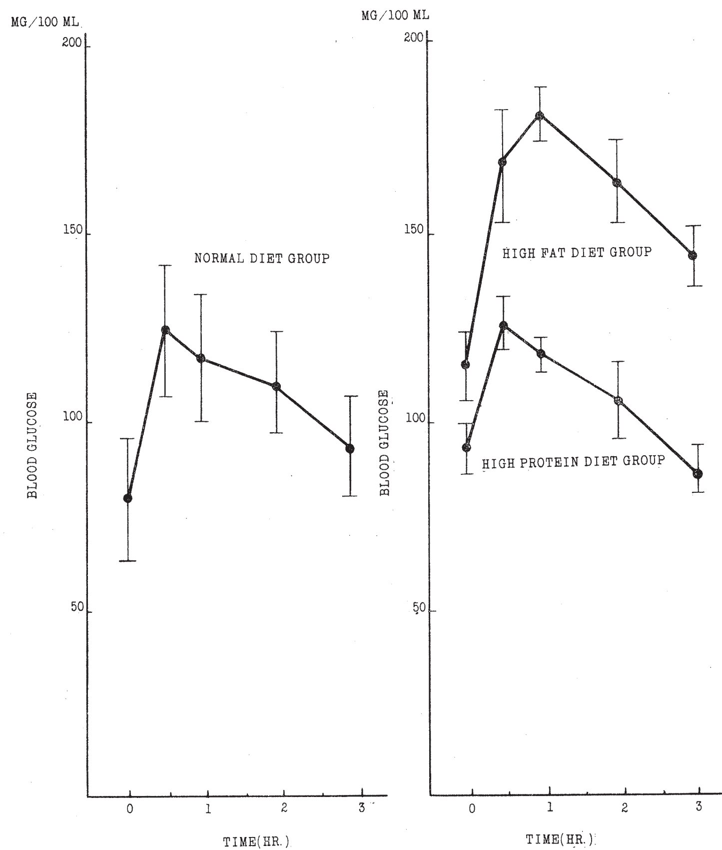


FIG. 2. Changes in glucose tolerance curves by the composition of diet. Each point and bar represents mean±S.D. of measured blood glucose values of seven rats in each group. Rats were fed for 100 days.

3. Liver fat and glycogen contents and serum NEFA level

In Fig. 4-a is shown the mean±S.E. of liver total lipid and glycogen on the 100th day. There was no significant difference detectable among the groups; however, Fig. 4-b and Fig. 4-c show that the total lipid of liver and the serum content of NEFA in the high fat diet group on the 400th day were both much higher than those of the normal or the high protein diet group. Fig. 4-b shows that the liver glycogen of the high fat group on the 400th day was less than that of the normal or the high protein diet group. The total weights of the livers were 10.3 g±2.3 g in the high fat group, 8.2±2.6 g in the normal group, and 9.1±2.4 g in the high protein group on the 400th day; therefore, changes in the total lipid and total glycogen of the liver of the rat on the high fat diet were significant.

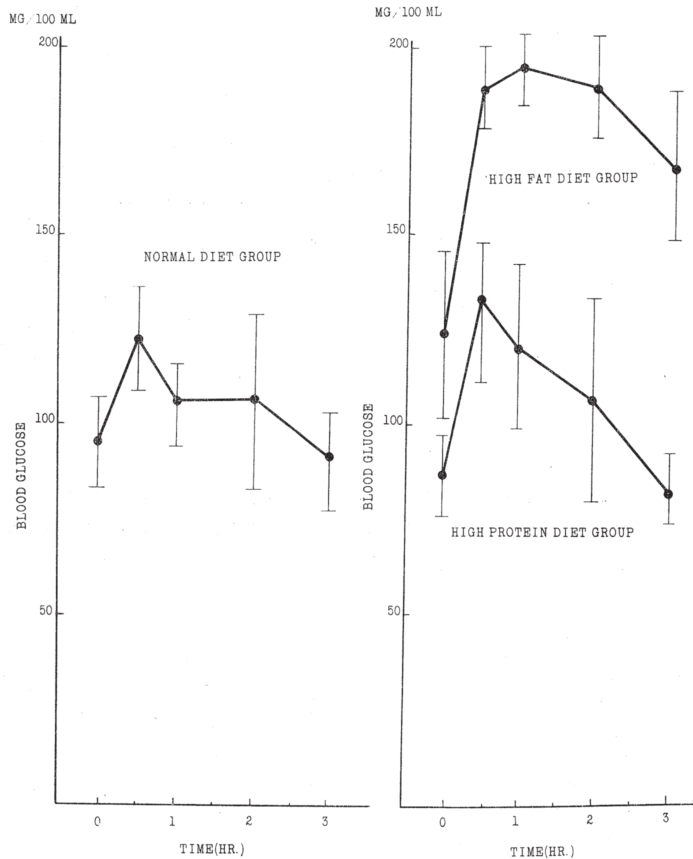


FIG. 3-a. Changes in glucose tolerance curves by the composition of diet.
Each point and bar represents mean±S.D. of measured blood glucose values of seven rats in each group. Rats were fed for 400 days.

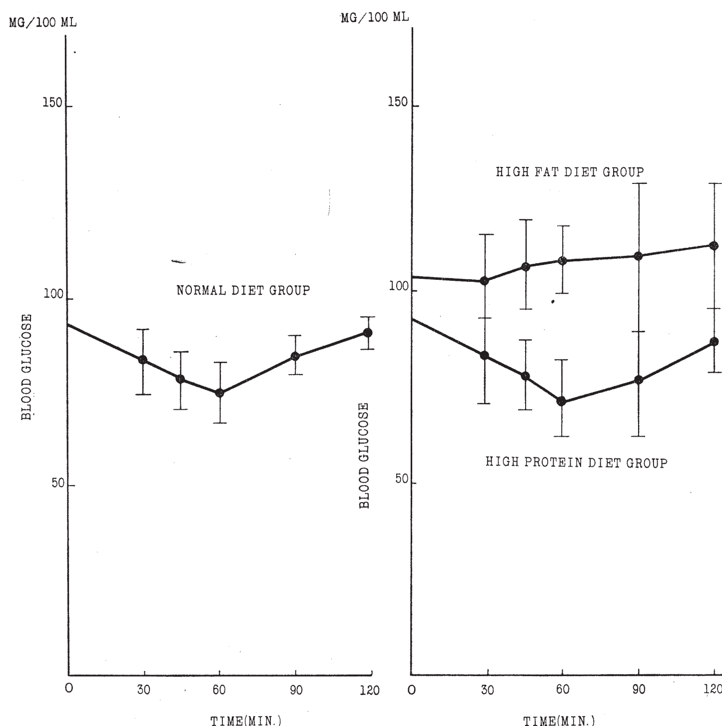


FIG. 3-b. Changes in insulin sensivitity curves by the composition of diets. Each point and bar represents mean±S.D. of measured blood glucose values of seven rats in each group. Rats were fed for 398 das.

4. Experiments in diaphragm and adipose tissue

a) Glucose uptake

In Fig. 5-a is shown the effects of insulin on the glucose uptake of adipose tissue or diaphragm. A significant increase of glucose uptake by insulin was observed in the normal diet group but not in the high fat diet group, and basal glucose uptakes and insulin effects on adipose tissue and diaphragm were apparently decreased in the high fat diet group.

b) Incorporation of glucose-U-C^{14} into CO_2

Fig. 5-b shows that the incorporation of glucose-U-C^{14} into CO_2 markedly increased in the normal diet group when insulin was added, but to a lesser extent in the high fat diet group regardless of the addition of insulin.

c) The effect of papain addition

Fig. 5-c shows changes in $C^{14}O_2$ production from glucose-U-C^{14} in the medium containing the diaphragms and the adipose tissues of the normal and high fat diet group with or without papain. Papain digested diaphragms showed

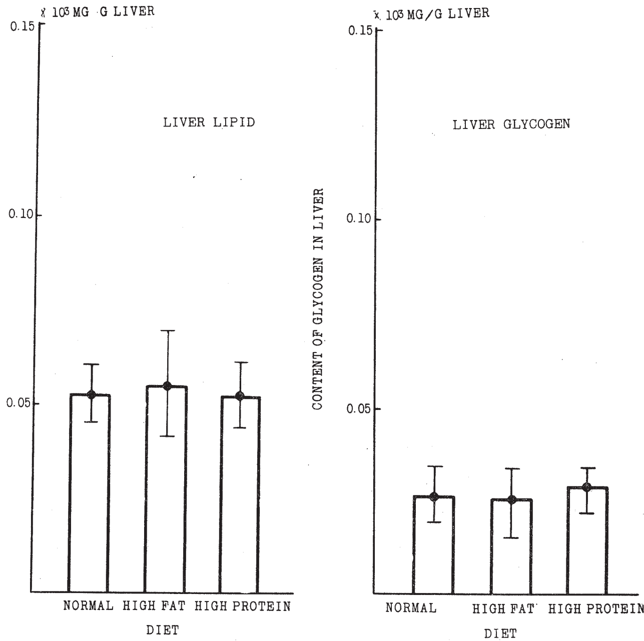


FIG. 4-a. Changes in total fat and glycogen contents in liver by the composition of diet.

Each point and bar represents mean±S.D. of glycogen or fat in liver in seven rats of each group. Rats were fed for 100 days.

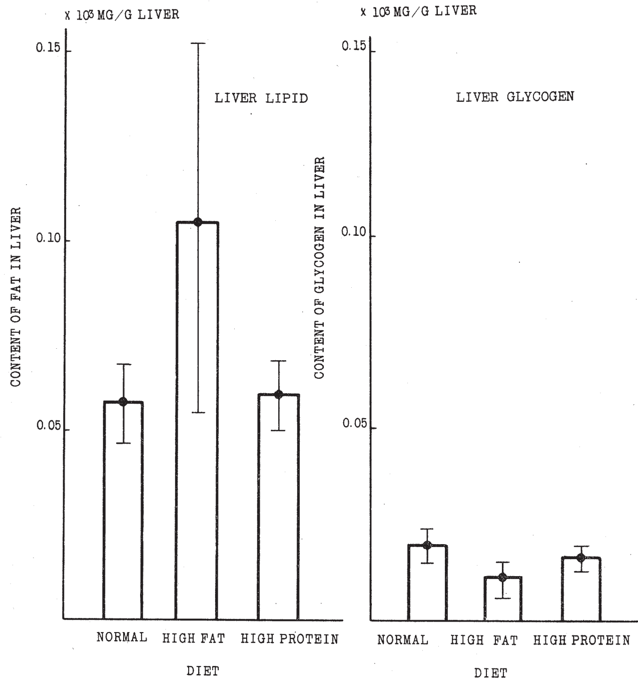


FIG. 4-b. Changes in total fat and glycogen contents in liver by the composition of diet.

Each point and bar represents mean value±S.D. of glycogen or fat in liver in seven rats of each group. Rats were fed for 400 days.

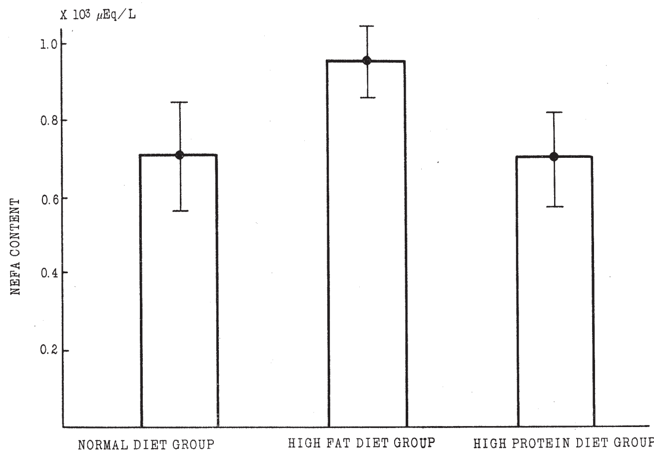


FIG. 4-c. NEFA content in serum of normal, high fat, and high protein diet groups.

Each point and bar represents mean NEFA content±S.D. of seven rats in each group. Rats were fed for 400 days.

decreased incorporated radioactivity. Papain digested adipose tissue in normal diet group showed increased radioactivity, that was, insulin like action. On the contrary, papain digested adipose tissues of high fat diet group showed decreased incorporated radioactivity.

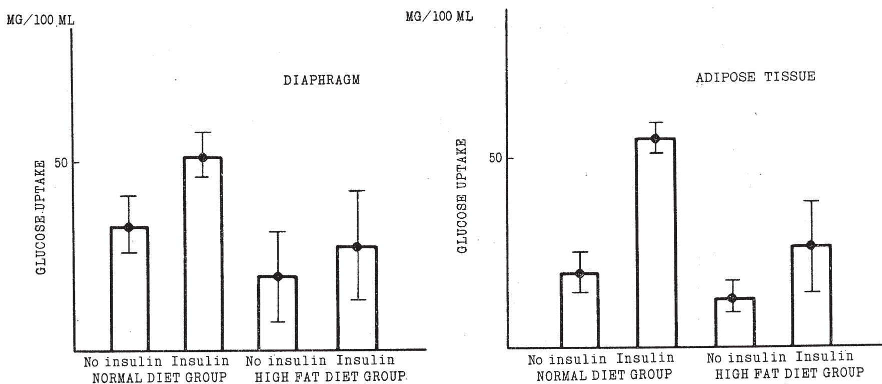


FIG. 5-a. Glucose uptake in diaphragm and adipose tissue with or without insulin addition *in vitro*.

Each point and bar represents mean values±S.D. of glucose uptake in diaphragms and ten adipose tissues of ten rats in each group. To the medium was added to be 0.5 mμ insulin/ml of medium, 50 mg of each, tissue was suspended in two ml of medium. Rats were fed for 105 days.

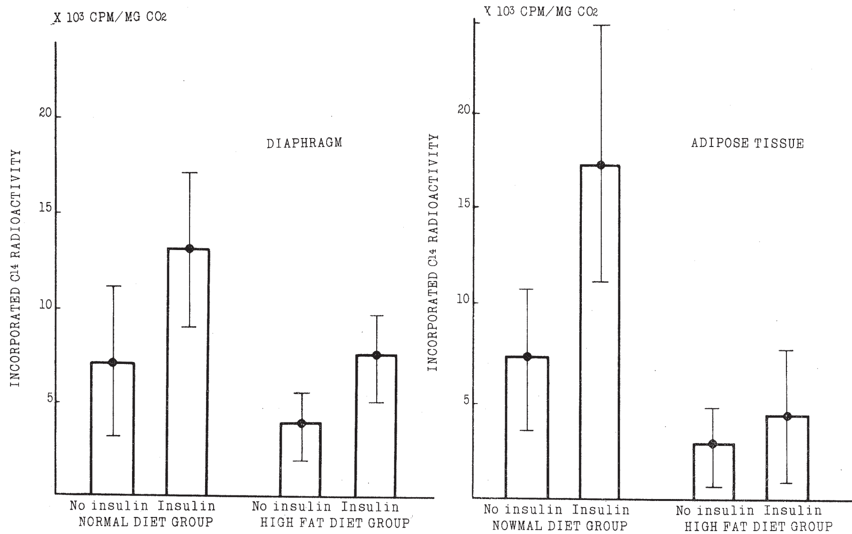


FIG. 5-b. Incorporation of glucose-U-C^{14} into carbon dioxide with or without insulin addition *in vitro.*

Each point and bar represents mean incorporated C^{14} radioactivity in ten tissues of ten rats in each group. To the medium was added 0.5 mμ insulin/ml of medium. Each tissue was suspended in two ml of medium. Rats were fed for 105 days.

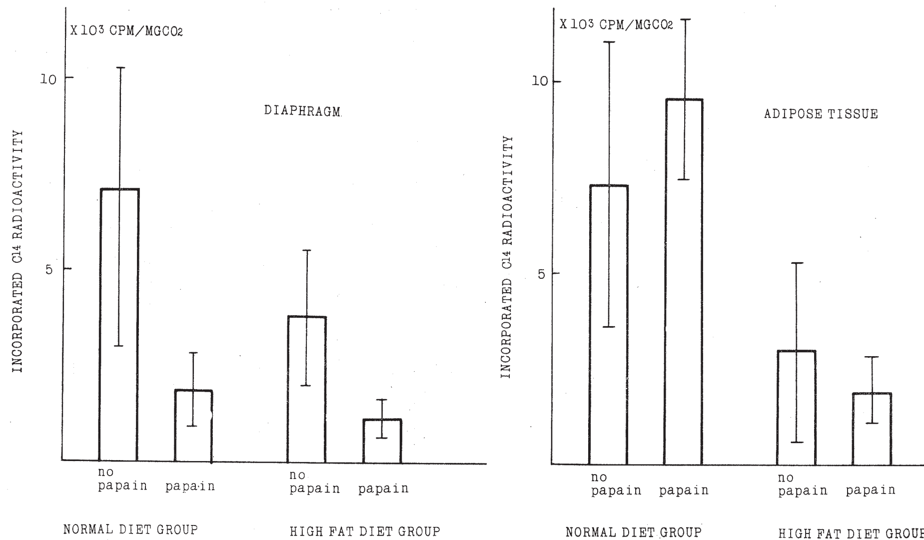


FIG. 5-c. Incorporation of glucose-U-C^{14} into carbon dioxide with papain addition *in vitro.*

Each point and bar represents mean incorporated C^{14} radioactivity in ten tissues of ten rats in each group. To the medium was added 8 mg papain. Each tissue was suspended in two ml of medium. Rats were fed for 105 days.

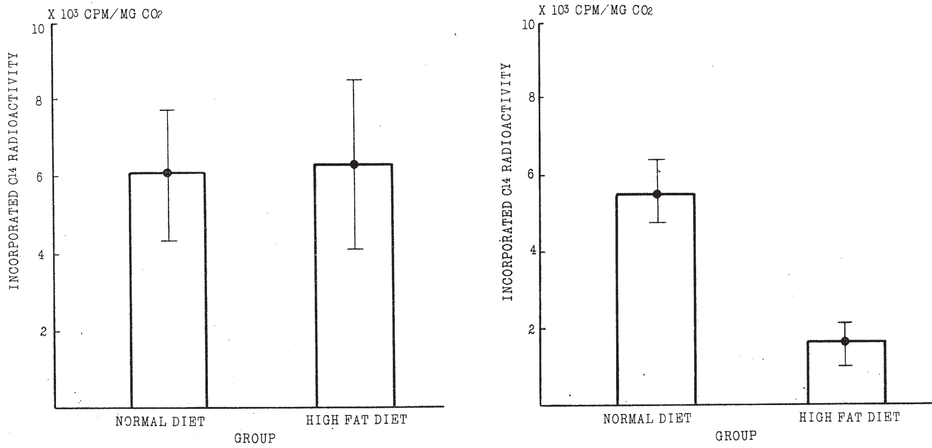


FIG. 6-a. Incorporation of glucose-U-C^{14} into carbon dioxide *in vitro.*

Each point and bar represents mean value±S.D. of incorporated C^{14} radioactivity in liver slices of five rats in each group. 200 mg of liver slice in each group was suspended in two ml of Krebs ringer bicarbonate buffer or Krebs ringer phosphate buffer. Rats were fed for 115 days.

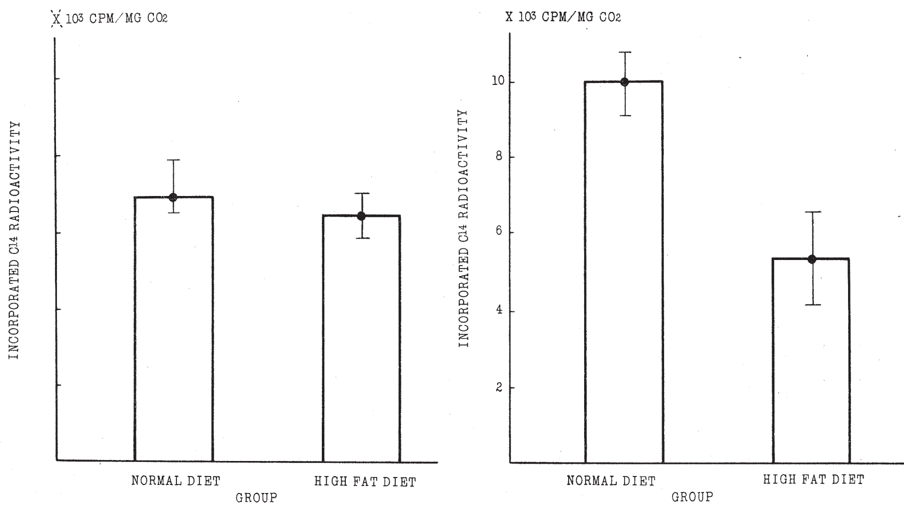


FIG. 6-b. Incorporation of sodium pyruvate-U-C^{14} into carbon dioxide *in vitro.*

Each point and bar represents mean±S.D. of five rats in each group. 200 mg slices of liver in each group was suspended in two ml of Krebs ringer bicarbonate buffer or Krebs ringer phosphate buffer. Rats were fed for 115 days.

5. Experiments in liver slices

a) Incorporation of glucose-U-C^{14} into CO_2

Fig. 6–a shows changes of $C^{14}O_2$ produced from glucose-U-C^{14} in the medium containing the liver slices of the normal and the high fat diet groups. In the medium of Krebs Ringer bicarbonate buffer, no significant difference was observed, but in the medium of Krebs Ringer phosphate buffer the production of $C^{14}O_2$ was less in the high fat diet group than in the normal diet group.

b) Incorporation of pyruvate-U-C^{14} into CO_2

Fig. 6–b shows that $C^{14}O_2$ produced from sodium pyruvate-U-C^{14} was less in the high fat diet group than that in the normal diet group, with Krebs Ringer phosphate buffer, but no significant change was observed with Krebs Ringer bicarbonate buffer.

6. Incorporation of alanine-U-C^{14}, sodium acetate-1-C^{14}, and glucose-U-C^{14} into various substances

a) Incorporation of alanine-U-C^{14} into liver glycogen and blood glucose

As shown in Fig. 7–a, the incorporation of alanine-U-C^{14} into liver glycogen and blood glucose was greater in the high fat rats than in the normal rats on the 110th day, which means that increased gluconeogenesis was observed in the high fat diet group.

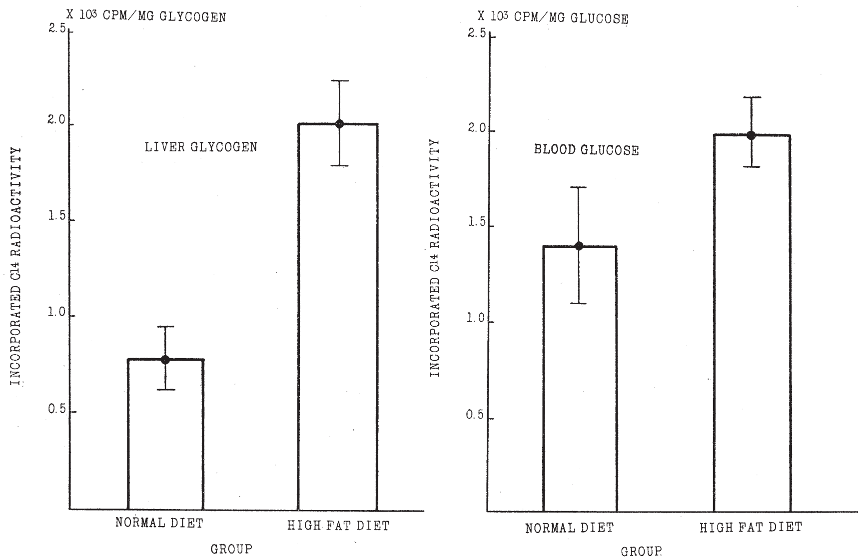


FIG. 7–a. Incorporation of alanine-U-C^{14} into liver glycogen and blood glucose. Each point and bar represents mean values of incorporated C^{14} radioactivity in liver or blood glucose of five rats$\pm$S.D. Rats were fed for 110 days.

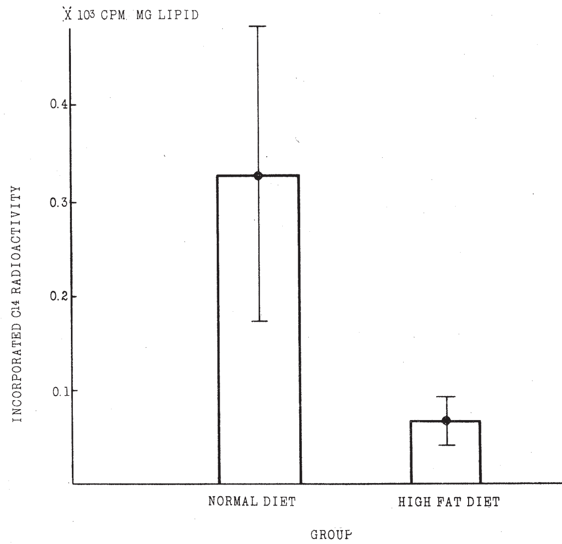


FIG. 7-b. Incorporation of sodium acetate-1-C^{14} into liver fat.
Each point and bar represents mean incorporated C^{14} radioactivity in the livers of five rats in each group±S.D. Rats were fed for 110 days.

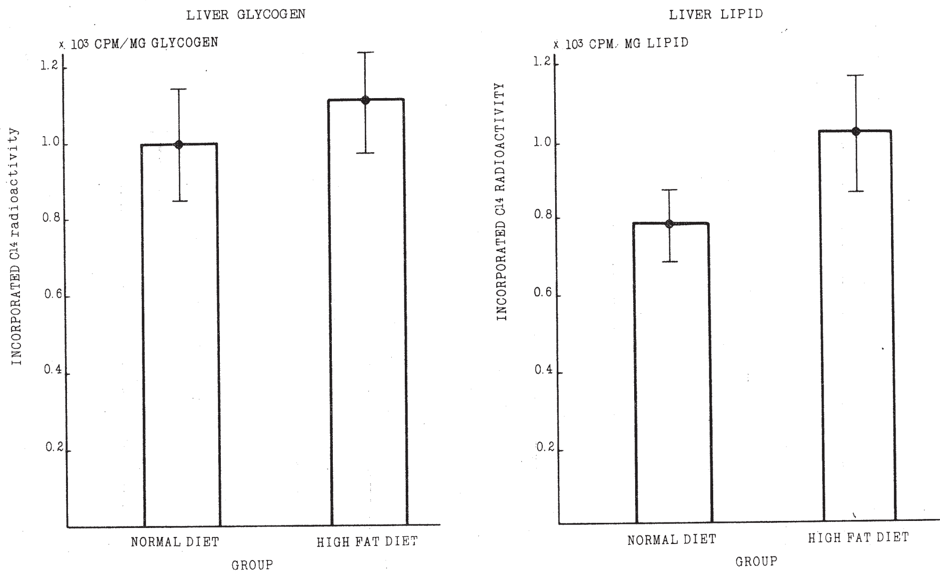


FIG. 7-c. Incorporation of glucose-U-C^{14} into glycogen and fat in liver.
Each point and bar represents mean value of incorporated C^{14} counts per MG glycogen or lipid in the livers of five rats in each group±S.D. Rats were fed for 110 days.

b) Incorporation of sodium acetate-1-C^{14} into liver fat

Fig. 7–b shows that the incorporation of sdium acetate-1-C^{14} into liver fat in the high fat group greatly decreased on the 110th day in comparison with the normal diet group.

c) Incorporation of glucose-U-C^{14} into liver glycogen and liver fat

The incorporation of glucose-U-C^{14} into liver glycogen and liver fat on the 110th day increased more in the high fat diet group than in the normal diet group, as seen in Fig. 7–c. Because the content of liver glycogen was less in the high fat diet group, glycogenolysis in the high fat group seems to have been increased.

7. Insulin content on the pancreas

Table 3 shows the data of the pancreatic insulin content of the rats in the three groups on the 405th day. Insulin content was determined by biological means using rabbits[10]. As shown in Table 3 the amount of pancreatic insulin of the high fat diet group was larger than that of the normal or the high protein diet group.

TABLE 3. Insulin Content of the pancreas

Group	Insulin Content Unit/g of tissue
Normal diet	1.37
High fat diet	1.52
High protein diet	1.36

Pancreatic insulin content in each group was determined using ten pancreases in every group as a pooled sample. Rats were fed for 405 days.

Pancreatic insulin was extracted by method of Best, extracted insulin was injected into rabbits. By changes of blood glucose, pancreatic insulin was estimated.

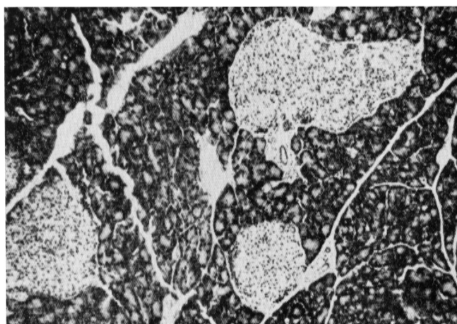
8. Histological finding of the pancreas

Table 4 shows the histological finding of the pancreas. As seen in Fig. 9, the Langerhan's island in the pancreas of the high fat rats on the 400th day shows apparent hypertrophy and their parenchymal cells increased. There was also a remarkable fibrosis in the exocrine gland of the high fat rats (Fig. 10). On the other hand, there was no change in the pancreas of normal diet groups (Fig. 11) and high protein diet groups.

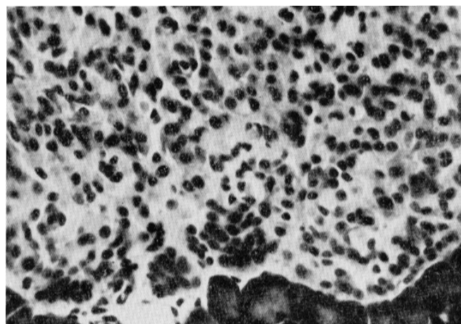
TABLE 4. Histological Finding of Pancreas

Findings	Fibrosis of exocrine gland										Hypertrophy of Langerhan's island									
Rats → Group ↓	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Normal diet	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
High fat diet	⧻	⧺	⧻	−	−	+	⧺	⧻	−	⧺	⧺	⧺	⧻	⧺	+	+	⧺	+	+	+
High protein diet	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

−: means no histological change
+: means small abnormal findings
⧺: is moderate abnormal findings
⧻: is distict abnormal changes
Fig. 9, 10, 11 are histological changes of No. 3 rats of three groups.

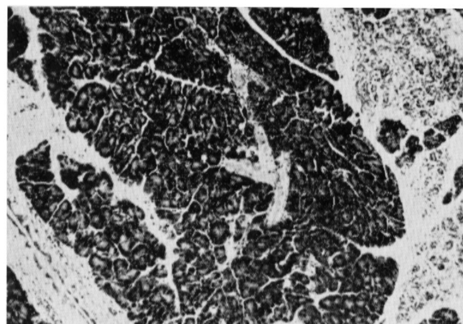


A

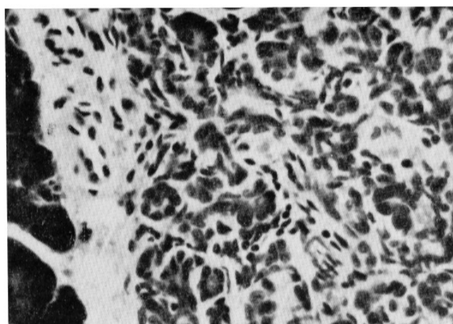


B

FIG. 9. Hypertrophy of Langerhan's island in rats belonging to high fat diet group. A (×100), B (×400)



A



B

FIG. 10. Fibrosis of exocrine glands in pancreas of rats belonging to high fat diet group. A (×100), B (×400)

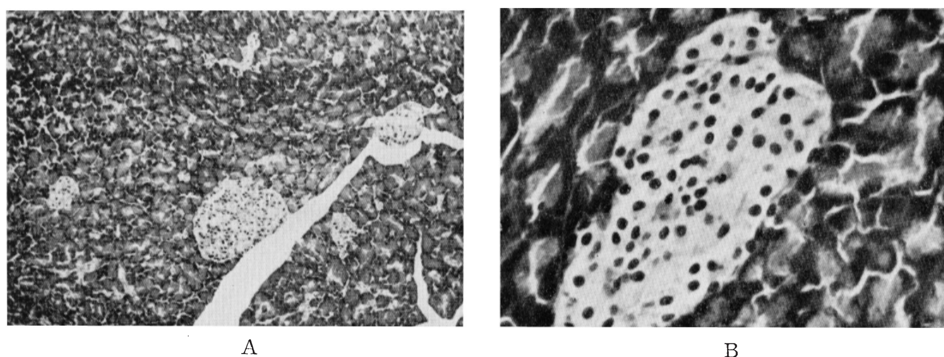


FIG. 11. Normal aspect of Langerhan's island in rats belonging to normal diet group. A (×100), B (×400)

DISCUSSION

The authors[3] have previously reported in the Journal of the Japan Diabetic Soceity that in male albino rats fed various diets having the same calorie contents rats fed high fat diets showed impaired carbohydrate metabolism. This time the author fed rats with a special fat diet having an increased amount of fat, as shown in Table 1, and found in those rats a remarkable increase of body weight, diabetic changes of G.T.T. in the early stage of feeding, and low insulin sensitivity. The low content of glucose in this high fat diet might have great influence on the metabolic changes of rats. But in the rats fed a high protein diet having the same low glucose content as that of the high fat diet, no corresponding change of carbohydrate and fat metabolism was found. This may indicate that not low glucose, but high fat in the diet significantly influenced the metabolic change of animals.

Much work has been done on disturbed carbohydrate metabolism of rats fed high fat diet and the disturbance of glycolysis has been considered as an important pathogensis of disturbed carbohydrate metabolism. Most of these studies[1][2], however, have been performed using excessive amount of fat in the diet for a short period of time, resulting only in a small disturbance of the carbohydrate metabolism.

Gonzlenz, C.[1] reported that low activity of liver glucokinase caused a disturbance of carbohydrate metabolism. In that case his rats were fed for 7 days with the extremely high fat diet which contained no carbohydrate, the essential element of the substrate of glucokinase. His data were based on absence of glucose rather than on high fat diet. On the other hand, Yugari[11] found, in his rats which were fed a high fat diet for 4 days, a decrease in the several liver enzymes in glycolysis, but no change in the liver enzyme in gluconeogenesis.

In this experiment the author fed a high fat diet for a longer period of time than in other experiments. These rats grew remarkably fat, showing abnormal carbohydrate metabolism. This is similar to the condition of the maturity onset type of human diabetes. In the following, the author gives some consideration concerning the pathogensis of the abnormal carbohydrate metabolism of high fat rats. The incorporation of glucose-U-C^{14} or pyruvate-U-C^{14} into CO_2 of liver slices in the high fat diet group was normal in the medium of Krebs Ringer bicarbonate buffer, but it was apparently decreased in the medium of Krebs Ringer phosphate buffer.

The experimental results mentioned above suggest that glycolysis was impaired under the aerobic condition, but that glycolysis showed no change under the anaerobic condition, even though CO_2 fixation is taken into considertion. Since lipolysis in the liver takes place so rapidly under the aerobic condition, glycolysis might be impaired. For this reason, the incorporation of glucose-U-C^{14} into triglyceride increased and that of sodium acetate-1-C^{14} into fat decreased, while a part of the fatty acid was incorporated into triglyceride. The decreased incorporation of sodium acetate-1-C^{14} into liver fat means that the synthesis of fatty acids from acetyl CoA decreased markedly. The reason why the total lipid of liver did not increase too much in the high fat rats means that the lipolysis in the liver was great in the high fat fed rats. On the other hand, the incorporation of alanine-U-C^{14} into liver glycogen and blood glucose increased, which indicates that there was an increase of gluconeogenesis. It appears that this increase was caused partly from the decreased activities of liver enzymes of glycolysis due to the increased breakdown of fatty acid and partly to the increased pyruvate carboxylase activity due to the increased level of acetyl CoA.

In the feeding of the high fat group, the rats body weight markedly increased in the early stage of feeding, reaching the peak of increase on the seventh or the eighth month and finally maintaining approximately constant weight. From the tests made on the 105th day on the high fat diet group, the glucose uptake, the incorporation of glucose-U-C^{14} into CO_2, and the effect of insulin at the fat pad and diaphragm showed low values. Papain treated diaphragms of normal and high fat diet groups showed the decrease in incorporated radioactivity. In the case of the adipose tissues of normal diet group, papain had an insulin like action, but papain digested adipose tissue in high fat diet groups showed the decrease in incorporated radioactivity, suggesting some difference in membrane structure of adipose tissue between both groups. In rats fed high fat diet keeping their stout condition for a certain period of time, their adipose tissue mainly consists of accumulated lipid, the activity of metabolism per unit weight might have decreased and its membrane structure might have suffered to such an extent that lower glucose uptake and insulin effects might be observed in the adipose tissue. On the other hand, the glycolytic system

of the diaphragm can be inhibited by the increased oxidation of NEFA, resulting both in the lowered insulin effect and lowered glucose uptake, which, if hypothesized so, the is equivalent to Randle's glucose fatty acid cycle. It is probable that because membrane structure of diaphragms in both groups seem to be identical, the decreased incorporation of glucose-U-C^{14} into CO_2 at the diaphragms might have resulted from the decreased glucose utilization due to the vigorous oxidation of fatty acid. The impaired oxidation of glucose in the diaphragm is considered to be one of the important factors which disturbed the carbohydrate metabolism. The lowered oxidation of glucose, lowered insulin sensitivity, impaired glycolysis in the liver, and the increased gluconeogenesis is considered to be an adaptive phenomena because of the vigorous oxidation of fatty acids. And it appears that no remarkable change of carbohydrate metabolism is induced by high fat feeding beyond the limit of adaptation, as long as animals have intact pancreases. It is reasonable from the point of adaptation that in the high fat group the increased secretion of pancreatic insulin and the hypertrophy of the Langerhan's island were observed. The mechanism of disturbance of carbohydrate metabolism in high fat rats would also be affected by other endocrine glands than the pancreas and the autonomic nerve system. Fig. 8 illustrates a simplified mechanism of disturbed carbohydrate metabolism.

The disturbed carbohydrate metabolism of high fat rats resembles the maturity onset type of human diabetes mellitus, which means that a high intake of fat could be one of the causes of human diabetes mellitus.

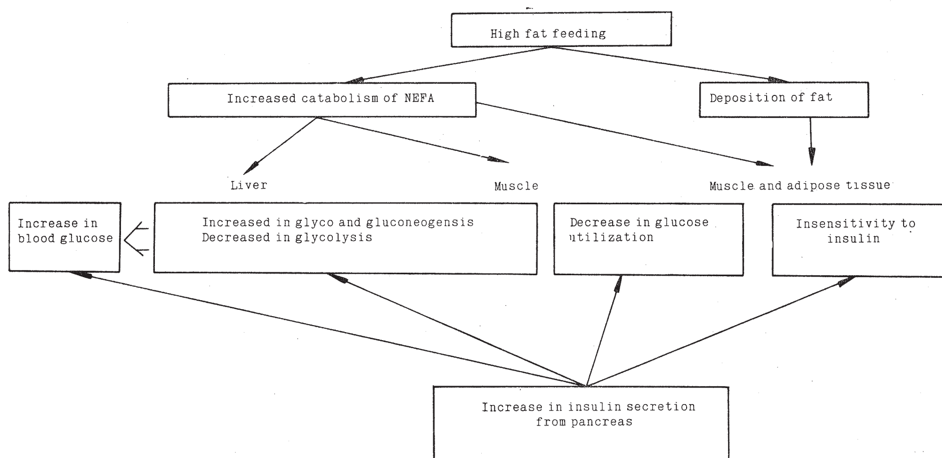


FIG. 8. Mechanism of disturbed carbohydrate metabolism induced by high fat diet.

SUMMARY

1) Rat fed with high fat diet showed abnormal glucose tolerance test and insulin sensitivity test.

2) The decrease of glucose utilization and the increase of gluconeogenesis were observed in the tested liver slices of rats in the high fat diet group.

3) Glucose utilization and insulin sensitivity of adipose tissues and diaphragms of rats fed with high fat diet were significantly reduced comparing with other groups.

4) The changes on the glucose utilization of a adipose tissues with the addition of papain in the medium were different in both groups indicating the different membrane structure of adipose tissues in high fat diets groups. The changes of the membrane structure of adipose tissue in high fat group may induce the lower glucose uptake and lower insulin sensitivity at the adipose tissue.

5) Pancreases of rats in the high fat diet group contained large Langerhan's island and fibrosis of exocrine glands. Author discussed the pathogenesis of distubed carhohydrate metabolism in high fat diet groups.

ACKNOWLEDGEMENT

The author thanks Prof. Kozo Yamada for his kind advices.

REFERENCES

1) Gonzalenz, C. Ureta, T., Sanchez, R. and Numeyer, H., Multiple molecular forms of ATP hexose phosphate transfuse from rat liver, *Biochem. Biophys. Res, Commum.*, **16**, 347, 1964.
2) Randle, P. J., Garland, P. B., Hales, C. N. and Newsholme, E. A., The glucose fatty acid cycle, *Lancet* **I**, 785, 1963.
3) Yamada, K. and Katsumata, K., Studies on the impaired carbohydrate metabolism induced by high fat diet. *J. Jap. Diabet. Soc.*, **9**, 112, 1966 (in Japanese).
4) Bragdon, J. H., Colorimetric determination of blood lipids, *J. Biol. Chem.*, **190**, 513, 1951.
5) Dole, V. P., A relation between none esterified fatty acid in plasma and metabolism of glucose, *J. Clin. Invest.*, **35**, 150, 1956.
6) Hassid, W. Z. and Abraham, S., Determination of glycogen, Method in Enzymology III, Academic Press, New York, 1967, p. 34.
7) Dubois, M., Determination of polyscharide, *Anal. Chem.*, **28**, 350, 1956.
8) Melton, S., Schrunpad, R., Wagle, M. and Ashmore, J , Studies on experimental diabetes, *J. Biol. Chem.*, **238**, 12, 1963.
9) Best, C. H., Jehcott, C. M. and Scott, D. A., Insulin in tissue other than pancreas, *Amer. J. Physiol.*, **100**, 285, 1932.
10) Commentary of the Japanese pharmacopeia, Nanko Co., Tokyo (in Japanese), Ed VI, 1951, p. 791.
11) Yugari, Y., Effect of fat containing diet on the sugar metabolism in rat liver, *Proc. Symp. Chem. Physiol. Path.*, **4**, 43, 1964.